bij
- Invasieve candidiasis[1]
- Invasieve aspergillose[2]
- Empirische antifungale therapie[3]

- Bewezen effectiviteit[1]
- Gunstig veiligheidsprofiel[4]
- Bij volwassenen en kinderen[5,6]

Referenties:
1. Mora-Duarte J.: Comparison of caspofungin and amphotericin B for invasive candidiasis. *N Eng J Med* 347;2020-9, 2002. 2. Maertens J.: Efficacy and safety of caspofungin for treatment of invasive aspergillosis in patients refractory to or intolerant for conventional antifungal therapy. *CID* 2004;39:000-000. 3. Walsh T.J.: Caspofungin versus Liposomal Amphotericin B for empirical antifungal therapy in patients with persistent fever and neutropenia. *N Eng J Med* 2004; 351:1391-402. 4. David W. Denning: Echinocandin antifungal drugs. *The Lancet* 362: 1142-51, 2003. 5. Walsh TJ: Pharmacokinetics, safety and tolerability of caspofungin in children and adolescents. *AAC* 49: 4536-4545, 2005. 6. Zaoutis TE: A prospective, multicenter study of caspofungin for treatment of documented candida or aspergillus infections in pediatric patients. *Pediatrics* 123:877-884, 2009.

1110CAN09NL539J1109

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NEDERLANDS TIJDSCHRIFT VOOR

Medische Microbiologie

Supplement bij negentiende jaargang, april 2011

Voorjaarsvergadering van de Nederlandse Vereniging voor Medische Microbiologie (NVMM)
en de Nederlandse Vereniging voor Microbiologie (NVvM)

Papendal, 18-20 april 2011
Programma-overzicht
Abstracts
Auteursindex

19e jaargang . april 2011 . Supplement

STRIKE!

Candida lusitaniae
Candida dubliniensis
Candida kefyr
Candida rugosa
Candida albicans
Candida krusei
Candida tropicalis
Candida glabrata
Candida parapsilosis
Candida guilliermondii
MYCAMINE®

Wereldwijd al meer dan 600.000 patiënten behandeld[1]

Hoog gebruiksgemak
- Geen oplaaddosis[2]
- Geen dosis aanpassing[2]
- Geen specifieke bewaarcondities[2]

Voor alle leeftijden (0-99)[2]

Productinformatie: zie elders in deze uitgave. MYC2010-652

Ruim een decennium focus op infectieserologie

Geautomatiseerd betrouwbaar meten door middel van ELISA techniek

Klopt dit wel?

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The Scientific spring meeting 2011 is organized by the Dutch Society of Medical Microbiology (NVMM) and the Dutch Society of Microbiology (NVvM).

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Snijders Scientific
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Tritium Microbiologie
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Westburg

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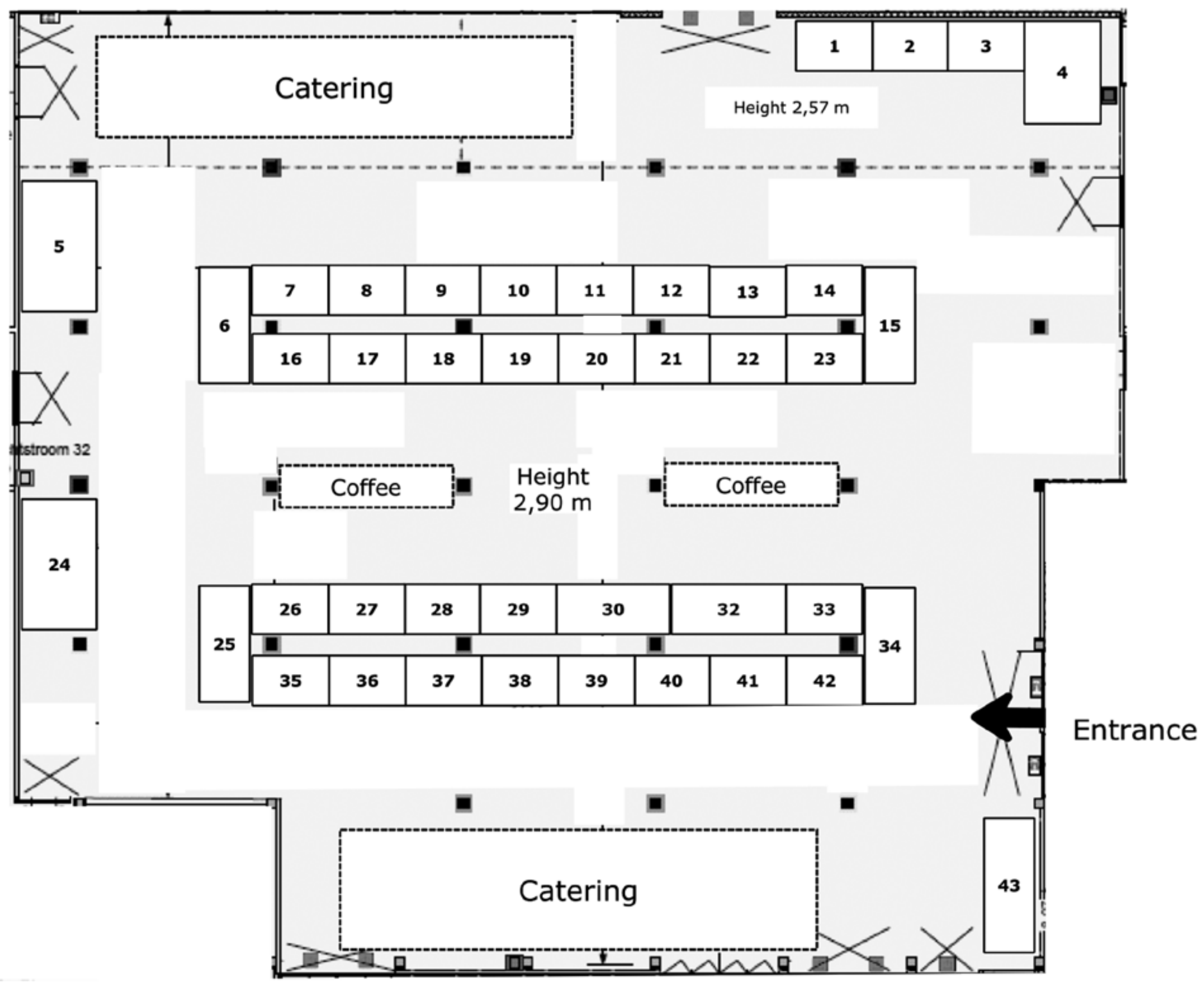
EXHIBITION - ROOM SYDNEY



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2	Meridian Bioscience
3	Bruker Daltonics
4	i2a Benelux
5	Abbott Molecular
6	Bio Rad Laboratories
7	Biolegio
8	Snijders Scientific
9	Carl Zeiss
10	New Brunswick Scientific Benelux
11	Astellas Pharma
12	MLS
13	Labolutions
14	Cepheid Benelux
15	Siemens Healthcare Diagnostics
16	Beldico
17+18	DiaSorin
19	Tritium Microbiologie
20	Menarini Diagnostics

Booth number	Company
21	MIPS
22	Alere Health
23	Technidata Benelux
24	Pfizer
25	Roche Diagnostics
26	Beckman Coulter
27	Lucron Bioproducts
28	R-Biopharm AG
29	Luminex
30	Qiagen Benelux
32	BD
33	Kiestra Lab Automation
34	Minigrip Nederland
35	Bodégro
36	Check-Points
37	Pathofinder
38	Appled Maths
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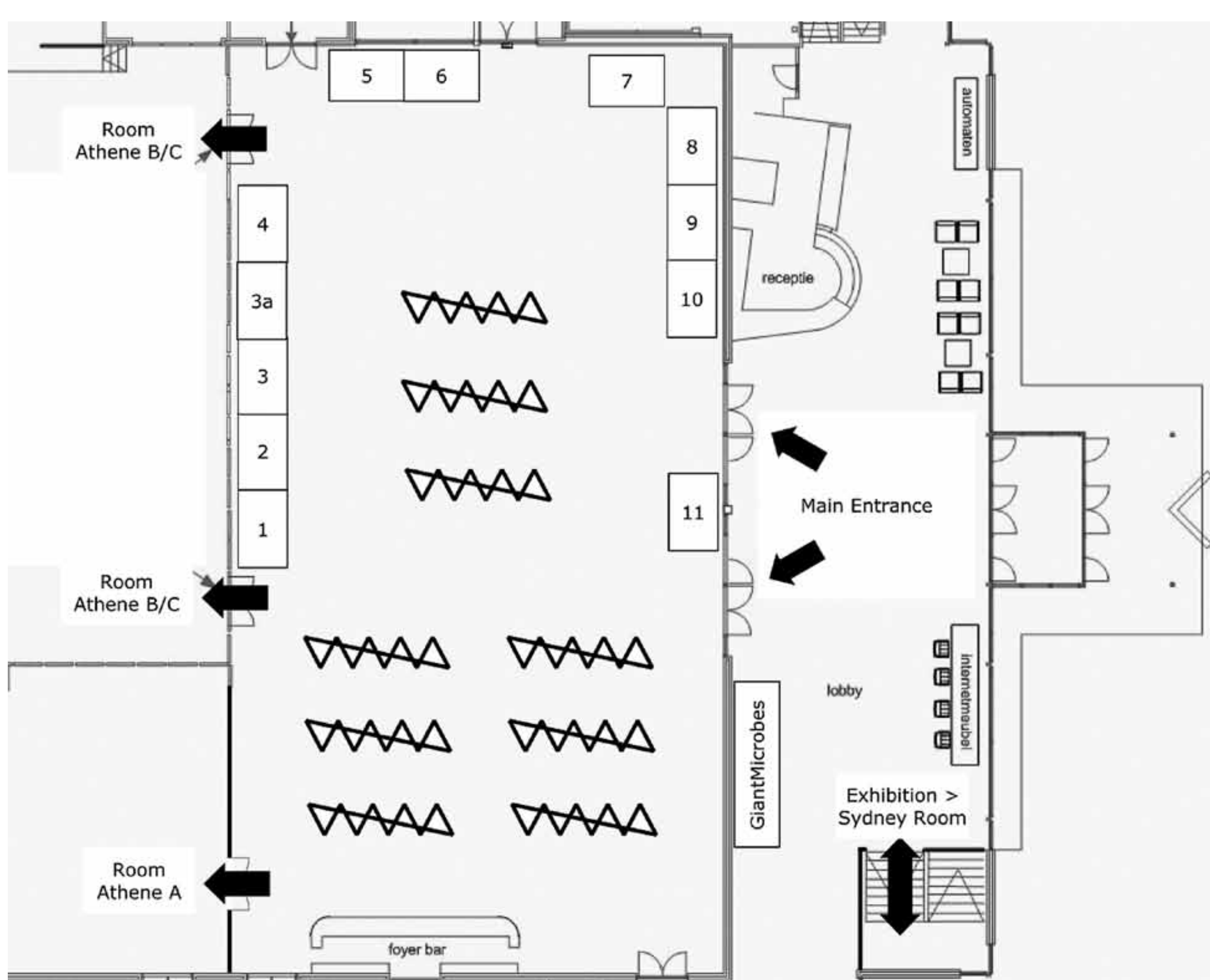
43	Oxoid



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3a	BioMérieux
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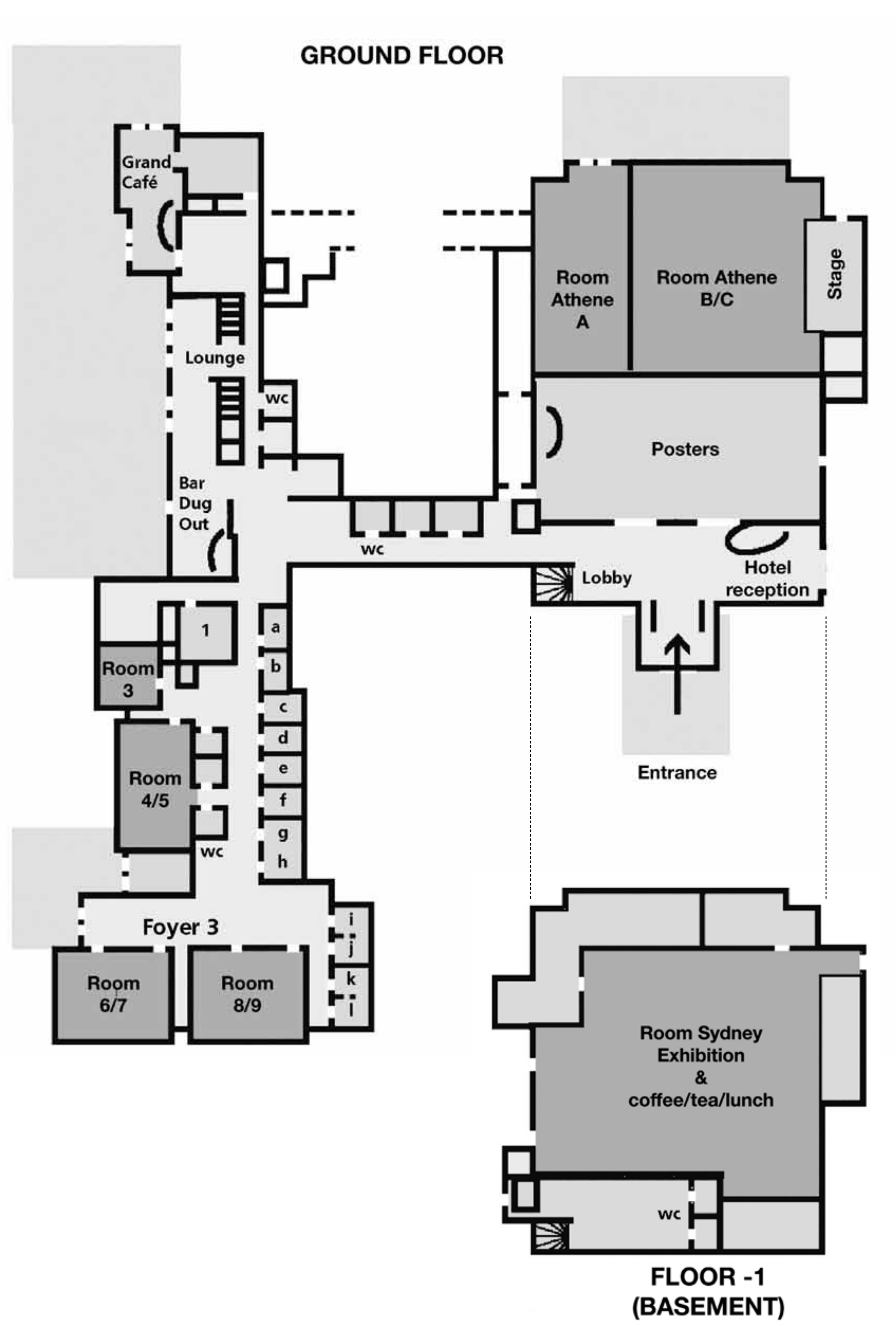
GROUND FLOOR
Grand Café
Lounge
Bar Dug Out
wc
Room Athene A
Room Athene B/C
Stage
Posters
wc
Lobby
Hotel reception
Entrance
1
Room 3
Room 4/5
wc
a
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g
h
Foyer 3
Room 6/7
Room 8/9
i
j
k
l
Room Sydney Exhibition & coffee/tea/lunch
wc
FLOOR -1 (BASEMENT)

MONDAY APRIL 18 2011

09:00 - 10:00 **Registration**

10:00 - 10:20 **Opening ceremony with his Royal Highness the Prince of Orange**

10:20 - 10:25 **Introduction to Joan W. Bennett**
Jos van Strijp

10:25 - 10:45 **History of Dutch Microbiology**
Joan W. Bennett (USA)

10:45 - 11:15 **Coffee/tea break**

11:15 - 11:25 **Antonie van Leeuwenhoek (1632-1723): Discovery of microorganisms**
Gijs Kuenen

11:25 - 12:15 **The contemporary van Leeuwenhoek**
J. Craig Venter (USA)

12:15 - 13:15 **Lunch**

13:15 - 13:20 **FEMS**
Bernhard Schink, FEMS President

13:20 - 13:25 **Martinus Beijerink (1851-1931): Discovery of viruses**
Lesley Robertson

13:25 - 14:15 **The contemporary Beijerink**
Harald zur Hausen (Germany)

14:15 - 14:25 **Johanna Westerdijk (1883-1961): Microbial Interactions**
Francine Govers

14:25 - 15:15 **Origin of the eukaryotic cells in the mid-Proterozoic Eon**
Lynn Margulis (USA)

15:15 - 15:45 **Coffee/tea break**

15:45 - 15:55 **Christiaan Eijkman (1858-1930): Bacteriology**
Jos van Strijp

15:55 - 16:55 **The contemporary Eijkman**
Barry Marshall (Australia)

16:55 - 17:05 **Albert Kluyver (1888-1956) and Cornelis van Niel (1897-1985): Unity in Biochemistry**
Jack Pronk

17:05 - 18:05 **The contemporary Kluyver & van Niel**
Paul Nurse (United Kingdom)

18:05 - 18:10 **Closing ceremony**
Jos van Strijp

18:10 - 18:25 **Future of Dutch microbiology and the NVvM**
Huub Schellekens

Sydney/Exhibition

18:25 - 19:30 **Reception**

Restaurant

19:30 - 21:30 **Dinner**

Athene

21:30 - 23:00 **Poster session - even poster numbers**

TUESDAY APRIL 19 2011

08:30 - 09:00 **Registration**

09:00 - 11:00 **Parallel sessions**

Athene B/C **Gut commensals - the good, the bad and the unknown**
Chairs: W. van Schaik & R. Willems

09:00 - 09:30 **Enterococcal Diversity: Understanding the emergence of multidrug resistant strains by knowing their roots**
O001 M.S. Gilmore (USA)

09:30 - 10:00 **Our other genome - the MetaHIT catalog of intestinal bacterial genes**
O002 S.D. Ehrlich (France)

10:00 - 10:30 **Lactobacilli in the small intestine, and how we respond to them**
O003 M. Kleerebezem

10:30 - 10:45 **IS-pro: fully automated analysis of the human intestinal microbiota**
O004 A.E. Budding

10:45 - 11:00 **Human *in vivo* responses to commensal and probiotic lactobacilli**
O005 P. van Baarlen

Athene A **Antibiotics and the bacterial cell envelope, a mixed blessing**
Chair: B.J. Appelmelk

09:00 - 09:30 **Molecular transport across porins: Rate limiting interactions**
O006 M. Winterhalter (Germany)

09:30 - 10:00 **Intervention opportunities in the mycobacterial cell envelope**
O007 L. Kremer (France)

10:00 - 10:30 **Lipid II as target for (l)antibiotics**
O008 E.J. Breukink

10:30 - 10:45 **Expression of multidrug resistance ABC transporter genes in *Bacillus subtilis***
O009 E. Reilman

10:45 - 11:00 **Comprehensive identification of genes required for antibiotic resistance and bile tolerance in the nosocomial pathogen *Enterococcus faecium***
O010 X. Zhang

Room 4/5 **Molecular microbiology**
Chair: H.A.B. Wösten

09:00 - 09:30 **The membrane potential is important for bacterial cell division**

O011 L.W. Hamoen

09:30 - 09:45 **Isolation of a prokaryotic cell organelle from the uniquely compartmentalized anammox bacteria**

O012 S. Neumann

09:45 - 10:00 **Mapping the interactions of the Twin-arginine translocation system (Tat) in *Bacillus subtilis***

O013 C.G. Monteferrante

10:00 - 10:15 **A model to study drug-induced mitochondrial dysfunction**

O014 R. de Boer

10:15 - 10:30 **Gel-free proteomic identification of the *Bacillus subtilis* insoluble spore coat protein fraction.**

O015 W. Abhyankar

10:30 - 10:45 **Protein complexes involved in membrane-bound electron transport of anammox bacteria**

O016 N. de Almeida

10:45 - 11:00 **Heterogeneity in micro-colonies of *Aspergillus niger* in liquid shaken cultures**

O017 G.J. Veluw

Room 6/7 WOGIZ: Public health consequences of molecular diagnosis in gastroenteritis

Chairs: S.B. Debast & M. Scholing

09:00 - 09:30 **Consequences of implementation of moleculair diagnostics of gastroenteritis outside and inside the laboratory**

O018 J.F.L. Weel

09:30 - 10:00 **Improved detection of five major gastrointestinal pathogens by use of a molecular screening approach**

O019 R.F. de Boer

10:00 - 10:15 **About STEC Working Group and Shigella outbreaks**

O020 J.H. van Zeijl

10:15 - 10:30 **Case control study gastroenteritis**

O021 L.E.S. Bruijnesteijn van Coppenraet

10:30 - 10:45 **Realtime PCR reveals the presence of three distinct Brachyspira species in human spirochaetosis**

O022 L.J. Westerman

10:45 - 11:00 **Reduction of workload of microbial gastroenteritis diagnostics by molecular pre-screening**

O023 W.A. Reijden

Room 8/9 Polymicrobial interactions during human health and disease

Chair: D.A. Diavatopoulos

09:00 - 09:30 **Interactions between respiratory bacteria and pneumovirus infections: Consequences for the pathogenesis of RSV- and HMPV-mediated severe disease**

O024 R.L. de Swart

09:30 - 10:00 **Recognition of peptidoglycan from the microbiota by Nod1 enhances systemic innate immunity**

O025 T.B. Clarke

10:00 - 10:30 **The human GI tract microbiota in health and disease**

O026 E.G.Z. Zoetendal

10:30 - 11:00 Discussion

Room 3 Antimicrobial resistance 1

Chair: M.A. Leverstein - van Hall

09:30 - 09:45 **Phosphatidylglycerol derived lipids are key to weak organic acid stress resistance in *Bacillus subtilis***

O029 J.W.A. Beilen

09:45 - 10:00 **Serotype related variation in *Streptococcus pneumoniae* susceptibility to human antimicrobial peptide LL-37 cathelicidin**

O030 P. Huizinga

10:00 - 10:15 **Macrolide resistance determination and molecular typing of *Mycoplasma pneumoniae* by pyrosequencing**

O031 E.B.M. Spuesens

10:15 - 10:30 **Retrospective analysis of candidemia in a tertiary medical center, 2004-2010 and its implications for empirical treatment.**

O032 P.R. Goswami

10:30 - 10:45 **Functional metagenomic analysis of the reservoir of antibiotic resistance genes in intensive care unit patients**

O033 E.B. Buelow

10:45 - 11:00 **Colistin resistance in gram-negative bacteria (GNB) during prophylactic colistin use in Intensive Care Units (ICU)**

O034 E.A.N. Oostdijk

11:00 - 11:15 **High rates of intestinal colonization with extended-spectrum beta-lactamase-producing Enterobacteriaceae in patients at a tertiary-care hospital in Israel**

O035 M. Gazin

11:15 - 11:30 Discussion

11:00 - 11:30 Coffee/tea break

11:30 - 13:30 Parallel sessions

Athene B/C ®evolution in vaccines

Chairs: A.J.W. van Alphen & A.D.M.E. Osterhaus

11:30 - 12:00 **Influenza vaccines**

O037 A.D.M.E. Osterhaus

12:00 - 12:30 **Vaccines against vector-borne diseases**

O038 G. Sutter (Germany)

12:30 - 13:00 **Polio eradication by vaccination**

O039 P.D. Minor (United Kingdom)

13:00 - 13:15 **Possible important differences in cellular immune responses after the change in the vaccination programme from the whole cell pertussis vaccine to an acellular vaccine**

O040 A.M. Buisman

13:15 - 13:30 **Long-lasting sterile protection and cellular immune responses against *P. falciparum* malaria in human volunteers**

O041 M.B.B. McCall

Athene A	**Biofilms**
	Chairs: T. Abee & O.P. Kuipers
11:30 - 12:00	**Biofilms of Gram-negative pathogens**
O042	J.M. Ghigo (France)
12:00 - 12:30	**Biofilm formation in *Salmonella enterica* serovar Typhimurium – more than a bacterial lifestyle**
O043	U. Römling (Sweden)
12:30 - 13:00	**Architecturally complex colony development in *Bacillus subtilis***
O044	A.T. Kovacs
13:00 - 13:15	**Mechanisms in *Listeria monocytogenes* biofilm formation and disinfectant resistance**
O045	S. van der Veen
13:15 - 13:30	**Biofilm development on new and cleaned membrane surfaces**
O046	L.A. Bereschenko

Room 4/5	**Fishing for parasites**
	Chair: L.M. Kortbeek
11:30 - 12:00	**Codfish cooked slowly**
O047	L.M. Kortbeek
12:00 - 12:30	**Fever and eosinophilia after visiting Italy**
O048	A.M. Vondeling
12:30 - 13:00	**Epidemiological and clinical aspects of opisthorchiasis in Italy**
O049	E. Pozio (Italy)
13:00 - 13:15	**Salmon surprise**
O050	J.J. Verweij
13:15 - 13:30	**Toxocara and Ascaris seropositivity among patients suspected of visceral and ocular larva migrans in the Netherlands: trends from 1998 to 2009**
O051	E. Pinelli

Room 6/7	**Eco-physiology**
	Chair: M.S.M. Jetten
11:30 - 12:00	**Ecophysiology and genomics of key nitrite-oxidizing bacteria**
O052	H. Daims (Austria)
12:00 - 12:15	**Analysis of bacterial and archaeal diversity in coastal microbial mats by using massive parallel 16S rRNA gene tag-sequencing**
O053	H.B. Henk
12:15 - 12:30	**Enrichment of nitrite-dependent methane oxidizers from an acidic peatland**
O054	B. Zhu
12:30 - 12:45	**Symbiosis in marine sponges: intimacy or flirts?**
O055	D. Sipkema
12:45 - 13:00	**Metabolic engineering of the cyanobacterium Synechocystis sp. PCC 6803: Modification of its carbon metabolism for the synthesis of fermentation products**
O056	S.A. Angermayr
13:00 - 13:15	**Biopolymer utilization by planktonic and biofilm bacteria in oligotrophic freshwater environments**
O057	E.L.W. Sack
13:15 - 13:30	**Methylacidiphilum fumariolicum SolV uses the Calvin cycle for carbon assimilation**
O058	A.F. Khadem

Room 8/9	**Microbiology in International Public Health: Dutch Input**
	Chair: E. Bowles
11:30 - 12:00	**International collaboration: a necessity in public health and especially in infectious diseases**
O059	R.A. Coutinho
12:00 - 12:30	**Inappropriate antibiotic use and resistance in Asia and it's global impact; a delicate balance of antibiotic accessibility**
O060	H.F.L. Wertheim (Vietnam)
12:30 - 12:45	**Goulash and Boerenkool, an evaluation of the Netherlands-Hungary microbiology exchange program**
O061	G.J.H.M. Ruijs
12:45 - 13:00	**Clinical Microbiology from a Dutch and German perspective**
O062	W.E. Silvis
13:00 - 13:15	**Human Enterovirus 71: Europe versus Asia**
O063	S.M.G. van der Sanden
13:15 - 13:30	**Molecular surveillance of multidrug resistant tuberculosis in the European Union; identification of a major clone and quality of VNTR typing**
O064	J.L. de Beer

Room 3	**Clinical microbiology 1**
	Chair: M.J.H.M. Wolfhagen
11:30 - 11:45	**Discriminating Lyme neuroborreliosis from other neuro-inflammatory diseases using levels of CXCL13 in cerebrospinal fluid**
O065	N.D. van Burgel
11:45 - 12:00	**Increased frequency of positive Lyme serology in patients with aspecific skin lesions**
O066	A.P. van Dam
12:00 - 12:15	**Measuring T-cell Responses in Q fever using a newly developed ELISPOT assay**
O067	J.J.M. Bouwman
12:15 - 12:30	**Potential value of an ELISPOT interferon gamma release assay as a diagnostic tool in Q fever infection**
O068	G.J.M. Limonard
12:30 - 12:45	**Low prevalence of *Coxiella burnetii* endocarditis in patients with a history of valve surgery or cardiac valve prosthesis in a Q fever endemic area**
O069	L.M. Kampschreur
12:45 - 13:00	**QSZB study: a cross-sectional study of Q fever in patients with an aneurysm, vascular and/or heart valve prostheses in an endemic region**
O070	M.C.A. Wegdam-Blans
13:00 - 13:30	Discussion

13:30 - 14:30	**Lunch**

Athene A

13:30 - 14:30 **NVvM business meeting**

14:30 - 16:30 **Parallel sessions**

Athene B/C **Antimicrobial resistance in the environment and humans: Food for thought**

Chair: C.M.J.E. Vandenbroucke-Grauls

14:30 - 15:00 **Resistance in Gram-negative bacteria: An inconvenient truth**

O073 J. Kluytmans

15:00 - 15:30 **How little we know about gene dissemination!**

O074 A. Andremont (France)

15:30 - 16:00 **How little we know about epidemiology of *E. coli*!**

O075 N. Frimodt-Møller (Denmark)

16:00 - 16:15 **Prevalence of ESBL-producing Enterobacteriaceae (ESBL-E) in Raw Vegetables**

O076 E.A. Reuland

16:15 - 16:30 **Prevalence of extended-spectrum Beta-lactamase producing Enterobacteriaceae in faecal samples of patients in the community**

O077 I. Overdevest

Athene A **Viral zoonoses**

Chair: M.P.G. Koopmans

14:30 - 15:00 **New zoonoses from a birds- eye perspective**

O078 A. Osterhaus

15:00 - 15:30 **Of mice and mast: Epidemiology of hantaviruses in North-West Europe**

O079 C.B.E.M. Reusken

15:30 - 15:45 **Only two residues are responsible for the dramatic difference in receptor binding between swine and new pandemic H1 hemagglutinin**

O080 R.P. de Vries

15:45 - 16:00 **Immature Dengue virus: A veiled pathogen**

O081 I.A. Rodenhuis

16:00 - 16:15 **Emergence and spread of human adaptation markers in avian influenza viruses during an HPAI A(H7N7) virus outbreak**

O082 M. Jonges

16:15 - 16:30 Discussion

Room 4/5 **Under your skin: Parasites and skin problems**

Chair: L.M. Kortbeek

14:30 - 15:00 **Skin manifestations of parasitic diseases: What you should know, how to diagnose**

O084 P.J. de Vries

15:00 - 15:30 **Photodynamic therapy in cutaneous leishmaniasis**

O085 E.M. van der Snoek

15:30 - 16:00 **How do maggots operate?**

O086 G. Cazander

16:00 - 16:15 **Presence of *Pneumocystis jiroveci* colonization in patients with chronic obstructive pulmonary disease**

O087 M.J. Vanspauwen

16:15 - 16:30 **A real-time PCR on the SSU rRNA (18S) gene for cestode detection**

O088 J.H. Roelfsema

Room 6/7 **Morphogenesis of bacteria**

Chair: D. Claessen

14:30 - 15:00 **Transport of peptidoglycan units across the bacterial membrane**

O089 E.J. Breukink

15:00 - 15:30 **Regulation of peptidoglycan synthesis by outer membrane proteins**

O090 T. den Blaauwen

15:30 - 16:00 **Positive control of cell division: FtsZ is recruited by SsgB during sporulation of *Streptomyces***

O091 G.P. van Wezel

16:00 - 16:15 **Regulation of FtsZ ring formation**

O092 D.-J. Scheffers

16:15 - 16:30 **Activity and localization of the pneumococcal Ser/Thr protein kinase StkP is controlled by its PASTA domains**

O093 J.W. Veening

Room 8/9 **®evolution in microbial education (www.nvvmonderwijs.nl)**

Chair: A.J.W. van Alphen

14:30 - 15:00 **Is microbiology gendered?**

O094 J.W. Bennett (USA)

15:00 - 15:30 **Tele-microbiology for developing countries**

O095 M.D. de Jong

15:30 - 16:00 **How to build a bacterium**

O096 O.P. Kuipers

16:00 - 16:30 **Microbiology outreach – how the mushroom got its spots and other stories**

O097 S. Assinder (United Kingdom)

Room 3 **Microbial pathogenesis 1**

Chair: P.W.M. Hermans

14:30 - 14:45 **The hypothetical proteases PA0572 of *Pseudomonas aeruginosa* cleaves P-selectin glycoprotein ligand-1**

O098 B.W. Bardoel

14:45 - 15:00 **Epstein-Barr virus protein BNLF2a exploits host tail-anchored protein integration machinery for T cell evasion**

O099 D. Horst

15:00 - 15:15 **Nuclease expression by *Staphylococcus aureus* promotes escape from neutrophil extracellular traps**

O100 E.T.M. Berends

15:15 - 15:30 **Pneumococcal meningitis: interactions between *Streptococcus pneumoniae* and the blood-brain barrier**

O101 G. Molema

15:30 - 15:45 **TroA of *Streptococcus suis* is required for efficient manganese acquisition and for full virulence**

O102 P.J. Wichgers Schreur

15:45 - 16:00	**Host - pathogen interactions with *Streptococcus pneumoniae* during colonization and infection in an elderly mouse model**
O103	C.L. Krone
16:00 - 16:15	**Structural insights on the *Mycobacterium tuberculosis*: the EspB substrate component**
O104	M. Sani
16:15 - 16:30	**Metabolites of commensal bacteria modulate the TLR response in epithelial cells**
O105	M.Y. Lin
16:30 - 17:00	**Coffee/tea break**

Room 6/7

17:00 - 17:30	**A Clubhouse for Microbiologists: Special lecture on the The Microzoo, a future initiative of Artis Zoo Amsterdam**
	H. Balian & K. Greven
17:30 - 18:00	**Award ceremony: SKMM Kwaliteit Prijs Medische Microbiologie**

Room Sydney

18:00 - 19:00 **Drinks**

Athene B/C

19:00 - 21:00 **Dinner**

Foyer

21:00 - 22:30 **Poster session odd posternumbers & poster award**

Athene C

22:30 - 01:30 **Party**

0:00 **100th Anniversary NVvM**

WEDNESDAY APRIL 20 2011

08:30 - 09:00 **Registration**

09:00 - 11:00 **Parallel sessions**

Athene B/C **Livestock associated MRSA: A sheep in wolveskin?**

Chair: J. Kluytmans

09:00 - 09:30	**Livestock-associated MRSA: The European situation**
O106	R. Skov (Denmark)
09:30 - 10:00	**Pig MRSA (ST398) - a sheep in wolf skin and the human MSSA (ST398) - a wolf in sheepskin?**
O107	F. Lowy (USA)
10:00 - 10:30	**MRSA carriage and occurrence of disease in swine veterinarians**
O108	E.J.M. Verkade
10:30 - 10:45	**Methicillin-resistant coagulase-negative *Staphylococci* isolated from pig farms in the Netherlands are a potential reservoir of mecA for *Staphylococcus aureus***
O109	P. Tulinski
10:45 - 11:00	**Typing of methicillin-resistant *Staphylococcus aureus* sequence type 398 isolates with a new optical mapping technique**
O110	T. Bosch

Athene A **New human retroviruses**

Chair: C.A.B. Boucher & J.M.D. Galama

09:00 - 09:30	**Human tumor viruses and rumor viruses**
O111	R.A. Weiss (United Kingdom)
09:30 - 10:00	**Beneficial and detrimental effects of human endogenous retroviruses**
O112	R. Kurth (Germany)
10:00 - 10:15	**No XMRV in a well established cohort of CFS patients in the Netherlands**
O113	J.M.D. Galama
10:15 - 10:30	**RNAi gene therapy for HIV-1: escape and countermeasures**
O114	B. Berkhout
10:30 - 10:45	**HIV-1: old foe repackaged?**
O115	H. Schuitemaker
10:45 - 11:00	Discussion

Room 4/5 **The granuloma in infectious diseases**

Chair: A.M. van der Sar

09:00 - 09:30	**The role of macrophages in granuloma formation**
O117	S. Gordon (United Kingdom)
09:30 - 10:00	**The granuloma in tuberculosis: a host-pathogen collusion**
O118	S. Ehlers (Germany)
10:00 - 10:30	**Schistosoma mansoni egg glycoproteins induce type-2 granulomas *in vivo* by a glycan-dependent mechanism**
O119	C.H. Hokke
10:30 - 10:45	**Zebrafish embryo screen to identify mycobacterial genes involved in granuloma formation**
O120	E.J.M. Stoop
10:45 - 11:00	**Disruption of *M. marinum* ESX-5 leads to increased granuloma formation and bacterial growth in adult zebrafish**
O121	E.M. Weerdenburg

Room 6/7 **Stressing microbes**

Chair: S. Brul

09:00 - 09:30	**Lipid signaling regulated by pH: Phosphatidic acid as a pH biosensor**
O122	C.J.R. Loewen (Canada)
09:30 - 10:00	**Regulation of cellular signaling and cell growth through cytosolic pH**
O123	R. Dechant (Switzerland)
10:00 - 10:15	**Intracellular pH controls yeast growth**
O124	G.J. Smits
10:15 - 10:30	**Weak acid stress in *Bacilli***
O125	A.S. ter Beek
10:30 - 10:45	**Prediction of stress induced robustness using molecular biomarkers**
O126	H.M.W. Besten

10:45 - 11:00 **How dead is dead? A multiparameter viability toolbox applied to *Listeria monocytogenes***

O127 R. Kort

Room 8/9 **Fungal biodiversity and genomics**

Chair: R.P. de Vries

09:00 - 09:30 **Genomic approaches for the characterization of classical genetic mutants and metabolic pathways in filamentous fungi**

O128 S.E. Baker (USA)

09:30 - 10:00 **Comparative transcriptome analysis between the opportunistic pathogen *Aspergillus fumigatus* and the rarely pathogenic *Aspergillus nidulans***

O129 G.D. Robson (United Kingdom)

10:00 - 10:15 **Meiotic recombination in sexual progeny of *Aspergillus fumigatus***

O130 S.M.T. Camps

10:15 - 10:30 **Azole-resistance in *Aspergillus fumigatus*: collateral damage of fungicide use?**

O131 E. Snelders

10:30 - 10:45 **A cosmopolitan Burkholderia terrae equipped as universal migrator along different fungal hyphae**

O132 R. Nazir

10:45 - 11:00 **Daqu - a fermentation starter for Chinese liquor fermentation**

O133 M.J.R. Nout

11:00 - 11:30 **Coffee/tea break**

11:30 - 13:30 **Parallel sessions**

Athene B/C **Microbial pathogenesis 2**

Chair: J.A.G. van Strijp

11:30 - 11:45 **Profiling of global interactions between human serum proteins and the *Staphylococcus aureus* cell surface**

O134 G. Buist

11:45 - 12:00 **Identification of genes essential for *Moraxella catarrhalis* survival under iron-limiting conditions**

O135 S.P.W. Vries

12:00 - 12:15 **Secretion of virulence factors in pathogenic mycobacteria: Type VII substrates are recognized by multiple secretion signals**

O136 H. Daleke

12:15 - 12:30 **Staphylococcal secreted protease aureolysin mediates immune evasion by cleaving complement C3**

O137 A.J. Laarman

12:30 - 12:45 **Comparison of effectiveness of oral, nasal and subcutaneous infection routes of *Coxiella burnetii* in goats**

O138 H.I.J. Roest

12:45 - 13:00 **Serum lipoproteins neutrolize the virulence of Staphylococcal Phenol-Soluble-Modulins**

O139 B.G.J. Surewaard

13:00 - 13:15 **Exploring the transcriptome and proteome of *Bordetella pertussis*, the causative agent of whooping cough**

O140 D. de Gouw

13:15 - 13:30 **The DNA-binding and -cleavage activities of the *Mycoplasma genitalium* Holliday junction resolvase (RecU) are biochemically uncoupled**

O141 C. Vink

Athene A **Varicella Zoster virus: Known and unknown aspects**

Chair: Ph.H. Rothbarth

11:30 - 12:00 **Varicella Zoster Virus and the Central Nervous System**

O142 M.A. Nagel (USA)

12:00 - 12:30 **Vaccination against herpes zoster, pros and cons.**

O143 W. Opstelten

12:30 - 12:45 **Guideline varicella 2010 - what's new?**

O144 Ph.H. Rothbarth

12:45 - 13:00 **Diagnosis of Neurological VZV Disease**

O145 M.A. Nagel (USA)

13:00 - 13:15 **Compartmentalization of aciclovir-resistant Varicella Zoster Virus: implications for sampling in molecular diagnostics**

O146 A.A.T.P. Brink

13:15 - 13:30 **How to keep varicella out of the hospital**

O147 N. Hartwig

Room 4/5 **Hand hygiene: Why, when and how!**

Chairs: R.R. Beumer & W.C. Hazeleger

11:30 - 12:00 **Improvement of hand hygiene in hospital and health care settings**

O148 D. Pittet (Switzerland)

12:00 - 12:30 **Hand hygiene: when, why, how**

O149 E. Todd (USA)

12:30 - 13:00 **The virucidal efficacy of hand disinfectants**

O150 E. Tuladhar

13:00 - 13:30 Discussion

Room 6/7 **System biology**

Chair: B. Teusink

11:30 - 12:00 **Microbial systems biology**

O153 B.O. Palsson (USA)

12:00 - 12:30 **Flexibility of metabolic networks: genome-scale stoichiometric analysis of single species and a yoghurt consortium**

O155 F. Bruggeman

12:30 - 12:45 **Multivariate approach for detecting interactions between of environmental parameters and composition of microbial communities**

O157 D. Bogaert

12:45 - 13:00 **Curli fimbriae and cellulose production are influenced by environmental conditions and affect biofilm formation of *Salmonella enterica* subsp. enterica serovar Typhimurium**

O158 G.A.A. Castelijn

13:00 - 13:15 **Interactions amongst marine archaeal and bacterial nitrifiers and anammox bacteria under oxygen limitation in a lab-scaled model system**

O159 J. Yan

13:15 - 13:30 **How models can help to understand and improve the production of protein based bio-ingredients by the lactic acid bacterium *Lactococcus lactis***

O160 L. Sijtsma

Room 8/9 **Clinical microbiology 2**

Chair: B.J.M. Vlaminckx

11:30 - 11:45 **Identification of biochemically inert non-fermentative bacteria derived from cystic fibrosis patients by matrix-assisted laser ionization/desorption time-of-flight mass spectrometry (MALDI-TOF MS)**

O161 S.Q. van Veen

11:45 - 12:00 **Quantitative Mass Spectrometry reveals new therapeutic targets against the human fungal pathogen *Candida albicans***

O162 C.J. Heilmann

12:00 - 12:15 **Correlation between bacterial DNA load and severity of disease in *S. aureus* bacteraemia**

O163 W. Rozemeijer

12:15 - 12:30 **Period of increased risk for *Clostridium difficile* infection after exposure to antibiotics**

O164 M.P.M. Hensgens

12:30 - 12:45 **Male urinary tract infections in Dutch general practices**

O165 C.D.J. den Heijer

12:45 - 13:00 **Comparison of two matrix-assisted laser desorption ionisation-time of flight mass spectrometry methods for the identification of clinically relevant anaerobic bacteria**

O166 M. Knoester

13:00 - 13:30 Discussion

13:30 - 14:30 **Lunch**

Athene A

13:30 - 14:30 **BBC-MMO business meeting**

14:30 - 16:30 **Parallel sessions**

Athene B/C **Microbial pathogenesis 3**

Chair: W. Bitter

14:30 - 15:00 **C-type lectins in infection and immunity**

O169 T.B. Geijtenbeek

15:00 - 15:15 **Immune modulating properties of mycobacterial type VII secretion systems**

O171 J. Bestebroer

15:15 - 15:30 **Regulation of the energy metabolism in *C. jejuni***

O172 M.M.S.M. Wosten

15:30 - 15:45 **The pervasive effects of a *Citrobacter rodentium* infection on mouse gut microbial diversity**

O173 C. Belzer

15:45 - 16:00 **Identification of immune modulators using a phage display library displaying *S. aureus* secreted proteins**

O174 C. Fevre

16:00 - 16:15 **Bacterial evasion of Neutrophil Extracellular Traps: *Staphylococcus aureus* inhibits neutrophil elastase and myeloperoxidase**

O175 D.A.C. Stapels

16:15 - 16:30 **Translocation into the cytosol novel pathogenicity factor mycobacteria**

O176 N.N. van der Wel

Athene A **Polyomaviruses, known and new causes of infection in immunocompromized hosts**

Chair: M.C.W. Feltkamp

14:30 - 15:00 **Pathogenesis of JC-virus reactivation leading to PML**

O177 E.O. Major (USA)

15:00 - 15:30 **Polyomavirus BK: Opportunity makes a Pathogen**

O178 H. Hirsch (Switserland)

15:30 - 15:45 **Identification and prevalence of a new human polyomavirus associated with trichodysplasia spinulosa**

O179 E. van der Meijden

15:45 - 16:00 **The seroprevalence of seven high-risk HPV types in the Dutch population**

O180 M. Scherpenisse

16:00 - 16:15 **Detection of Merkel cell polyomavirus in chronic lymphocytic leukemia cells by fluorescent in situ hybridization (FISH)**

O181 A. Haugg

16:15 - 16:30 **Clinical features of wuv and kiv; pathogens or passengers?**

O182 A. Riezebos-Brilman

Room 4/5 **Spatial spread of emerging clones and the consequences for typing**

Chair: P.H.M. Savelkoul & B. Duim

14:30 - 15:00 **Rapid pneumococcal evolution in response to clinical interventions**

O183 S. Bentley (United Kingdom)

15:00 - 15:30 **Clonal expansions of MRSA**

O184 U. Nübel (Germany)

15:30 - 16:00 **Continental scale dynamics**

O185 H. Grundmann

16:00 - 16:15 **A quantitative account of genomic island acquisitions in prokaryotes**

O186 M.W.J. van Passel

16:15 - 16:30 **Characterization of the penA mosaic gene in *Neisseria gonorrhoeae* strains with decreased susceptibility to cephalosporins in Amsterdam, the Netherlands**

O187 A.P. van Dam

Room 6/7 **Antimicrobial resistance 2**

Chair: M.A. Leverstein - van Hall

14:30 - 14:45 **Evaluation of the Dutch surveillance on carbapenemase producing Enterobacteriaceae**

O188 M. Leverstein - van Hall

14:45 - 15:00 **First detection of an Ambler class D OXA-48-type ß-lactamase in a *Klebsiella pneumonia* strain in The Netherlands**

O189 M. van Apeldoorn

15:00 - 15:15 **Prevalence of tobramycin-resistant Enterobacteriaceae in a Dutch hospital: implications of the harmonised clinical breakpoints of the European Committee on Antimicrobial Susceptibility Testing**

O190 E.M.L. Verhaegh

15:15 - 15:30 **Eradication of Extended-Spectrum Beta-Lactamases (ESBLs) during Selective Digestive tract Decontamination (SDD)**

O191 E.A.N. Oostdijk

15:30 - 15:45 **Dutch patients, retail chicken meat and poultry share the same ESBL genes, plasmids and strains**

O192 M.A. Leverstein - van Hall

15:45 - 16:00 **Comparison of ESBL contamination in organic and conventional retail chicken meat**

O193 J.W.T. Cohen Stuart

16:00 - 16:15 **Characteristics of extended-spectrum cephalosporin-resistant clinical isolates from companion animals and horses**

O194 E. van Duijkeren

16:15 - 16:30 Discussion

Room 8/9 **Interactive session on the field of medical microbiology & infectious diseases**

Chairs: M.J.M. Bonten & B. Mulder

14:30 - 16:00 **Interactive session**

O196

16:00 - 16:30 **Interactive presentations of cases in infectious diseases by residents in training**

16:30 - 17:00 **Coffee/tea break**

Athene B/C

17:00 - 18:00 **NVMM business meeting**

O001

Enterococcal diversity: understanding the emergence of multidrug resistant strains by knowing their roots

M.S. Gilmore

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The enterococci are highly evolved commensal microbes of the gastrointestinal tracts of all branches of the animal kingdom. As a result, they also occur in the environment where they are often used as indicators of fecal contamination. Beginning in the late 1960s, multidrug resistant strains of enterococci began to emerge, and by the 1980s they began to be recognized as leading causes of hospital acquired infection. Our research has aimed to understand the selective forces and genetic events that led to the evolution of a gastrointestinal tract commensal into a hospital pathogen. Multidrug resistant hospital adapted enterococci typically have genomes 25% larger than commensal strains that are replete with mobile elements including plasmids, phages, transposons, and fitness islands. Strains with a large complement of mobile elements also lack recognizable, functional CRISPR defenses of the genome, which we suggest facilitates their ability to acquire exogenous DNA including antibiotic resistances. We recently showed that recombination across repeated IS elements within a hospital isolate permits highly conjugative plasmids to recombine into the chromosome and mobilize markers from anywhere on the chromosome (including antibiotic resistances, capsule genes, MLST markers, a pathogenicity island) into recipient strains. Moreover, in the process of transferring the pathogenicity island, the recipient resident CRISPR element was displaced, creating more virulent strains with reduced genome defense. Based on these findings a coherent picture of the recent evolution of enterococci has emerged.

O002

Our other genome - the MetaHIT catalog of intestinal bacterial genes

S.D. Ehrlich

INRA, MICA Department, Jouy en Josas, France

The main objective of the MetaHIT consortium is to explore associations of the bacterial genes, genomes or communities from the human gut with the chronic diseases such as obesity and inflammatory bowel diseases (IBD). To reach this objective we have developed a Quantitative Metagenomics gene profiling pipeline. The pipeline is based on two main elements, the reference gene catalog of the gut bacterial genes and the high throughput sequencing of the total stool DNA.

To establish the reference gene catalog, we carried out Illumina sequencing of total fecal DNA from 124 individuals of European origin. From some 0.6 Tb of sequence we have assembled contigs of high quality and revealed 3.3 million non-redundant genes, 150-fold more than encoded by our own genome. The gene catalog captures over 85% of the genes from our cohort and includes about 80% of the sequences determined in previous studies of smaller scope, carried out in Japan and the US. This indicates that it represents well the human intestinal metagenome and deserves to be considered as our other genome. About 99% of the genes are of bacterial origin, indicating that the catalog includes at least 1000 bacterial species, given that an average bacterial genome encodes about 3300 genes (Qin et al., Nature 2010).

To establish the bacterial gut gene profiles, we carry out high throughput sequencing of total stool DNA, generating some 30 million reads for each sample and match the reads of high quality to the catalog genes. The profiles reveal the presence and the abundance of the catalog genes in each of the individuals we study. We shall present the results of two case/control studies, one of obese and lean individuals of Danish origin (n=177) and the other of the ulcerative colitis (UC) patients and healthy individuals of Spanish origin (n=62), which reveal that bacterial species are associated with the chronic diseases.

O003

Lactobacilli in the small intestine, and how we respond to them

M. Kleerebezem

NIZO food research, Health Dept., Ede

The human gastrointestinal tract harbors a complex community of microbes, which plays a prominent role in human health. Recent metagenomics efforts have generated the first genetic catalogue of genes of the fecal microbiota (Qin et al. 2010), which is complemented with an increasing number of complete genome sequences of representative intestinal isolates (Nelson et al. 2010). These efforts start to create an inventory of the function-repertoire of the human intestine microbiota that can be used for microbiota function-modeling in relation to health and disease, which may support the rational design of dietary interventions aimed to improve consumer's health via modulation of the intestine microbiota.

Despite these advances, we should realize that the microbiota composition varies between different locations

in the GI tract and most studies have been targeting the large intestine (fecal) microbiota. Our knowledge is especially limited when it comes to the small intestine microbiota, which is a consequence of its limited accessibility. At the same time, it is clear that the small intestine is the site where initial interactions between food and intestinal microbes take place, while also important host metabolic- and immune-functions are coordinated by small intestinal interactions with the residing microbiota as well as dietary microbes like probiotics. This presentation will focus on efforts aiming to elucidate the human small intestine microbiome at composition and functional level, using metagenome and metatranscriptome approaches. The combinatorial interpretation of these data, allowed the reconstruction of the human small intestine microbial community and its functional repertoire. These analyses revealed that the microbiome of the human small intestine fluctuates in time, possibly as a function of dietary intake habits. Nevertheless, various species belonging to the streptococci could be shown to consistently be among the prominent inhabitants of the human small intestine, where they are involved in metabolic networks that also involves Veillonella spp., as well as members of *the Clostridium cluster* XIV and relatives of *Escherichia coli*. In view of the observed complexity and dynamics of the human small intestine microbiota, one can raise the question whether small intestine mucosa of different individuals may be expected to launch a conserved and coherent response to dietary microbes like probiotics. The second part of this presentation will focus on the elucidation of the molecular response patterns to probiotic species using metagenomics approaches. Biological interpretation of the observed response patterns has provided molecular insights that support certain clinical effects observed with these health-benefit cultures, but also provides clues to potential novel health-benefit applications of these bacteria.
Overall, the worldwide attention for human intestine microbiomics should include appropriate attention for the small intestine microbiota, since this may be the intestinal community that is most responsive to dietary interventions. Moreover, coherent mucosal responses to microbial community changes in the small intestine can be measured and may explain the proposed health effects of certain dietary interventions, like probiotic supplementations (Van Baarlen et al. 2009; 2010).

References

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2. Nelson, et al. Science. 2010;328:994-99.
3. Van Baarlen, et al. PNAS 2009;106:2371-6.
4. Van Baarlen, et al. PNAS. 2010; in press.

O004

IS-pro: fully automated analysis of the human intestinal microbiota

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The human large intestine is one of the most densely populated microbial ecosystems on earth, with bacterial cell counts of up to 10^{11}/gram luminal content. An especially intriguing feature of this ecosystem is its commensalism with the human host. It has been shown that the intestinal microbiota is strictly species specific, implying that host and microbiota have co-evolved for a long time. The relatively recent adoption of a modern westernized' lifestyle, coincides with a dramatic increase in a number of previously rare diseases, including inflammatory bowel disease, asthma, diabetes mellitus, rheumatoid arthritis, multiple sclerosis and others. Many of these diseases have now been shown to be characterized by an altered intestinal microbiota. Analysis of the exact nature of the often complex changes in the intestinal microbiota may greatly enhance our understanding of the etiology of these diseases. Moreover, as these changes are often disease specific, analysis of fecal microbiota may be used as a non-invasive diagnostic tool. Currently however, the techniques employed for these analyses are typically expensive, laborious or both. This has restricted research in this field to small patient numbers and has prohibited implementation of microbiota analysis in clinical diagnostics.
Here we present IS-pro: an inexpensive and fast method for high-throughput analysis of the human intestinal microbiota which has been fully validated in silico, *in vitro* and *in vivo* in human samples. The method combines species identification by 16S-23S interspace (IS) length with phylum identification by colour labelling of primers. The entire process of IS-pro consists of a single PCR followed by fragment analysis by capillary gel electrophoresis and automated analysis of digital profiles. For the automated analysis we developed a web-based software tool that first calibrates profiles and identifies peaks and then translates profiles into a list of bacterial species by means of a large library of IS sequence data that we built by means of second generation sequencing. An internal amplification control consisting of multiple DNA fragments of varying lengths is used for quality control of the PCR process over the entire range of fragment lengths. IS-pro is currently optimized for the human intestinal microbiota, but may easily be adapted for use in other microbial communities.

O005

Human *in vivo* responses to commensal and probiotic lactobacilli

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It is now widely accepted that humans coexist with a plethora of microbial species, collectively termed the microbiota. The cellular pathways that mediate appropriate, tolerant responses to these extremely diverse bacteria during homeostasis are unknown. Several intestinal diseases including inflammatory bowel diseases (IBD) are co-determined by disproportionate proinflammatory immune responses to gut microbiota. We are therefore interested in characterising responses of healthy persons to common microbiota and investigate the genetic basis of tolerance.

To study *in vivo* responses of healthy humans to common representatives of the microbiota, we performed whole-genome gene expression profiling of 8 humans, 6 hours after consumption of three different species of *Lactobacillus*, lactic acid bacteria that are commonly sold as probiotics: *L. acidophilus* Lafti-L10, *L. casei* CRL-431 and *L. rhamnosus* GG. To investigate specificity of human responses, we also investigated responses of 8 humans to three different growth stages of a fourth bacterial species, *L. plantarum*. Both studies used a randomised double-blind, placebo-controlled crossover design. RNA was extracted from duodenal biopsies, taken by standard duodenoscopy. Histology was performed to compare the biopsies and check for abundance of immune cells. We found that human transcriptional responses to all four bacterial *Lactobacillus* species were different in terms of pathway activation, induced gene regulatory networks and upregulation of cytokine genes. Moreover, responses to the three different growth stages of the species *L. plantarum* were clearly different, with exponentially growing bacteria promoting proliferation-related pathways, and stationary viable or heat-killed bacteria promoting immune response pathways. Intruigingly, some modulated pathways play roles in IBD, for example interleukin (IL)-17 signalling modulated by *L. casei* and the IL-23 signalling pathway that was induced by *L. acidophilus*. The modulated gene networks and pathways contribute to understanding why probiotic application is sometimes successful. The inter-individual transcriptome variation helped to explain why persons are frequently nonresponding to consumption of probiotics, or are responding in a variable way.

We conclude that:

1. different lactic acid bacterial species are perceived as different by the human intestinal mucosa;
2. lactic acid bacteria induce pathways and processes that play roles in lipid metabolism, immunity and tolerance, cell proliferation and mucosal homeostasis;
3. investigating the modulated pathways with known involvement in IBD could contribute to understanding how these pathways are dysregulated in persons suffering from IBD;
4. it is possible to perform human *in vivo* studies to investigate intestinal tolerance to the microbiota using well-characterised bacterial species and functional genomics approaches.

O006

Molecular transport across porins: rate limiting interactions

M. Winterhalter

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The permeation of water soluble molecules across cell membranes is controlled by channel forming proteins and particularly the affinity to the channel surface determines the selectivity. An adequate method to study properties of these channels is electrophysiology and in particular analysing the ion current fluctuation in the presence of permeating solutes provides information on possible interactions with the channel surface. As the affinity of antibiotic molecules to the inside of the channels is significantly weaker than that of preferentially diffusing nutrients in substrate-specific pores, the resolution of conductance measurements has to be significantly increased to be able to resolve the events in all cases. We demonstrate that miniaturization of the lipid bilayer; varying the temperature or changing the solvent may enhance the resolution. We tested our approach on OmpF from *E. coli* and measure the temperature dependent rates for a number of antibiotics (β-lactams and fluoroquinolones). From the temperature dependent rates we may conclude on the energy barrier. The latter can be compared with all-atom molecular dynamics and allows to identify the rate limiting molecular interaction. For example for OmpF and Ampicillin the main interaction is with the D113 and the NH_3^+ side-chain. Combining electrophysiology with all atom computer modelling provides atomic details of solute permeation.

References

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O007

Intervention opportunities in the mycobacterial cell envelope

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Tuberculosis, which was declared a global emergency by the WHO in 1993, still kills more people than any other bacterial disease. The emergence of *Mycobacterium tuberculosis* strains resistant to some or all-current antitubercular drugs seriously hampers control of the disease. Generation of new drugs requires identification of novel mycobacterial targets and characterization of biochemical pathways specific to mycobacteria. Many unique metabolic processes occur during the synthesis of the lipid-rich mycobacterial cell wall, which accounts for its unusually low permeability and thus contributes to resistance towards common antibiotics and chemotherapeutic agents. Mycolic acids are major components of the mycobacterial cell envelope and are direct modulators of interactions between mycobacteria and the infected host. Mycolic acids are targeted by first-line and second-line antitubercular drugs such as isoniazid and ethionamide. Because the enzymes participating in mycolic acid biosynthesis are unique, they represent an attractive reservoir of targets for new drugs discovery.

Recent studies indicated that thiocarbamide-containing drugs, including the second-line antitubercular drug thiacetazone (TAC), are pro-drugs that all require to be activated by the monooxygenase EthA, supporting the earlier observation that clinical isolates with mutations in *ethA* are co-resistant to these drugs. Importantly, through a combination of genetic, biochemical and structural studies, it was demonstrated that TAC affects mycolic acid biosynthesis by inhibiting the mycolic acid cyclopropane synthases PcaA, CmaA2 and MmaA2. These enzymes are *S*-adenosylmethionine (SAM)-dependent methyltransferases that introduce cyclopropane rings in mycolic acids, which have been reported to participate in persistence of *M. tuberculosis* in infected mice. In addition, the chemical synthesis of a new generation of TAC analogues allowed to select more active compounds, particularly attractive in the context of emergence of multidrug resistance. These studies open new perspectives in the development of innovative antitubercular chemotherapy.

O008

Lipid II as target for (l)antibiotics

E.J. Breukink

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Lipid II is a membrane-anchored cell-wall precursor that is essential for bacterial cell-wall biosynthesis. The effectiveness of targeting Lipid II as an antibacterial strategy is highlighted by the fact that it is the target for at least five different classes of antibiotic, including the clinically important glycopeptide antibiotic vancomycin. However, the growing problem of bacterial resistance to many current drugs, including vancomycin, has led to increasing interest in the therapeutic potential of other classes of compound that target Lipid II. I will review progress in understanding of the antibacterial activities of these compounds, which include lantibiotics, vancomycin derivatives and even the eukaryotic (and human) defensins and consider factors that will be important in exploiting their potential as new treatments for bacterial infections.

O009

Expression of multidrug resistance ABC transporter genes in *Bacillus subtilis*

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UMCG, Medical Microbiology, Groningen

Introduction: Since the clinical introduction of antibiotics, pathogenic bacteria have evolved efficient mechanisms to neutralize them. This resulted in the emergence of several multidrug resistant (MDR) bacterial pathogens, which are a serious threat for public health since effective treatment of infections with these bacteria is difficult. An efficient mechanism to achieve MDR is the (over) expression of efflux pumps such as ATP-binding cassette (ABC) transporters. In mammals, ABC transporters are notorious for their role in MDR. In bacteria however, their function in resistance has remained largely elusive. The non-pathogenic soil bacterium *Bacillis subtilis* is an excellent model for studying ABC transporters and their roles in development of MDR. In this study, promoter-GFP fusions were used to monitor the responses of potential MDR-ABC transporters genes to antibiotic stress.

Methods: Promoter regions of selected ABC-transporter genes (e.g. *yheI/yheH* and *ykpA*) were cloned into BaSysBioII, an integrative plasmid for generating transcriptional GFP fusions (Botella *et al.* Microbiology. 2010;156:1600-8). Chromosomal integration via single cross-over of resulting plasmids ensured that the promoter-

GFP fusions are controlled by all upstream regulatory sequences, while expression of the intact ABC transporter gene remained unaffected. The promoter-GFP reporter strains were grown in a microplate reader allowing real-time measurements of growth (OD_{600}) and GFP fluorescence at 5 min intervals. Antibiotic stress was induced during exponential growth by the addition of sub-inhibitory concentrations of lincomycin, chloramphenicol, erythromycin, kanamycin, or gentamycin. The recorded data was corrected for background fluorescence determined from the parental strain (*Bacillus subtilis 168*) before calculating promoter activity: $(GFP^{t}-GFP^{t-1})/OD_{600}^{t}$.

Results: Promoter yheI/yheH: These genes encode a heterodimeric ABC transporter, which has been linked previously to drug efflux (Torres *et al.* Biochim Biophys Acta. 2009;788:615-22). In our experiments we demonstrate that the expression of GFP is clearly induced by erythromycin, chloramphenicol or lincomycin, while activity of the *yheI/yheH* promoter remains unaffected by gentamycin or kanamycin. An interesting feature of the response is a significant delay observed between the time point of antibiotic addition and the induction of the promoter, which is postponed for approximately 100 minutes.

Promoter *ykpA*: The gene encodes an uncharacterized ABC transporter system with 2 duplicated ATP-binding domains and no transmembrane domain. Although the promoter controlling *ykpA* expression is active during growth its activity is not affected by the antibiotics.

Conclusions: The use of promoter-GFP fusions allows fast, sensitive and real-time expression analyses of genes for potential MDR-ABC transporters. Importantly, the analysis of promoter activity in real-time reveals critical parameters of the expression dynamics of ABC transporter genes under antibiotic stress conditions, including response time, expression level, and duration of induction. This system can also be used to study regulatory mechanisms involved in the expression of MDR-ABC transporter genes. In addition, promoter activity can be studied by flow cytometry and live-cell microscopy, extending the studies from the population level to single cells. Ultimately, we aim to use a similar system for studies on the role of ABC transporters in the development of MDR in *Staphylococcus aureus*, a clinically relevant pathogen that readily develops resistance to antibiotics.

O010

Comprehensive identification of genes required for antibiotic resistance and bile tolerance in the nosocomial pathogen *Enterococcus faecium*

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Enterococcus faecium is a gram-positive commensal bacterium of the mammalian gastro-intestinal tract. In the last two decades it has emerged as a multi-resistant nosocomial pathogen. Despite its increasing clinical significance, existing molecular tools for the genetic manipulation and genome-wide analysis of *Enterococcus faecium* are limited.

In this study, we developed a transposon delivery vector (pZXL5) that was composed of both a ColE1 and a gram-positive thermo-sensitive replicon, a gentamicin resistant mariner transposon with two outward-facing T7 promoters, a nisin-induced mariner transposase, and a chloramphenicol resistance marker. A highly saturated transposon insertion mutant library was generated in *E. faecium* E1162 using pZXL5. Transposon insertions were distributed around the entire chromosome in a random fashion and these insertions could be reproducibly mapped by a microarray-based hybridization approach we termed Microarray-based Transposon Mapping (M-TraM). The relevance of genes identified by M-TraM was confirmed by generating targeted mutations and subsequent phenotypic testing. By comparing the mutant library following growth in the presence or absence of β-lactam antibiotics (ampicillin and cefoxitin), we identified genes that are involved in resistance to β-lactams, leading to the identification of a β-lactam insensitive peptidoglycan crosslinking pathway and a novel putative β-lactamase. In addition we identified genes that contribute to bile tolerance, as this is a trait required for colonization of the gastrointestinal tract and determined that a transporter of compatible solutes is crucial for efficient growth of *E. faecium* in the presence of bile.

In conclusion, we have developed a fast and powerful method for the identification of conditionally essential genes in *E. faecium*, which will greatly improve our understanding of this important nosocomial pathogen.

O011

The membrane potential is important for bacterial cell division

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Many cell division related proteins are located at specific positions in the bacterial cell, and this organized distribution of proteins requires energy. Here we report that the proton motive force, or more specifically the (trans-) membrane potential, is directly involved in protein localization. It emerged that the membrane potential modulates the distribution of several conserved cell division proteins such as MinD, FtsA, and the bacterial cytoskeletal protein MreB. We show for MinD that this is based on the membrane potential stimulated binding of its C-terminal amphipathic helix. This novel function of the membrane

potential has implications for how these morphogenetic proteins work, and provide an explanation for the effects observed with certain antimicrobial compounds.

O012

Isolation of a prokaryotic cell organelle from the uniquely compartmentalized anammox bacteria

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The bacteria capable of anaerobically oxidizing ammonium (anammox) have been discovered only quite recently.[1] Since then their significance for the global nitrogen cycle has become apparent due to their large contribution to the oceanic nitrogen loss[2] and they are already applied for the removal of ammonium from municipal wastewater. Like other members of the phylum Planctomycetes, anammox bacteria exhibit a cell compartmentalization that is otherwise unique for prokaryotes.[3] The cells are subdivided into three compartments. The outermost compartment is the paryphoplasm and has an unknown function, but is presumably not analogous to the periplasmic space in gram-negative bacteria. It is separated by an intracytoplasmic membrane from the riboplasm, which harbors the RNA as well as DNA of the cell. The innermost compartment is the anammoxosome and is hypothesized to be the side of catabolism and energy generation, analogous to eukaryotic mitochondria.[4,5] Isolation of this prokaryotic cell organelle from the anammox bacterium *Kuenenia stuttgartiensis* was attempted by various physical and chemical disruption techniques and led to separation of two subcellular fractions by Percoll density centrifugation. These were investigated with immunofluorescence microscopy and transmission electron microscopy for their outer appearance, DNA content and hybridization with an antibody targeting the anammoxosome. Future studies will include organelle proteomics and activity assays.

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O013

Mapping the interactions of the twin-arginine translocation system (Tat) in *Bacillus subtilis*

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Introduction: Studies on the mechanisms through which bacteria regulate protein traffic from the cytoplasm to extracytoplasmic locations is very important to understand their interactions with the surrounding environment. Moreover, such studies provide important leads to allow the application of these systems for recombinant protein production. In bacterial cells there are several protein transport systems that are responsible for different functions. One of these is the Twin-arginine translocation system (Tat). The Tat system has two main features: 1) It is able to select proteins with a cleavable N-terminal signal peptide that contain a key twin-arginine motif together with other determinants, and 2) it allows the secretion of proteins in a folded state. While in gram-negative bacteria the three subunits TatA, TatB and TatC are the essential pieces that are required for the translocation process, gram-positive bacteria posses a minimal Tat system based on 'just' the TatA and TatC subunits. In the present studies, we focused our attention on the Tat system of *Bacillus subtilis*, a well known cell factory and a paradigm for studies on gram-positive bacteria. In this bacterium there are two minimal TatAC systems that operate in parallel for the translocation of at least three substrate proteins, namely YwbN, QcrA and PhoD. To date, relatively little is known about the regulation of this system and the interactions that occur between the different Tat components.
Methods: To obtain a deeper insight into these processes, we performed a Yeast two-hybrid (Y2H) screen to identify interactions between the different Tat subunits and other *B. subtilis* proteins. The results of this Y2H screen were subsequently verified in two different ways: 1) All the genes for proteins that showed relevant interactions were mutated and the impact of these mutations on the secretion of the YwbN protein was checked, and 2) the interaction between the subunits of the translocase were verified using bimolecular fluorescence complementation (BiFC), a technique based on the reconstitution of a split fluorescent protein fused to two interacting partner proteins.
Results: The Y2H analyses revealed an intricate network of interactions of the different Tat proteins of B. subtilis. Subsequent BiFC studies confirmed the identified interactions between different TatA subunits of *B. subtilis*.

Moreover, at least one novel determinant, WprA, was identified that is needed for the secretion of YwbN via the Tat pathway.

Conclusion: Y2H screens can be successfully used to identify interactions between membrane-embedded Tat complexes for protein translocation and other membrane-associated or secreted proteins. WprA was identified as a novel determinant for the secretion of the YwbN protein.

O014

A model to study drug-induced mitochondrial dysfunction

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To address fundamental questions concerning drug-induced mitochondrial dysfunction, *Caenorhabditis elegans* was developed as a model organism. Several studies have shown the occurrence of mitochondrial dysfunction as a consequence of therapeutic drug use. Behind most of these side-effects appears to be a common mechanism: a decreased mitochondrial energy-generating capacity putatively caused by the secondary inhibition of mitochondrial DNA polymerase, resulting in the depletion of mitochondrial DNA (mtDNA). However, the exact mechanism remains unknown. Since most of these results have been obtained in patient- or cell culture studies, it poses limitations on the experiments that can be performed. Progress in this field is highly dependent on the development of a good model system.

With our model system we could show a concentration dependent absolute and relative, compared to nuclear DNA, decline in mtDNA copies when the organisms are cultured in the presence of various anti-retroviral drugs. In addition, exposure to these drugs results in increased ROS production and/or a morphologically discernible disruption of the mitochondrial network. The severity of the effects is drug-specific and concentration dependent. The observed biochemical and morphological effects are not necessarily provoked by the same compounds. Interestingly, some of the effects can be alleviated by supplementation of compounds active on the mitochondrial respiratory chain. Given the similarity of the observed effects in *C. elegans* to effects seen in patients on anti-retroviral therapy, we conclude that *C. elegans* is a highly suitable model organism to study drug induced mitochondrial dysfunction and search for compounds to alleviate the toxicities. Preliminary results suggest the beneficial effect of supplementation as a way of counteracting and alleviating some of these pernicious side-effects.

O015

Gel-free proteomic identification of the *Bacillus subtilis* insoluble spore coat protein fraction

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The genus *Bacillus* has the ability to form endospores, dormant cellular forms that are able to survive heat and acid preservation techniques commonly used in the food industry. Resistance characteristics of spores towards various environmental stresses are attributed to their coat proteins. Previously, seventy proteins have been assigned to the spore coat of *Bacillus subtilis* using Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE), Two-Dimensional Electrophoretic (2-DE) gel approaches, and protein localization assumptions inferred from genome-wide transcriptome studies. Here we present a 'gel-free' protocol, addressing the insoluble coat fraction that is capable of comprehensive *B. subtilis* spore coat protein extraction. Using Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) we identified from the insoluble *B. subtilis* spore coat fraction 58 covalently linked proteins, of which 23 are putative novel spore coat proteins not assigned to the spore coat until now. Identification of spore coat proteins from a *B. subtilis* food spoilage isolate corroborated a generic and applied use of our protocol.

Conclusions:

1. To develop specific and sensitive spore detection and/or purification systems from food stuff or patient material, suitable protein targets can be derived from our proteomic approach.
2. Finally, the protocol can be extended to study cross linking amongst the spore coat proteins as well as for their quantification.
3. Our analytical strategy will also be a starting point in future research on quantitative analysis of coat proteins to compare differences between strains or stress conditions.

O016

Protein complexes involved in membrane-bound electron transport of anammox bacteria

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Anaerobic ammonium oxidizing (anammox) bacteria conserve energy from the oxidation of ammonium to dinitrogen gas with nitrite as the electron acceptor. This process involves a cyclic electron flow with hydrazine and nitric oxide as the intermediates. Energy conservation proceeds by a chemiosmotic mechanism featuring the quinol:cytochrome c oxidoreductase (bc1 complex). Still, the genome of the anammox bacterium *Candidatus Kuenenia stuttgartiensis* shows an unprecedented redundancy of respiratory genes suggesting intricate and only partly understood cellular electron transport systems. Moreover, for carbon fixation, electrons are withdrawn from the anammox process that have to be replenished through the oxidation of nitrite to nitrate by the nitrate reductase (NAR) system. As nitrite is a relatively poor reductant, the electrons have to be energized to enter the bc1-complex or to feed a quinone pool, which implies reverse electron transport (RET).
In order to understand the metabolic processes involved in energy conservation in anammox bacteria, respiratory membrane-bound enzyme complexes, including the NAR system, were separated by Blue Native – PAGE and identified by specific in-gel activity assays and LC-MS/MS analysis.
Protein correlation profiling using LC-MS/MS data from consecutive Blue Native gel slices enabled the identification of almost all membrane-bound protein complexes suggested by the genome, including three types of bc1 complexes and three distinct ATPases, as well as the NAR system.
The NAR gene cluster in the genome of *K. stuttgartiensis* is the most complex one known to date coding for the catalytic subunits of nitrate reductase (narGH) and a series of electron carriers, apparently mediating electron flow and bifurcation associated with RET. These include genes encoding six putative heme-containing proteins, two putative blue-copper proteins and a putative anchor to the membrane showing homology to a cytochrome bd oxidase subunit (cydA), together covering almost the full natural repertoire of electron carriers. Using reduced methyl viologen as artificial electron donor and NO3- as electron acceptor, in-gel activity assay showed a single band with NAR activity. The reversed reaction, the oxidation of nitrite to nitrate could be shown with whole membrane preparations with ferrocene as a high-redox-potential electron acceptor. In the membrane preparations, the complete NAR system was recovered as two subcomplexes, the smaller one containing the catalytic subunits and the larger subcomplex containing the membrane bound part of the complex.
In conclusion, we assume that the nar operon in the genome of *K. stuttgartiensis* encodes for a nitrate reductase that is able to catalyze the oxidation of nitrite to nitrate. An unusual cluster of associated heme and copper proteins thereby might play a mayor role in the RET in order to feed the cyclic electron flow of the anammox reaction.

O017

Heterogeneity in micro-colonies of *Aspergillus niger* in liquid shaken cultures

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Colonies of the filamentous fungus *Aspergillus niger* secrete large amounts of proteins. On solid media only part of the colony is involved in secretion. We assessed whether heterogeneity can also be found in micro-colonies of *A. niger*.
Strains expressing *GFP* from the glucoamylase (*glaA*) or the ferulic acid esterase (*faeA*) promoter were grown in liquid medium. We used the Complex Object Parametric Analyzer and Sorter (COPAS) to analyze the diameter and fluorescence of the micro-colonies. Two populations of micro-colonies were distinguished that differed in their diameter. The population of small micro-colonies of strains expressing *GFP* from the *glaA* or *faeA* promoter comprised 39% and 25% of the culture, respectively. Two populations of micro-colonies could also be distinguished when expression of *GFP* in these strains was analyzed. The population lowly expressing *GFP* consisted of 68% and 44% of the whole population, respectively.
Here, the COPAS was used for the first time to analyze *A. niger* micro-colonies. Our results show that there is heterogeneity in size and gene expression between micro-colonies within a liquid shaken culture. This implies that protein production in an industrial fermentation can be increased by reducing the heterogeneity between the micro-colonies.

O018

Consequences of implementation of moleculair diagnostics of gastroenteritis outside and inside the laboratory

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More than 5 years ago, Tim Schuurman clearly showed that in contrast to *Salmonellae*, detection of *C. jejuni* in patients suffering from gastroenteritis significantly improved when culture of *C. jejuni* was preceded by molecular detection of *C. jejuni* DNA. He published his results in his thesis.[1] Since patients, transport of the faecal specimens, and culture method was fairly identical in his study as in our routine situation, in our laboratory a discussion was started whether in general culture of bacteria causing gastroen-

teritis should not be preceded by molecular screening of these specimens. And if we agreed to change our bacterial work flow, what would be the consequences for diagnosing parasitic and viral causes of gastroenteritis. We, microbiologists, molecular microbiologists, technical analysts, and management had frank discussion but we agreed. In our situation we should go for it. It took us two years before we could start. Multiplex PCR's were designed and tested, extraction methods were compared, a whole new molecular lab had to built and finances had to be found. A lot of technical analysts, until then working as routine analysts in the routine bacteriology had to be trained in molecular microbiology. General practioners sending in faecal specimens were informed on the fact that they got their results in 48h in stead of 5 days. Furthermore they should no longer send the TFT set but only one faecal specimen. By going over the results of a set of PCR tests, run in our laboratory, all our microbiologists were confronted with the advantages and problems of molecular microbiology, the so called pro's and contra's of implementing this technique in routine microbiology.

Summer 2008, we started and of course we encountered a lot of surprises. Some were positive, others led to much debate, and a few were challenging. First some of the positive findings. At first, adjusted for the increased number of faecal specimens send to our lab, due to increased alertness among the technical analysts, a significant increase in the number of cultured *C. jejuni* (25%) and *Shigella* species (400%) was found.[2] Secondly, no extra positives were found if a next faecal specimen of a patient was analysed. So only one specimen was analysed. Thirdly in weekdays over 95% of the specimens could be reported within 48 hr. Fourthly, since we now isolated many more Shigella, we could clearly show the enormous percentage of TMP-SMZ resistance in these strains (90%), indicating that on this issue the SWAB richtlijn should be adapted. Regarding the debate issue, we had lengthy discussions on the best platform for diagnosing *C. difficile* infections. Should we remain stick to detection of toxins or should we go for the toxin genes by performing PCR's. Even more difficult were the facts that i) we also found a lot of Shiga toxin gene positive samples, reported to the public health service doctors and their staff that remain negative in culture causing a lot of unforeseen debate ii) we had a lot more work to do in the pre-analysis setting of our lab, iii) we missed cyclospora and possibly some other parasites by no longer performing routine microscopy, iv) we found a staggering increase in the number of *D. fragilis* positive patients and v) we encountered a raise in the costs for the hospitals. In our presentation, we will zoom in on the points raised above.

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O019

Improved detection of five major gastrointestinal pathogens by use of a molecular screening approach

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Objectives: The detection of bacterial and parasitic gastrointestinal pathogens through culture and microscopy is laborious and time-consuming. We evaluated a molecular screening approach (MSA) for the detection of five major enteric pathogens: *Salmonella enterica*, *Campylobacter jejuni*, *Giardia lamblia*, Shiga toxin-producing *Escherichia coli* (STEC), and *Shigella* spp., Enteroinvasive *E. coli* (EIEC), for use in the daily practice of a clinical microbiology laboratory.

Methods: The MSA consists of prescreening of stool specimens with two real-time multiplex PCR (mPCR) assays, which give results within a single working day, followed by guided culture/microscopy of the positive or mPCR-inhibited samples. In the present 2-year overview, 28,185 stool specimens were included.

Results: The MSA was applied to 13,974 stool samples (49.6%), whereas 14,211 samples were tested by conventional methods only (50.4%). The MSA significantly increased the total detection rate compared to that of conventional methods (19.2% versus 6.4%). The detection of all included pathogens, with the exception of *S. enterica*, significantly improved. MSA detection frequencies were as follows: *C. jejuni*, 8.1%; *G. lamblia*, 4.7%; *S. enterica*, 3.0%; STEC, 1.9%; and *Shigella* spp./*EIEC*, 1.4%. The guided culture/microscopy was positive in 76.8%, 58.1%, 88.9%, 16.8%, and 18.1% of mPCR-positive specimens, respectively. Of all mPCRs, only 1.8% was inhibited. Other findings were that detection of mixed infections was increased (0.9% versus 0.02%) and threshold cycle (CT) values for MSA guided culture/microscopy-positive samples were significantly lower than those for guided culture/microscopy-negative samples.

Conclusions: In conclusion, an MSA for detection of gastrointestinal pathogens resulted in markedly improved detection rates and a substantial decrease in time to reporting of (preliminary) results.

O020

About STEC working group and *Shigella* outbreaks

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Molecular techniques have improved sensitivity and speed in microbiological diagnostics dramatically in the last two decades. Initially introduced for the identification of organisms showing fastidious growth on ordinary media, or no growth at all, these techniques are used more and more in multiplex algorithms for screening of multiple targets in routine samples.

In the Netherlands several microbiology laboratories introduced multiplex PCR for the screening of fecal samples on the presence of bacterial (and parasitic) agents, with subsequent culture only performed on PCR-positive samples. This new strategy resulted in some remarkable findings.

First, laboratories have noticed a steady increase in the number of samples. Apparently physicians seem to be willing to postpone empirical treatment and send stool samples in exchange for rapid screening combined with early recognition of positives and feedback of results.

Second, the number of positive samples has increased dramatically mostly due to positive PCR results. The high sensitivity of molecular techniques and the ability to detect (DNA) targets either in samples from pre-treated patients or in samples with a prolonged transport time seem to be responsible for this increase. Studies clearly show many PCR-positive results in the higher Ct-value ranges whereas culture-positive samples match PCR results with lower Ct-values. This finding applies very strongly for STEC and *Shigella* spp./EIEC, less for *Campylobacter* spp., whereas for *Salmonella* spp. there seems to be no advantage of PCR when compared to culture.

Third, the number of cultured isolates increased. This phenomenon is probably due to the increased awareness of technicians that some microbial agent must be present in these selected stool samples.

However, each technique has its drawback. For certain bacterial species the amount of PCR+/culture- samples outnumbers the amount of culture+ samples, giving fuel to the discussion on whether these PCR+/culture- results are either true or false positives.

In case of STEC or *Shigella* spp./EIEC PCR positivesamples results have to be reported to the Public Health Agencies who should perform a search for contacts of the index patient as well as for a probable source of the infection. The increased number of PCR+/culture- STEC results increases the workload of these Agencies substantially, and doubt is expressed about the relevance of contact and source screening in these cases.

A working group of microbiologists and public health physicians have suggested that laboratories handle a Ct-value of less than 35 for STEC positive PCR results as threshold for reporting the result to the Public Health Agency. Studies should be started to review the consequences for patients with results on both sides of this threshold.

For *Shigella* spp./EIEC we recognised a similar problem with positive PCR results for Shiga-toxin gene containing samples that could not be detected on specific growth media for *Shigella* spp.

We will discuss the consequences of the use of Ct-value thresholds for reporting to Public Health Agencies, and conclude that for microbiology laboratories there is still a lot of work to do in order to support physicians with a correct and suitable result. Laboratories should continue to invest in both molecular and traditional techniques and should interpret results with great care.

O021

Case control study gastroenteritis

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Recently, the diagnosis of protozoal causes of gastroenteritis by molecular methods has been shown to be more sensitive than microscopic examination. Also a recent development is diagnosing bacterial gastroenteritis by screening the fecal samples for pathogenic bacteria by molecular methods, in combination with subsequent culturing the positive samples.

Highly sensitive multiplex real-time PCR assays have been developed for the most common protozoal and bacterial pathogens causing gastroenteritis, such as *Salmonella*, Dientamoeba, *Giardia*, *Campylobacter*, pathogenic *Escherichia coli* and *Shigella*.

However, by implementing a more sensitive molecular method detecting different diagnostic targets, the relevance of the diagnostic test results needs to be redefined for these organisms. This applies especially to samples that are weakly positive in the real-time PCR assays, but culture negative.

Therefore, a case control study is currently being performed by 5 regional laboratories for medical microbiology to assess the diagnostic value of real-time PCR results for these micro-organisms.

In particular, the prevalence of pathogenic protozoa and bacteria in asymptomatic subjects, in comparison to that in patients with complaints, is being investigated.

O022

Realtime PCR reveals the presence of three distinct *Brachyspira* species in human spirochaetosis

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Introduction: Infections with *Brachyspira* species results in a wide range of intestinal disorders in both humans and animals. While there is vast knowledge on the impact of *Brachyspira* infections in animals, little is known regarding human infections. Human intestinal spirochaetosis is thought to be caused by two *Brachyspira* species: *Brachyspira aalborgi* and *Brachyspira pilosicoli*. Prevalence rates are estimated between 1-32% in the general population and up to 63% homosexuals. Diagnosis is based on histopathology of colon-biopsies but does not allow for definitive species identification.

Aim: To design a real-time PCR that allows for the diagnosis of human intestinal spirochaetosis and identification of the infecting *Brachyspira* species.

Methods: Fifty colon-biopsy samples from histologically positive patients collected between 2001 and 2010 were identified from the archival collection of Tergooiziekenhuizen (n=17 patients) and UMC Utrecht, (n=8 patients). In addition, 24 spirochete negative colon-biopsy samples were selected. Biopsy samples were deparafinized, hydrated and spiked with phocine herpes virus as internal control. DNA was isolated on a Roche MP96 and Realtime PCR and SYBR-green melt-curve analysis of the PCR products were performed on a Roche LC 480. Species designation was confirmed by DNA sequence analysis of the PCR fragments.

Results: Small subunit rRNA sequences from the public databases were downloaded and aligned and primers amplifying a 136 bp region in the 5'end of the rRNA gene that is specific for *Brachyspira* species were designed.

All 25 histologically positive patients were positive in the PCR and all 24 negative controls were negative. SYBR-green based meltcurves of the positive samples suggested that next to the two *Brachyspira* species known to infect humans, a third *Brachyspira* species was occasionally present in these samples. Sequence analysis confirmed the presence of single infections with *B. aalborgi* (15/25), *B. pilosicoli* (2/25), and a thus far unreported Brachyspira species (4/25). In addition, there were four double infections: 2/25 *B. aalborgi* with the novel *Brachyspira* species, and 2/25 *B. pilosicoli* with the novel *Brachyspira* species.

Conclusions: To our knowledge this is the first real-time PCR that allows for simultaneous detection and species discrimination of *Brachyspira* in routine biopsy materials. The PCR revealed that in human spirochaetosis: 1) a surprisingly high number of double infections is present, 2) a thus far unknown *Brachyspira* species is involved, and 3) not *B. pilosicoli* but *B. aalborgi* is the most common infecting species in human intestinal spirochaetosis.

O023

Reduction of workload of microbial gastroenteritis diagnostics by molecular pre-screening

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Introduction: Fecal samples from patients with diarrhoea form a substantial part of the workload in diagnostic microbiology laboratories. The diagnosis of the bacterial and parasitic pathogens in gastrointestinal infections is routinely performed by a combination of culture, microscopy and antigen detection techniques. To reduce the workload of this combined approach, we emphasized that molecular methods can be used to screen the stool samples for positivity. PCR negative stool samples can be discarded from further analysis, leading to a reduction of workload and costs.

Objective: To determine the reduction of workload of gastrointestinal diagnostics at the parasitology and bacteriology labs using a PCR screening method.

Methods: A total of 267 stool samples from patients with gastroenteritis were subjected to PCR screening. From these samples, 89 were send in for parasitolgy, 97 were send in for bacteriology and 81 were send in for both. A multiplex PCR was used for the gastroenteritis-associated pathogens: *Campylobacter* spp., *Salmonella enterica*, *Shigella* spp., *Giardia lamblia*, *Dientamoeba fragilis*, *Cryptosporidium* spp., and *Entamoeba histolytica*. DNA extractions of the samples were carried out on an EasyMag DNA-extractor (bioMrieux) and three multiplex PCRs per sample were performed on a LightCycler 480II (Roche Diagnostics).

Results: The prevalence of *Giardia lamblia*, *Cryptosporidium*, *Entamoeba histolytica*, *Salmonella enterica*, and *Shigella* by PCR were low: 1.76%, 0.6%, 0%, 1.1% and 0.6% respectively. From a total of 170 samples send for parasitology, 33 samples (19.4%) were detected by *Dientamoeba fragilis*-PCR and microscopy/ELISA, 14 (8.2%) were detected by *Dientamoeba fragilis*-PCR only resulting in a prevalence of *Dientamoeba fragilis* by PCR of 27.6%. From the samples send for bacteriology (n=178), 12 (6.7%) were positive by *Campylobacter*-PCR and culture techniques, whereas 22 (12.4%) were positive by *Campylobacter*-PCR only. The discrepancy between PCR and culture techniques for *Campylobacter* was due to very low bacterial loads (P<0.0001), in contrast to the

discrepancy between PCR and microscopy/ELISA for *Dientamoeba fragilis* (p=0.106).

Conclusion: Without specific anamnestic data, pre-screening of 267 stool samples by PCR resulted in a reduction to 29.4% positive samples only that are eligible for confirmation by microscopy/ELISA for parasitology and a reduction to 19.7% positive samples only that have to be cultured e.g. for antibiotic susceptibility.

O024

Interactions between respiratory bacteria and pneumovirus infections: consequences for the pathogenesis of RSV- and HMPV-mediated severe disease

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Respiratory syncytial virus (RSV) and human metapneumovirus (HMPV) are members of the family *Paramyxoviridae*, subfamily *Pneumovirinae*. Both viruses are considered important causes of acute respiratory tract infections in infants, immunocompromised subjects and the elderly. However, in the majority of cases primary infections with RSV or HMPV remain restricted to the upper respiratory tract and are associated with mild clinical symptoms. By the age of 2 to 3 years virtually all children have seroconverted to both viruses. It remains largely unknown which factors determine the clinical outcome of RSV and HMPV infections.

Since RSV and HMPV hospitalization have been associated with respiratory bacterial co-infections, we have tested if bacterial Toll-like receptor (TLR) agonists influence RSV or HMPV infections in human primary cells or cell lines. The bacterial lipopeptide Pam3CSK4, the prototype ligand for the heterodimeric TLR2/1 complex, enhanced RSV infection in primary epithelial, myeloid and lymphoid cells. Surprisingly, enhancement was optimal when lipopeptides and virus were added simultaneously, whereas addition of Pam3CSK4 immediately after infection had no effect. We identified two structurally related lipopeptides without TLR-signaling capacity that also enhanced RSV infection, whereas Pam3CSK4-reminiscent TLR2/1 agonists did not. A similar TLR-independent enhancement of infection could also be demonstrated for HMPV, measles virus and HIV-1. The effect of Pam3CSK4 was primarily mediated by enhanced virus binding to target cells, for which the N-palmitoylated cysteine in combination with the cationic lysines were shown pivotal.

In a follow-up study, normal human bronchial epithelial cells (NHBE) were cultured on transwell-plates. Air-liquid interface (ALI) was created by removing medium from the apical side to promote mucocilliary differentiation. Well-differentiated (wd-)NHBE cells were pre-incubated with *Haemophilus influenzae*, *Moraxella catarrhalis*, *Staphylococcus aureus* or *Streptococcus pneumoniae* for one hour at 37C and then infected with HMPV encoding enhanced green fluorescent protein (EGFP). EGFP-positive cells were counted visually at 24h post-infection. Of 57 children of the Generation R cohort study nasopharyngeal swabs and blood samples were collected at 0, 6, 14 and 24 months of age. All swabs were cultured to determine the colonization status of the children for *H. influenzae*, *M. catarrhalis*, *S. aureus* and *S. pneumoniae*. Serum was used to acquire seroconversion rates to HMPV using ELISA which were related to bacterial carriage. Pre-incubation of wd-NHBE with *S. pneumoniae* resulted in increased susceptibility to infection with HMPV-EGFP as determined by enumeration of EGFP-positive cells. This was not the case for cells pre-incubated with *S. aureus*, *H. influenzae* or *M. catarrhalis*. In the paediatric cohort study, frequent nasal carriage of *S. pneumoniae*, but not of the other respiratory bacteria, was associated with increased seroconversion rates of infants to HMPV.

Our combined *in vitro* and *in vivo* data suggest a specific interaction between *S. pneumoniae* and HMPV. We conclude that bacterial colonization may play an important role in the pathogenesis of HMPV- and RSV-related disease.

O025

Recognition of peptidoglycan from the microbiota by Nod1 enhances systemic innate immunity

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Humans are colonized by a large and diverse bacterial flora (the microbiota) essential for the development of the gut immune system. A broader role for the microbiota as a major modulator of systemic immunity has been proposed; however, evidence and a mechanism for this role have remained elusive. In this work we show that the microbiota are a source of peptidoglycan that systemically primes the innate immune system, enhancing killing by bone marrow–derived neutrophils of two major pathogens: *Streptococcus pneumoniae* and *Staphylococcus aureus*. This requires signaling via the pattern recognition receptor nucleotide-binding, oligomerization domain-containing protein-1 (Nod1, which recognizes *meso*-diaminopimelic acid (*meso*DAP)-containing peptidoglycan found predominantly in gram-negative bacteria), but not Nod2 (which

detects peptidoglycan found in gram-positive and gram-negative bacteria) or Toll-like receptor 4 (Tlr4, which recognizes lipopolysaccharide). We show translocation of peptidoglycan from the gut to neutrophils in the bone marrow and show that peptidoglycan concentrations in sera correlate with neutrophil function. *In vivo* administration of Nod1 ligands is sufficient to restore neutrophil function after microbiota depletion. *Nod1-/-* mice are more susceptible than wild-type mice to early pneumococcal sepsis, demonstrating a role for Nod1 in priming innate defenses facilitating a rapid response to infection. These data establish a mechanism for systemic immunomodulation by the microbiota and highlight potential adverse consequences of microbiota disruption by broad spectrum antibiotics on innate immune defense to infection.

O026

The human GI tract microbiota in health and disease

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The microbiota in the human gastrointestinal (GI) tract contains a very diverse collection of microbes from various phylogenetic groups that outnumbers the body cells by a factor of ten. It is evident that it remains a challenging task to decipher the functional role of all these microbes in the GI tract and their interactions with the host under conditions of health and disease, especially since only approximately 20% of GI tract microbes has successfully been cultured to date. A major breakthrough in overcoming limitations of culturing procedures has been gained by the application of molecular approaches, especially those based on the genetic diversity of ribosomal RNA (rRNA) and its corresponding genes which have become an established marker for routine taxonomic classification and phylogeny of microbes. Currently, more than 1,400,000 small subunit or 16S rRNA sequences are available in public DNA databases, which is far more than what has been deposited for any other gene. The use of 16S rRNA sequence information to study the GI tract microbiota has provided considerable new insights, including that the microbiota is host-specific, affected by the host genotype, stable in time in healthy adults and dominated by the phyla Firmicutes, Bacteroidetes and Actinobacteria. Compared to healthy subjects the microbiota in subjects suffering from GI disorders is often unstable and distinct in composition. Despite these findings, the exceptional complexity of the microbiota hampers establishment of links between the microbiota and GI disorders. This makes it extremely difficult to generate hypotheses about the role of specific microbial groups in the etiology of the various GI disorders. In order to pinpoint specific microbial groups that can be linked to GI disorders, high throughput characterization of the microbiota at various phylogenetic levels is needed. This can be done by barcoded pyrosequencing of 16S rRNA genes and/or phylogenetic microarraying using the Human Intestinal Tract Chip (HITChip) as will be demonstrated with some examples. It has to be realized that functional data cannot be extrapolated from 16S rRNA data. To obtain insight into the microbiota at the functional level and how this can be correlated to health and disease, omics approaches, such as metagenomics (the study of genomes recovered from environmental samples) are needed. Metagenomics will provide insight into the genetic potential within an ecosystem and since its introduction, the field of metagenomics is rapidly growing, which is by part due to the ever increasing capacity of next generation sequencing technologies. This has in the past few years already resulted in the generation of hundreds of genome sequences from intestinal isolates as well as hundreds of fecal metagenomes from healthy individuals and patients suffering from GI disorders, which in total consists of more than 5 million microbial genes. This enormous catalogue of genes from GI tract microbes and metagenomes is a very useful reference set that can be used to study the microbiota and its function in health and disease.

O029

Phosphatidylglycerol derived lipids are key to weak organic acid stress resistance in *Bacillus subtilis*

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Sorbic acid (trans, trans-2,4-hexadienoic acid) is widely used as an antimicrobial agent in foods. As resistance to its action in *Bacillus* is known and *Bacilli* are a key problem in microbial food stability (both safety and spoilage), we set out to identify its molecular basis in order to provide new antimicrobial targets. We used transposon mutagenesis to identify mutants with an altered susceptibility. Random mutant libraries were created in *Bacillus subtilis* wild-type strain PB2 and were screened for altered sorbic acid sensitivity. Disruption of rodZ (formally known as ymfM) turned out to cause the greatest sensitivity of all mutants screened for sorbic acid. In addition this strain was hypersensitive to the more hydrophilic acetic acid as well as NaCl induced stress. Because rodZ lies upstream of the essential phosphatidylglycerol synthase gene pgsA, we compared the sensitivity of rodZ::Tn10 to a Pspac-pgsA strain in which pgsA expression was controlled reduced. Such reduction was found to cause sensitivity to sorbic acid, acetic acid and NaCl, similar to that observed for the rodZ::Tn10 strain. Overexpression of rodZ or of the rodZ-pgsA operon from

the pDG 148 plasmid did not restore wild-type sensitivity for the stresses tested in respective transformants of the rodZ::Tn10 strain. It should be noted that expression from the pgsA position in the transformant bearing the rodZ-pgsA containing vector was significantly impaired. Overexpression of pgsA in the rodZ::Tn10 strain did partially restore the sensitivity for sorbic acid to wild-type levels. However, it did not restore the altered morphology of the mutant strain.

Phosphatidylglycerol synthase is committed to the biosynthesis of the phospholipids: phosphatidylglycerol (PG), cardiolipin (CL) and lysyl-phoshpatidylglycerol (LPG). Phospholipid analysis shows PG, CL, and LPG were all severely depleted in both the rodZ::Tn10 as well as the Pspac-pgsA strain. In addition increased PG-fatty acid chain length was observed for the residual PG molecules in both mutants. PG-fatty acid chain length was also increased in the wild-type strain upon sorbic acid stress adaptation. Our results show that the presence of PG-derived phospholipids and increased PG-fatty acid chain length correlates with resistance against sorbic acid. We propose that pgsA and rodZ are functionally intertwined and needed for normal phospholipid homeostasis, cell morphology and weak acid stress adaptation.

O030

Serotype related variation in *Streptococcus pneumoniae* susceptibility to human antimicrobial peptide LL-37 cathelicidin

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Introduction: Antimicrobial peptides (AMPs) are considered to be essential players in the host innate immune responses against a broad spectrum of pathogens. Yet the exact mechanism of interaction between AMPs and the gram-positive bacterium *Streptococcus pneumoniae* are not fully elucidated. It was previously suggested that positively charged AMPs kill pneumococci by disrupting bacterial membranes and that the negatively charged capsular polysaccharides may play either a protective role (Llobel et al. Microbiol. 2008) or to the contrary, sensitize bacterial cell to AMP mediated killing (Beiter *et al.* Infect Immun. 2008).

Aim: The aim of this study was to get insight into the impact of the capsule on *S. pneumoniae* resistance/susceptibility to AMPs.

Methods: Susceptibility to LL37 was tested for a range of 19 isogenic capsular variants of a serotype 4 strain TIGR4, and 6 and 4 variants respectively of serotype 6B strains 603 and 618, plus unencapsulated mutants of all three. In addition, LL37 susceptibility was tested for all clinical isolates that were used as capsule donors in variants construction. Susceptibility was tested by applying fixed assay condition of phosphate buffer supplemented with 0.5% Tryptic Soy Broth with or without 2 M of LL37, using an initial culture density of 106 CFU/ml. The effect of LL37 was measured by dividing number of CFUs recovered from LL37-supplemented by number of CFUs recovered from the peptide-free culture after one hour of incubation at 37 °C and 5% CO_2.

Results: We observed large variation in susceptibility to LL37 among isogenic capsular variants of TIGR4 with over to 2 log^{10} difference between mutants. Serotypes 19A and 19F were most susceptible and 3, 4, 12F, 35B most resistant to LL37. Capsular mutants of serotype 6B, 14 and 19F were tested in all three genetic backgrounds. The rank was identical for all three strain sets with serotype 14 being most resistant, serotype 6B intermediate and serotype 19F being most susceptible. Interestingly, the unencapsulated mutants were relatively resistant to LL37 suggesting that indeed certain capsular polysaccharides sensitize bacteria to LL37. There was a trend toward significant correlation between TIGR4 variants resistance to LL37 and the number of carbons per capsule oligosaccharide unit for all 19 serotypes (Spearman test, ρ=0.36, p=0.13). The correlation becomes significant after exclusion of serotype 3, the only serotype that is not covalently linked to a cell wall (ρ=0.57, p=0.014). However, when donor strains were tested for susceptibility to LL37, the correlation between serotype and LL37 susceptibility was absent, indicating an additional effect of serotype independent factors to pneumococcal resistance to LL37 as well. In addition, there was a significant (ρ=0.62, p=0.003) correlation between TIGR4 mutants resistance to LL37 and serotype-associated invasiveness as estimated for 17 serotypes for which attack rates were published by Sleeman et al. (J Infect Dis. 2006).

Conclusion: Results of this study suggest that serotype-dependent variation in *S. pneumoniae* susceptibility to LL37 may be linked to a clinical outcome of host-pathogen interaction. For the majority of serotypes this variation may be directly related to the chemical properties of the capsular polysaccharide.

O031

Macrolide resistance determination and molecular typing of *Mycoplasma pneumoniae* by pyrosequencing

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Introduction: The first choice antibiotics for treatment of *Mycoplasma pneumoniae (Mpn)* infections in children are macrolides. Several recent studies, however, have indicated that the prevalence of macrolide (ML)-resistance, which is determined by mutations in the bacterial 23S rRNA, is increasing among Mpn isolates. Consequently, it is imperative that ML-resistance in *Mpn* is rapidly detected to allow appropriate and timely treatment of patients. We therefore set out to determine the utility of pyrosequencing as a convenient technique to assess ML-resistance. In addition, we studied whether pyrosequencing could be useful for molecular typing of *Mpn* isolates.

Materials and methods: A total of four separate pyrosequencing assays were developed. These assays were designed such as to determine a short genomic sequence from four different sites, i.e. two locations within the 23S rRNA gene, one within the MPN141 (or P1) gene and one within the MPN528a gene. While the 23S rRNA regions were employed to determine ML-resistance, the latter two were used for molecular typing. The pyrosequencing assays were performed on a collection of 108 Mpn isolates, including 4 ML-resistant isolates, and a collection of 116 clinical samples positive for *Mpn* by standard PCR.

Results: The ML-resistant isolates within the collection were readily identified by pyrosequencing. Moreover, each strain was correctly typed as either a subtype 1 or subtype 2 strain by both the MPN141 and MPN528a pyrosequencing test, indicating a 100% correspondence between the two typing assays. From the collection of clinical samples, 88 samples could be confirmed as Mpn-positive by real-time PCR. In 75 (88%) of these samples, a genotype could be determined by one or both typing assays. ML-resistant genotypes were not found in the collection of clinical samples.

Discussion & conclusion: In conclusion, pyrosequencing is a convenient technique for ML-resistance determination as well as molecular typing of both *Mpn* isolates and *Mpn*-positive clinical samples. Since culturing of *Mpn* is insensitive and time-consuming, the direct determination of ML-resistance on clinical samples could have a significant impact on the antibiotic therapy of *Mpn*-infected patients.

O032

Retrospective analysis of candidemia in a tertiary medical center, 2004-2010 and its implications for empirical treatment

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Introduction: The number of patients at risk for developing candidemia increases with the scope, intensity, and duration of medical treatments. Studies from the 1990s indicate an increase of candidemia-associated crude mortality. Subsequently, empirical treatment of invasive yeast infections with azole derivates has been replaced with newer echinocandines due to the assumption of better outcomes. In order to provide the foundation for an advice for empirical treatment of invasive yeast infections in our hospital, we reviewed the laboratory records from 2004 till 2010 for blood cultures with yeasts.

Methods: Results from our laboratory medical information system GLIMS were searched for positive blood cultures with yeasts from 2004 until 2010. Identification of yeasts was completed using conventional lab techniques, API (Biomeriuex), FISH and recently Maldi-Tof. Susceptibility tests and cut-off values for anti-mycotic drugs were determined with the Sensititer Assay and E-test using Eucast breakpoints of 2010.

Results: From January 1 2004 until 1 December 2010, our laboratory received 102,745 blood with 106 patients having a blood culture positive with yeast. In 2004 14,409/15(cultures received/culture positive patients), 2005 14,903/16, 2006 15,623/19, 2007 15363/16, 2008 16,287/17, 2009 14,281/20, 2010 till dec 1 11,879/3). Fifty-six of 106 patients had *Candida albicans* (52%), 45 a non-albicans (42%), 3 were not determined (1%). Non-albicans species were 2 *Rhodotella* spp, 2 *Cryptococcus neoformans*,1 *Saccharomyces cerevisiae*. Forty-seven of the patients (44%) were in intensive care units (8 from the surgical ICU, 25 from the internal medicine ICU, 5 from the thoracic surgery ICU, 16 from the pediatric ICU). Eight patients (8%) came from the pediatric units (excluding neonatology), 12 from the surgical and ENT wards (11%), the remaining were distributed among the hematology, internal medicine wards, emergency, gynaecology, and dermatology. Susceptibility for fluconazol was found in 93% of *C. albicans*, 60% of *C. glabrata*, and 88% of *C. parapsilosis*. 93% of *C. albicans* were sensitive to voriconazol, 85% of the *C. glabrata*, and 100% of the *C. parapsilosis*. All of the cultured C. *albicans* and *C. parapsilosis* strains were sensitive to caspofungin using a proposed breakpoint of 2 mg/l.

Conclusion: The rate of culture positive invasive yeast infections in our tertiary medical center has shown a remarkably consistency in the years 2004-2009, at around 0.001% of all submitted blood cultures with a marked drop in 2010. Based on susceptibility data of isolated strains, empirical therapy with fluconazol was inappropriate in 12% (7 strains I,7 strains R against fluconazol,) of the identified agents from all invasive yeast infections, suggesting a more prominent place for echinocandines in empirical therapy.

O033

Functional metagenomic analysis of the reservoir of antibiotic resistance genes in intensive care unit patients

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In the last decade metagenomic approaches have revolutionized our understanding of the composition and diversity of microbiological communities in many environments.

Functional metagenomic screening of environmental DNA has revealed an enormous reservoir of antibiotic resistance genes ('the resistome') in the soil but also in the human gut microbiota.

The emergence of antibiotic resistant bacteria in hospitals presents a major threat to public health. It is of importance to generate novel insights into the diversity of antibiotic resistance genes in the human gastrointestinal tract. Antibiotic resistance genes detected in non-opportunistic bacteria may potentially transfer to opportunistic pathogens, thereby further exacerbating the burden of infections caused by antibiotic resistant bacteria.

We will use functional metagenomic approaches to identify and quantify antibiotic resistance genes from the microbiota of patients hospitalized in Intensive Care Units (ICUs). The main focus of our research is to evaluate the effect of antibiotic therapies that are applied in ICUs on the human resistome. ICUs are considered to be breeding grounds of novel resistance genes, since patients in ICUs are generally treated with high doses and a broad variety of antibiotics, either therapeutically or prophylactically.

We have developed protocols (using gradient centrifugation) for the efficient isolation of high-molecular weight DNA from human feces and have constructed fosmid libraries using the vector pCC1FOS (Epicentre). Subsequent screening of the fosmid library will lead to the identification of inserts that confer antibiotic resistance to *Escherichia coli*. Transposon mutagenesis will then pinpoint the resistance determinant. Sequencing of the insert will allow us to make predictions on the organisms that harbor the identified resistance genes. We also aim to generate metagenomic libraries using alternative hosts, which will allow for screening of genes to which *E. coli* shows intrinsic resistance or which are poorly expressed or translated in this standard cloning host.

The outcome of this study will be a detailed assessment of the impact that antibiotic therapy has on the reservoir of antibiotic resistance genes in the human gut. An in-depth functional characterization of the resistance reservoir of the human microbiome will increase our understanding of the emergence of antibiotic resistance and might open up avenues for the development of novel intervention strategies to combat the emergence of antibiotic resistance.

O034

Colistin resistance in gram-negative bacteria (GNB) during prophylactic colistin use in intensive care units (ICU)

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Introduction: Colistin is increasingly used in ICU patients as a last resort antibiotic to treat infections caused by multi-resistant GNB. Selective Digestive Tract Decontamination (SDD) and Selective Oropharyngeal Decontamination (SOD) are prophylactic regimens for ICU-patients consisting of topical application of tobramycin (TOB), colistin (COL) and amphotericin B applied every 6hr throughout ICU stay. Although reported sparsely, colistin use may lead to colistin resistance. As SDD and SOD are almost universally used in Dutch ICUs, we quantified the conversion rate from COL susceptibility to resistance (S-R conversion) among GNB in 2 Dutch ICU-cohorts.

Methods: Cohort-1 was a single-center observational study comprising all microbiological data from all ICU-patients between January 2008 and October 2010 in a tertiary hospital. In this ICU, SDD was used from January 2008 until August 2009 and SOD from September 2009 to October 2010. In cohort-2 only rectal carriage with GNB was assessed and consisted of all SDD-patients participating in a 13-center cluster-randomized trial with at least one rectal culture result (New Eng J Med. 2009;360:20). We identified all S-R conversions of colistin in *Escherichia coli* (EC), *Klebsiella* spp. (KS) or *Enterobacter* spp. (EB), occurring within a single bacterial species, during a single ICU-admission. As part of SDD and SOD rectal and respiratory cultures were obtained at admission and twice weekly. For each COL S-R conversion, the time between the first COL susceptible and resistant isolate was calculated. The COL S-R conversion rate was the number of events per 1,000 patientdays at risk.

Results: For cohort-1, in total 41,597 cultures were obtained in 6049 patients comprising 31,163 patientdays (1.3 culture per patientday). 2103 of 6049 patients had an ICU stay >48 hours resulting in 23,179 patient days. In cohort-2, 1917 patients were included resulting in 26,036 patientdays and 6,128 rectal culture results (0.24 per patientday). COL S-R conversion occurred in 18 of 2103 ICU-patients (0.86%) in cohort-1, and in 19 of 1917 patients (1.0%) in cohort-2. Median length of ICU stay of COL S-R converters was 29 days (range 8-96, IQR 30) and 19 days (range 3-268, IQR 49) and median time to conversion was 11 days (range 4-35, IQR 10) and 5 days (range 2-71; IQR 4) for cohort-1 and -2 respectively. Conversion rate was 0.79 per 1,000 ICU-patient days at risk for cohort-1 and 0.73 for cohort-2. Based on epidemiological linkage there was no evidence of cross-transmission of colistin resistant GNB.

Conclusion: In ICUs with low endemicity of antibiotic resistance and continuous exposure to topical COL, COL S-R conversion occurred in less than 1% of patients, yielding a COL S-R conversion rate of below 0.8 per 1,000 patient days at risk.

O035

High rates of intestinal colonization with extended-spectrum beta-lactamase-producing *Enterobacteriaceae* in patients at a tertiary-care hospital in Israel

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Objectives: Nosocomial infections caused by extended-spectrum beta-lactamase producing *Enterobacteriaceae* (ESBL-En) are increasing worldwide. The influx of these bacteria into hospitals has major implications for infection-control. We studied the prevalence and molecular epidemiology of ESBL-En isolated from stool samples of hospitalized patients at the Tel-Aviv Sourasky Medical Center, Israel.

Methods: Stool samples (n=120) were screened for presence of ESBL-En on the BLSE bi-plate (MacConkey/ Drigalski agar supplemented with ceftazidime and cefotaxime, respectively). Isolates growing on BLSE agar were purified and phenotypic presence of ESBLs confirmed by the double disc synergy test (DDST; cefotaxime, ceftazidime, cefepime, and amoxicillin/ clavulanate). Isolates were identified by conventional and semi-automated (Api20NE/E, bioMrieux; Ultraflex MALDI-TOF, Bruker Daltonics) biochemical testing. Beta-lactamase genes (blaCTX-M, blaTEM, blaSHV and blaOXA) were detected by consensus PCRs. blaCTX-M, blaTEM and blaSHV amplicons were typed by bidirectional sequencing. Subtypes were elucidated by multiple alignments (ClustalW2; DNASTAR) and assigned based on the Lahey (www.Lahey.org) and NCBI (www.ncbi.nlm.nih.gov) databases.

Results: Forty-four (36.7%) stool samples showed presence of ESBL-En. CTX-M, TEM, SHV and OXA genes were found in 35 (79.6%), 32 (72.7%), 15 (34.1%) and 4 (9.1%) stool samples, respectively. Upto 4 different ESBL-En species could be recovered from a single stool sample. One, 2, 3, and 4 different ESBL-En species were recovered from 22, 15, 6, and 1 stool samples, respectively. Majority of the stool samples carried Escherichia coli either alone (n=15, 34.1%) or in combination (n=10, 22.7%) with other ESBL-En. Of the 74 ESBL-En isolates studied, *E. coli* (n=30, 40.5%) was predominant followed by *Klebsiella pneumoniae* (n=22, 29.7%), and *Enterobacter cloacae* (n=7, 9.5%). CTX-M, TEM and SHV genes were present either alone or in combination in 52 (70.3%), 51 (68.9%) and 17 (23.0%) of the 74 ESBL-En analyzed, respectively. Of the 52 CTX-M genes, 43 (82.7%) were identified as CTX-M-15, 5 as CTX-M-2 (9.6%), 2 (3.8%) as CTX-M-27, 1 (1.9%) as CTX-M-24, and 1 (1.9%) as CTX-M-94. Of the 51 TEM genes, 44 (86.3%) were identified as TEM-1, 5 (9.8%) as TEM-116, 1 (2.0%) as TEM-2 and 1 (2.0%) as TEM-37. Of the 17 SHV genes, 6 (35.3%) were identified as SHV-12, 5 (29.4%) as SHV-11, 4 (23.5%) as SHV-98, 1 (5.9%) as SHV-5 and 1 (5.9%) as SHV-33. The majority (n=20, 27.0%) of the isolates carried CTX-M-15 in combination with TEM-1, followed by 12 (16.2%) carrying only CTX-M-15, 6 (8.1%) carrying TEM-1 and 4 (5.4%) carrying CTX-M-15 in combination with TEM-1 and SHV-11.

Conclusions: High rates of intestinal colonization with ESBL-En in hospitalized patients in Israel underscore the importance of screening for ESBL-En carriage on hospital admission. CTX-M-15 harbouring *E. coli* were the most prevalent ESBL-En in our study.

O040

Possible important differences in cellular immune responses after the change in the vaccination programme from the whole cell pertussis vaccine to an acellular vaccine

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From 2005 onwards, the whole cell pertussis (wP) vaccine component has been changed to an acellular pertussis (aP) component. Afterwards, infants are vaccinated at 2,3,4 and 11 months (primed) with high doses of purified proteins combined to an alum adjuvant followed by an aP booster at 4 years of age. Since antibody levels wane rapidly after pertussis vaccination, long-term B- and T-cell immune responses might play a major role in protection against pertussis. In addition, the roles of the various T-helper-cell immune responses (Th1, Th2, Th3 and Th17) regulating the balance between protection, allergy and auto-immunity are important.

In a cross-sectional study, we investigated B- and T-cell memory immune responses in wP or aP primed children of 4 years of age before and after the aP preschool booster. B-cells were purified from PBMCs and polyclonally stimulated by CpG and cytokines. After 5 days memory B-cells were detected by ELI-Spot assays specific for the pertussis vaccine components, pertussis toxin, filamentous heamagglutin, pertactin as well as tetanus toxoid. T-cells were stimulated with these proteins for 5 days, interferon-γ

producing cells were measured and T-helper cytokines (Th1, Th2, Th17 and Th3) were determined by fluorescent bead-based multiplex immunoassay (MIA). In addition to plasma IgG levels, subclasses of IgG and IgA and IgE levels were measured by MIA too.
Three years after the wP or aP vaccinations low IgG levels to the pertussis proteins were found. However, in these children pertussis protein-specific memory B-cells as well as memory T-cells were identified. After the preschool booster aP primed children showed high pertussis protein-specific IgG levels and high numbers of memory B-cells. In addition, also higher levels of protein-specific IgG4 and total IgE were induced compared to wP primed children.
Before the preschool booster, still very high pertussis protein-specific T-cell responses were found in aP vaccinated children. Surprisingly, these T-cell responses did not show a typical increase in cytokine production shortly (10 days) after the preschool booster. However, especially the Th2 cytokine concentrations and to a lesser extent the Th1 and Th17 cytokines remained high during the first 28 days after the booster. In contrast, wP vaccinated children showed low T-cell cytokine concentrations, but the preschool aP booster induced increases at 10 days followed by decreases at 28 days after booster.
This study shows the presence of long-term pertussis protein-specific memory B- and T-cells in children despite waning antibody levels and that aP vaccination resulted in higher B-cell memory responses compared to wP. However, aP vaccination resulted in very high Th2-cell responses in infants and unusual T-cell kinetics, high IgG4 and high IgE levels after the pre school booster which are associated with allergy. These responses might be correlated to the higher incidence of severe local side effects of vaccination reported after the fifth aP vaccination in children. We hypothesize that the high doses of pertussis proteins in combination with a Th2 skewing adjuvant in infants might overstimulate the immune system of infants indicating that vaccination schedules might be adapted.

O041

Long-lasting sterile protection and cellular immune responses against *P. falciparum* malaria in human volunteers

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Introduction: For lack of an effective vaccine, malaria remains to this day responsible for an intolerable burden of morbidity and mortality, not least in sub-Saharan Africa. Compounding the many technical hurdles in developing such a vaccine, it is commonly held that (naturally-acquired) immunity to malaria is both difficult to induce and quick to wane. Recently, however, we demonstrated the successful induction of sterile protection against experimental *P. falciparum* infection in human volunteers who had previously been exposed to patent sporozoites under cover of chloroquine prophylaxis. Here we have assessed the longevity of this protection and the immune responses associated with it.
Methods: Six out of the original ten protected volunteers were re-challenged by exposure to the bites of 5 *P. falciparum*-infected *A. stephensi* mosquitoes, almost 2 years after the original trial. Five malaria-nave volunteers served as controls. Volunteers were monitored daily for the development of parasitaemia by thick blood smear and retrospectively by PCR. As soon as thick smear-positive, or else presumptively on day 21 after challenge, volunteers were treated with a curative regimen of atovaquone-proguanil. Venous whole blood was drawn at various time points prior to, during and following infection for the assessment of parasite-specific immune responses. Serological responses against sporozoites and *P. falciparum*-infected erythrocytes (*Pf*RBC) were measured in serum by standard ELISA techniques. Cellular responses were assessed by incubating peripheral blood mononuclear cells*in vitro* with sporozoites or *Pf*RBC, followed by intracellular cytokine staining and flow cytometry.
Results: Four out of the six previously immunised volunteers remained sterilely protected following re-challenge, whereas all five control volunteers developed patent parasitaemia. The two other immunised volunteers also developed parasitaemia, but with a markedly delayed patency.
Serological responses were barely measurable against either sporozoites or *Pf*RBC in any of the volunteers at any time point. In contrast, cellular responses against both sporozoites and *Pf*RBC, in particular interferon-α production, were readily induced by the original immunisation and challenge and remained robust up till the point of re-challenge. The principle contributors to this response proved to be $\gamma\delta$T cells, $CD4^+$ effector memory T cells and NK cells.
Conclusions: These findings prove conclusively that protective immunity against malaria in humans can not only be readily induced, but also sustained over the course of years. Such protection appears associated with long-lived cellular immune responses against both pre-erythrocytic and blood-stage parasites. This realisation moreover greatly enhances the prospect of achieving implementable vaccination against malaria and advocates in particular for the further development of whole-parasite-based vaccines.

O043

Biofilm formation in *Salmonella enterica* serovar typhimurium – more than a bacterial lifestyle

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Biofilm formation, the ability of microorganisms to colonize surfaces, adhere to each other and build up multicellular communities, is the preferred form of life by most microorganisms. Investigation of this life style in bacteria has not only discovered novel fundamental physiological processes in bacteria, but also provided novel targets for antimicrobial treatment. For example, biofilm research has discovered novel principles of secondary messenger regulation, identified novel exopolysaccharides and quorum-sensing molecules. On the other hand, identification of biofilm extracellular matrix components provided targets for rationalized approaches to antimicrobial treatment.
The gastrointestinal pathogen *Salmonella typhimurium* has been one of the early model organisms to study the molecular mechanisms of biofilm formation. Investigation of the features of plate-grown cells, which express a peculiar colony morphology, the rdar morphotype, discovered the novel secondary messenger cyclic di-GMP and the common biofilm matrix component cellulose.
In *S. typhimurium*, biofilm formation on biotic and abiotic surfaces contributes to transmission, colonization, persistence, survival and virulence. The biofilm observed inside and outside the host is closely related to the rdar morphotype, a characteristic colony morphology observed when *Salmonella* grows on agar plates. Rdar morphotype formation is characterized by the production of the extracellular biofilm matrix components curli fimbriae and cellulose. Rdar morphotype xpression is activated by the major biofilm regulator CsgD which binds directly to the promoter region of the curli synthesis operon and the *adrA* gene encoding a di-guanylate cyclase which synthesizes the cyclic di-GMP for the activation of cellulose biosynthesis and thereby coordinates bacterial behavior and gene expression. Among many factors, expression of CsgD is tightly controlled by secondary messenger signaling through cyclic di-GMP. CsgD, however, does not only activate biofilm matrix components, but also represses virulence properties of *S. typhimurium* by unknown mechanisms, which involves cyclic di-GMP signalling. Consequently, biofilm formation of *S. typhimurium* dynamically also modules the interaction with the host which might affect disease outcome.

O044

Architecturally complex colony development in *Bacillus subtilis*

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Under certain conditions *B. subtilis* forms highly structured biofilms, which can be observed under laboratory conditions as pellicle or architecturally complex colony formation. While most genetically amenable laboratory strains have lost their ability to form structured colonies, few variants are still able to form complex colonies, providing a valuable tool to examine biofilm formation and the effect of regulators. Many regulators effecting biofilm formation are also participating in other processes in the cells. Biofilm formation is closely intertwined with motility and sporulation of *B. subtilis*.
We have observed reduced biofilm structure formation in *B subtilis* when the *rok* (regulator of *comK*) gene was mutated. Rok is a repressor of ComK, the master regulator of competence and also reduces the transcription of a family of genes that specify membrane-localized and secreted proteins, including a number of genes that encode products with antibiotic activity. Transcriptome analysis of the *rok* mutant under biofilm conditions revealed the upregulation of previously identified genes regulated directly by *Rok* or indirectly by *comK* in liquid cultures. The *yuaB*, *yobB* and *yoqLM* genes were found to be downregulated in the rok mutant. Biofilm formation was not affected by a *comK* mutation. When transcriptome analysis was performed on a KO of *comK* and *comK+rok*, a list of differentially regulated genes excluded the previously identified ComK regulated genes.
Mutant strains were generated in genes that were downregulated in the *rok* strain under complex colony development. Only a mutation in *yuaB* showed a reduction in biofilm structure, similar to that of the rok mutant. *YuaB*, a small secreted protein, was recently shown to be involved in biofilm formation. It is regulated by DegU, AbrB and dependent on biosynthesis of the polysaccharide component of the *B. subtilis* biofilm matrix. *Rok* regulates the expression of *yuaB* indirectly, but independently from the previously described regulators. Overexpression of YuaB in the *rok* strain restored biofilm formation indicating that Rok effects the development of *B. subtilis* biofilm solely via regulating the expression of *yuaB*. We further present the application of fluorescence microscopy to follow spatial gene expression in *B. subtilis* biofilms.

Reference

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O045

Mechanisms in *Listeria monocytogenes* biofilm formation and disinfectant resistance

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The food-borne pathogen *Listeria monocytogenes* is frequently encountered in food processing facilities on food contact surfaces, on floors, and in drains, resulting in (cross-) contamination of food products. Several studies have shown that certain strains of *L. monocytogenes* can be present in food processing environments for years. Their presence and survival of disinfection treatments is expected to be dependent on their ability to form (stress resistant) biofilms. Since biofilms are generally more difficult to eradicate during disinfection treatments, the capability of *L. monocytogenes* to form biofilms poses a major concern for the food industry. Possible mechanisms involved in the increased resistance of biofilms to disinfectants are the restricted penetration of the biofilm, the slow growth rate of organisms in the biofilm, and the induction of resistance mechanisms in the biofilm. So far, little is known on the function of stress resistance mechanisms in biofilm formation and their resistance to disinfectants. For *L. monocytogenes* biofilms, two distinct morphologies have been identified. *L. monocytogenes* static biofilms on polystyrene and glass consists of a homogeneous layer, while on stainless steel *L. monocytogenes* biofilms consist of single attached cells or microcolonies. Static biofilms contain the small rod-shaped morphology, which is very similar to the morphology of planktonic cells. However, *L. monocytogenes* continuous-flow biofilms consist of ball-shaped microcolonies, which are surrounded by a dense network of knitted chains composed of elongated cells.

In this study, we investigated the role of the stress genes *recA*, which encodes the transcriptional activator of the SOS response, *sigB*, which encodes a major transcriptional regulator of the class II stress response genes, *hrcA*, which encodes the transcriptional regulator of the class I heat-shock response, and *dnaK*, which encodes a class I heat-shock response chaperone protein, in *L. monocytogenes* static and continuous-flow biofilm formation and/or their function in the resistance of biofilm cells to disinfectants. We showed that continuous-flow biofilm formation and not static biofilm formation is dependent on RecA and the SOS response. Using Q-PCR analyses, promoter reporters, and in-frame *recA* and SOS response deletion mutants, we showed that *recA* and the SOS response are activated during continuous-flow biofilm formation and that deletion of the SOS response gene *yneA*, which is involved in cell elongation during SOS response activation, results in diminished biofilm formation under continuous-flow conditions. Furthermore, Q-PCR and promoter reporter studies showed that *sigB*, *hrcA*, and *dnaK* are activated in static and/or continuous-flow biofilms. Biofilm formation studies using in-frame deletion mutants and complementation mutants showed that the presence of SigB, HrcA, and DnaK is required to obtain wild-type levels of both static and continuous-flow biofilms. Finally, disinfection treatments of planktonic grown cells and cells obtained from static and continuous-flow biofilms showed that SigB, HrcA, and DnaK are involved in the resistance of both planktonic cells and biofilms to the disinfectants benzalkonium chloride and peracetic acid. In conclusion, our study highlighted the impact of stress resistance mechanisms on biofilm formation and resistance of biofilms against disinfectants.

O046

Biofilm development on new and cleaned membrane surfaces

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This study provides a basic insight in the microbiological aspects of biofouling in full-scale reverse osmosis (RO) systems. Biofouling is a process in which microorganisms attach to membranes and develop into a thick film that can block the entire RO system. Management of this problem requires basic understanding of the mechanism of this phenomenon. The basic questions of this research project therefore addressed the origin, succession and spatiotemporal development of biofilms in full-scale RO systems, in particular in relation to operational aspects of RO systems. The multifaceted research strategy involving acquisitions of representative samples and use of many molecular and microscopic analysis techniques in parallel was employed. The investigation showed that biofilms are able to grow on any surface in a full-scale RO plant. This gives local niches for detachment of biomass, either as single cells or cell clumps, and results in a spreading of bacteria to the further stages of the plant. In the RO membrane modules, the enriched bacteria might more easily colonise the surfaces since they will be better adapted to growth in the system than bacteria present in the feed water. Initially, the single cell colonizers (sphingomonads) form a number of flat and abundantly EPS-embedded cell monolayers over the entire membrane surface. The clumps-associated pioneers (mainly Beta- and Gammaproteobacteria) appear to be trapped mainly in the first part of the module, most likely due to a filtering action of the spacer. In time,

these bacteria develop in pillar-like structures and slowly spread throughout the whole membrane module on top of the established sphingomonads biofilm. The secondary colonisers (bacteria and eukaryotes) occur in the resulting biofilm formations. Although composition of the biofilm microbial community undergoes a succession in time, the architecture of an established biofilm appears to be rather stable. Conventional treatment of RO membrane modules with chemicals did not lead to cleaning: the sphingomonads cells can be detected under the collapsed but obviously not removed biofilm EPS matrix. After cleaning, the biofouling layer seemed to grow faster (within 6 days) than a fresh biofilm (16 days). To conclude, biofouling is a complex phenomenon with two appearances: a fouling layer on the membrane limiting the water flux and a fouling layer on the spacer limiting the water flow through the spacer channel and resulting in an increased pressure drop. It became clear that cleaning strategies should focus more on the removal of accumulated biomass and not only on the killing of cells. Moreover, the basal Sphingomonas layer requires further research to appropriately control biofouling in RO systems. It might also be possible to design the RO-membrane module in a different manner, leading to a different biofilm morphology which gives less rise to operational problems.

O048

Fever and eosinophilia after visiting Italy

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A 35 year old female presented with complaints of fever and malaise. She had no medical history. There were no respiratory or urinary tract symptoms. She had recently visited Northern Italy, but not the tropics. Her father had similar complaints.
On physical examination no fever was present and no other abnormalities were detected.. Laboratory results showed mildly elevated liver enzymes, CRPl 39 mg/L and leukocyte count 11.2 x 10^9/L, 30 percent eosinophiles. Chest X-ray and abdominal ultrasound showed no liver abscess or other abnormalities.
Because of the eosinophilia parasitological examination of the stool was performed. On microscopy one liverfluke egg was detected. PCR on the stools confirmed an *Opistorchis felineus* infection.
Opistorchis felineus is part of the family *Opistorchidae*, Class Trematodes (flukes). Fluke-infection is a common foodborne parasitic zoonose in Southeast Asia (*Clonorchis sinensis*). Worldwide the number of human infections with *Opisthorchis felineus* has been estimated to be estimated 1.2 million. Infection in Western Europe is extremely rare. The Trematode lives in the biliary duct of mammals where it reproduces. The eggs are excreted with the faeces. Water snails eat these eggs and develop into miracidia and free swimming cercariae. These cercariae invade freshwater fish such as the tench (member of the carp family). By eating raw fish mammals can be infected.
The incubation period is 2-4 weeks. Acute symptoms are abdominal pain, fever and malaise. Chronic complications include liver abscess and biliary duct carcinoma.
The diagnosis is made by microscopic detection of eggs in the faeces. It is impossible to differentiate microscopically between species of liver flukes, hence specific PCR testing is needed. The diagnosis can be confirmed by a positive antibody test.
Our patient had eaten raw fish in a restaurant in Italy, carpaccio of tench. Public health services in Italy report that a large percentage of tench in northern Italian lakes are colonised by *Opistorchis felineus*. It can also be found in the excrements of stray cats around the lakes. Fish can be decontaminated by heating up to 70 °C or freezing below -28 °C. Of the 52 people who had had diner in the restaurant concerned, 45 presented with symptoms at the local hospital. Eight of them were admitted. One patient developed a liver abscess. All patients were treated with praziquantel 75mg/kg/dd. Our patient made a full recovery. Doctors, patients and caterers should be aware that changing eating habits, e.g. consumption of raw fish in Europe, can lead to an incline of parasitic infections.

O049

Epidemiological and clinical aspects of opisthorchiasis in Italy

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The zoonotic trematode *Opisthorchis felineus* was first described in 1884 by Rivolta, who detected the trematode in cats and dogs in Italy (1884). This liverfluke helminth has a complex natural cycle which involves a freshwater snail of the genus *Bithynia* (first intermediate host), a freshwater fish of the family Cyprinidae (second intermediate host), and a fish-eating mammal (final host). The geographical range includes European and north-western Asian regions. Humans acquire the infection by eating raw fish infected with the larval stage of metacercaria. A high prevalence of human infection has been reported in Byelorussia, Russia and the Ukraine. In the European Union, sporadic human infections have been documented in Germany and Greece. In Italy, for over 100 years after the discovery of the parasite, the infection remained in oblivion. However, from 2003 to 2010, 105 human infections were documented, including cases occurring in seven outbreaks, as well as isolated cases. The duration of the incubation period is highly variable, from 10 to 30 days, and it mainly depends on the infective dose. The

symptoms of infection, which include fever, nausea, abdominal pain, and diffuse myalgia, are not pathognomonic. At the onset of symptoms, infected persons can also show marked leukocytosis with eosinophilia and elevated transaminases, though 3-4 months later these parameters can return to normal, even if adult worms are still present in the bile ducts. For this reason, clinical diagnosis in chronically infected persons can be very difficult if no epidemiological data are available. Diagnosis can be based on the microscope detection of eggs (15x30 μm) in stool or the detection of DNA in stool by PCR. Otherwise, anti-*Opisthorchis* IgG can be detected in the serum by ELISA using excretory/secretory antigens, though cross-reactions with other trematode infections can occur. Regarding treatment, the drug of choice is praziquantel (25 mg/kg *per os* tid, for 1 day); alternatively, albendazole (10 mg/kg daily *per os*, in 2 doses for 7 days) can be used, though it is less effective in persons harboring numerous worms and a second cycle of treatment may be necessary.

O050

Salmon surprise

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A gynecologist at the LUMC was invited by friends to dinner. On that evening fresh home-smoked wild salmon was served. The salmon was purchased at a food wholesaler in Leiden. Approaching the end of the meal someone saw something moving on their plate. Thereafter, several worm-like moving objects were observed. None of the dinner guests experienced physical complaints after the dinner. One day later, one of the 'worms' was brought to the laboratory of the LUMC and identified as an *Anisakis* species larva. In the presentation the lifecycle, risk-factors, clinical presentation, prevention and epidemiology of this parasite will be discussed.

O051

***Toxocara* and *Ascaris* seropositivity among patients suspected of visceral and ocular larva migrans in the Netherlands: trends from 1998 to 2009**

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Introduction: *Toxocara canis*, *Toxocara cati* and *Ascaris suum* are roundworms of dogs, cats and pigs respectively that can also infect humans. These zoonotic helminths have a worldwide distribution and are also endemic in the Netherlands. Infection with *Toxocara* sp. can result in visceral larva migrans (VLM), a syndrome caused by the migrating larvae and characterized by peripheral eosinophilia, fever, malaise, hepatomegaly, hyperglobulinemia, respiratory and nervous symptoms. Larvae can also migrate to the eye resulting in ocular larva migrans (OLM). Although *A. suum* has been reported to occasionally mature to the adult stage in humans, clinical cases of VLM suspected to be caused by *A. suum*, has also been described. To study the importance of exposure to these zoonotic helminths for the public health, we determined the trends of *Toxocara* and *Ascaris* seropositvity among patients suspected of suffering from VLM or OML over the past 12 years in the Netherlands. Age and gender distribution of the observed seropositivity was also investigated.

Methods: A total of 2845 serum samples from suspected VLM and OLM patients sent to our institution from 1998 to 2009 were used in this study. Diagnosis of these helminth infections relies mainly on serology. Detection of anti-*Toxocara* and *Ascaris* IgG antibodies was performed using an ELISA based on the excretory/secretory (E/S) antigen derived either from *T. canis* or *A. suum* larvae. In order to analyze seropositivity trends over the past twelve years and differences in age and gender of subjects testing positive for *Ascaris* spp. or *Toxocara* spp., univariate models were developed using logistic regression.

Results: Findings from this study indicate that for each year the *Ascaris* seropositivity was significantly higher compared to the *Toxocara* seropositivity. Furthermore, while *Toxocara* seropositivity decreased in time, the *Ascaris* seropositivity did not significantly changed for the past 12 years. The influence of age and gender on seropositivity was also investigated. Results indicate that both the *Ascaris* and *Toxocara* seropositivity increases with age and while gender had no influence on the *Ascaris* seropositivity, males showed higher *Toxocara* seropositivity.

Conclusion: The observed decrease in *Toxocara* seropositivity may be the result of implemented campaigns on deworming dogs and cats that exists for many years in the Netherlands, which is one of the recommended measures for control of animal and human toxocariasis. Very little is known however about ascariasis in humans due to *A. suum*. Epidemiological studies must be carried out in order to determine risk factors for infection and propose appropriate preventing measures against infection with this roundworm.

O052

Ecophysiology and genomics of key nitrite-oxidizing bacteria

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Nitrite-oxidizing bacteria (NOB) catalyze the second step of nitrification, the oxidation of nitrite to nitrate, and thus

represent an important functional group of nitrogen-cycle organisms. As most NOB are still uncultured and the few available cultures are difficult to maintain, this functional guild is barely studied and also dramatically undersampled by genomics. NOB are phylogenetically heterogeneous and novel representatives are still discovered. Interestingly, the different NOB are not equally distributed in major natural and engineered ecosystems. Only few ecophysiological factors determining this niche separation have been identified so far, although deeper insights into this phenomenon would be essential for a better understanding of nitrification ecology.

Recently, the complete genome of "*Candidatus Nitrospira defluvii*" was assembled from the metagenome of a nitrite-oxidizing enrichment. It represents the first genome of *Nitrospira*, which are the predominant NOB in many habitats. The analysis of this genome has revealed unexpected physiological features including a novel nitrite-oxidizing enzyme, the lack of common oxygen defense mechanisms, and the presence of complete heterotrophic pathways in this chemolithoautotroph. As indicated by a genomic comparison of *Nitrospira* to the well-known NOB of the genus *Nitrobacter*, the chemolithoautotrophic nitrite-oxidizing lifestyle has evolved more than once in the microbial world with different efficiencies of the underlying biochemical pathways. Intriguingly, the genome of *Ca. N. defluvii* also suggests a surprising evolutionary link between these NOB and anaerobic ammonium-oxidizing "anammox" organisms.

Meanwhile the genome of the closest cultured relative *of Ca. N. defluvii*, *Nitrospira moscoviensis*, has been sequenced and is currently being studied. Comparative analysis of the two *Nitrospira* genomes has shown that, despite a common core metabolism, these nitrite oxidizers clearly represent genetically and ecophysiologically distinct branches within the genus *Nitrospira*.

In conclusion, these results demonstrate that NOB are a complex functional group whose members share certain phenotypic traits, but differ dramatically in the required key enzymes and also in other features that may determine their environmental distribution and ecological success.

O053

Analysis of bacterial and archaeal diversity in coastal microbial mats by using massive parallel 16S rRNA gene tag-sequencing

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Coastal microbial mats are considered as the modern analogues of fossil stromatolites, which represent the most ancient ecosystem known. Microbial mats are small-scale and largely closed ecosystems in which a plethora of different functional groups of microorganisms are responsible for the biogeochemical cycling of the elements. Microbial mats play an important role in coastal protection and morphodynamics through the stabilization of the sediments and by initiating the development of salt-marshes. Little is known about the microbial diversity and how it contributes to the ecological functioning of coastal microbial mats. Knowledge of the microbial diversity in microbial mats may provide evolutionary clues and could result in biotechnological applications.

Here, we analyzed three different types of coastal microbial mats which are located along a tidal gradient. The mats were sampled during three different seasons and subjected to massive parallel tag sequencing of the V6 region of the 16S rRNA genes of Bacteria and Archaea. Analysis of this large dataset revealed that these coastal microbial mats are among the most diverse marine ecosystems that we know. We identified the key players and functional groups of microorganisms and interpreted their role in the mat ecosystem. The diversity between the different mat types was far more pronounced than the changes between the different seasons at one location. We concluded that the community composition and the microbial diversity were intrinsic of the mat type and seemed to depend on the location along the tidal gradient and are most likely a function of salinity.

O054

Enrichment of nitrite-dependent methane oxidizers from an acidic peatland

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Freshwater sediments which receive nitrate fluxes from agricultural runoff and methane from methanogenesis theoretically provide ideal conditions for the recently discovered process of anaerobic methane oxidation coupled to denitrification. *Candidatus Methylomirabilis oxyfera*, the responsible bacterium, employs a novel pathway, whereby N_2 and O_2 are formed from NO without N_2O as an intermediate; and the oxygen is used for canonical aerobic methane oxidation. To further our knowledge of the role of *M. oxyfera* in the environment, we determined methane and nitrate depth profiles of a minerotrophic peatbog at Brunssummerheide in Southern Limburg using gas chromatography and colorimetric methods. Methane was depleted at the same depth as nitrate, before reaching the oxic zone. Samples taken from this nitrate-methane transition zone displayed nitrite dependent anaerobic methane oxidation activity when incubated with ^{13}C labeled methane and ^{15}N labeled nitrite in batch

tests. Soil from this depth was used as an inoculum for a continuous anaerobic, methanotrophic enrichment culture using a mineral medium with nitrite and nitrate as electron acceptors, and a pH value of 6.2. During incubation, methane oxidation and nitrite conversion were regularly tested. Stable-isotope experiments showed that nitrite was preferred over nitrate, and methane oxidation ceased without either electron acceptor. FISH microscopy and PCR amplification of 16S rRNA (95% similarity) and pmoA gene (90% similarity) revealed that new *M. oxyfera*-like bacteria had been enriched. No clear population shift was observed after several months of further enrichment. Taken together, these results suggest that novel *M. oxyfera*-like bacteria are responsible for methane depletion in the anaerobic zone of the investigated peat land.

O055

Symbiosis in marine sponges: intimacy or flirts?

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Marine sponge gardens are biodiversity hotspots in the oceans. They are remarkable with respect to the wealth of bioactive compounds they harbour and the microbial diversity they are hosting. More than 7,000 bioactive compounds have been discovered in sponges and the number of active compounds being discovered still increasing every year (based on Blunt et al., 2009). The microorganisms inhabiting sponges can contribute up to 50% of the sponge's biomass and have a significant share in the unusual diversity richness of bioactive compounds that have been found in sponges.

We have studied the microorganisms inside the sponge to determine which microorganisms are consistently affiliated with sponges through time and space and how they are acquired. In general, it can be said that the majority of the microbes living in sponges are stably associated to their host and that they are sponge-specific (i.e. not found in other environments). Surprisingly, the microbial populations seem quite independent from the transmission strategy, because in cases of horizontal or vertical transmission, similar bacterial fingerprints were found.

We also assessed the associated microbes by cultivation. Three methods were examined to cultivate bacteria associated with the marine sponge Haliclona (Gellius) spp.: agar plate cultures, liquid cultures and floating filter cultures. A variety of oligotrophic media were employed, including the use of aqueous and organic sponge extracts, bacterial signal molecules and siderophores. More than 3900 isolates were analyzed and 205 operational taxonomic units (OTUs) were identified. Almost 89% of all isolates were a-Proteobacteria, however members of phyla that are less commonly encountered in cultivation studies, such as Planctomycetes, Verrucomicrobia and d-Proteobacteria, were isolated as well. The sponge-associated bacteria were categorized into three different groups. The first group represents OTUs that were also obtained in a clone library from tissue from the same sponge (group 1). Furthermore, we distinguished: OTUs that were obtained from sponge tissue, but not from sponge isolates (group 2), and OTUs that were not obtained from sponge tissue, but were obtained from sponge isolates (group 3). Seventeen OTUs categorized into group 1 represented 10-14% from all bacterial OTUs that were present in a large clone library from the sponge tissue, which is higher than previously reported cultivability scores for sponge-associated bacteria. Six of these seventeen OTUs were not obtained from agar plates, which underlines that the use of multiple cultivation methods is worthwhile to increase the diversity of the cultivable microorganisms from sponges. In addition, the use of alternative cultivation methods to agar plate cultivations was particularly rewarding for the isolation of previously uncultivated species or uncultivated genera, which underlines the importance of marine sponges for the discovery of new' microorganisms.

O056

Metabolic engineering of the cyanobacterium *Synechocystis* sp. PCC 6803: Modification of its carbon metabolism for the synthesis of fermentation products

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Cyanobacteria, including the model organism *Synechocystis* sp. PCC 6803, are oxygenic phototrophic microorganisms that can be utilized for biotechnological applications such as the production of carbon based fuel compounds. In such approaches, energy, ultimately derived from the sun, drives the synthesis of valuable biofuel compounds through the dark reactions of photosynthesis, thereby avoiding carbon dioxide emission, without the problems of mineral depletion associated with some alternative approaches. O_2 is the only byproduct formed. At the thylakoid membranes of cyanobacteria (sun)light provides the free energy for the synthesis of ATP and NADPH. These intermediates are required for CO_2 assimilation in the Calvin cycle, in wild type cells for the synthesis of the key building blocks for the growth of new cells. Evolution has adjusted the ratio at which the two high-energy intermediates ATP and NADPH are required in the Calvin cycle precisely to the

ratio at which they are formed at the thylakoid membrane. It would be of interest to use the methodology of synthetic biology to re-direct the C(3) sugars produced by the Calvin Cycle, towards fermentation products like ethanol and butanol.

Molecular genetic engineering makes it possible to construct synthetic operons, with genes originating from chemotrophic microorganisms, and introduce them into the oxyphototroph *Synechocystis* sp. PCC 6803. Accordingly, introduction of a heterologous fermentation pathway that uses the product of the Calvin cycle as its input substrate, will lead to the formation of the corresponding fermentation endproduct. Essentially, this type of recombinant metabolism allows formation of biofuel fermentation products from CO_2, H_2O and visible light only.

Preferably, these fermentation pathways are selected from oxygen tolerant microorganisms. The most prominent example of a heterologously expressed pathway results in the formation of (bio)ethanol with genes taken from the enthanologenic bacterium *Zymomonas mobilis*. Another example is the formation of lactic acid with genes taken from *Lactococcus lactis*.

Expression of heterologous genes in *Synechocystis* can be driven by selected promoters and their regulation enables the controlled expression of the encoded fermentation pathway upon reaching a pre-defined physiological state. Accordingly, the C(3) compounds from the central carbon metabolism of the cyanobacterium are then the central intermediates for the conversion of CO_2 and H_2O into a fermentation (end) product e.g. ethanol or lactate.

The construction of strains that express selected synthetic fermentation pathways can take advantage of the natural competence of *Synechocystis* sp. PCC 6803 to obtain stable site-directed insertions. As a result, the heterologously expressed pathways lead to the formation of the desired products. In conclusion, rational design parameters suggest the use of enzymes with suitable kinetic properties, to be expressed under control of strong and inducible promoters. The latter are particularly desirable in applications when product formation should be induced under selected, predefined conditions. Additionally, modifications within the (existing) biochemical pathways of *Synechocystis* sp. PCC 6803 can remove competing reactions and successively enhance the metabolic flux of CO_2 towards the desired (end) product(s).

O057

Biopolymer utilization by planktonic and biofilm bacteria in oligotrophic freshwater environments

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Oligotrophic aquatic environments contain low concentrations of biopolymers that can promote growth of heterotrophic bacteria and biofilm formation. Biofilm formation occurs in natural aquatic environments, but also in drinking water distribution systems where it may cause growth of bacteria that impair water quality and safety. The aim of this study was to characterize the ability of bacteria to utilize biopolymers in oligotrophic aquatic environments by determining the growth-promoting and biofilm-forming properties of biopolymers at g C per liter levels in water under static and dynamic conditions.

The recently isolated *Flavobacterium johnsoniae* strain A3 is specialized in utilizing polysaccharides and proteins at g C l^{-1} levels in natural and treated water. Batch experiments were conducted to investigate the growth kinetics of strain A3 with individual biopolymers at 0-200 g C l^{-1} in tap water. In addition to these batch experiments, the biofilm-forming properties of unchlorinated tap water enriched with individual biopolymers at 10 g C l^{-1} were assessed by analyzing ATP concentration, heterotrophic plate count, total cell count and T-RFLP fingerprint of a biofilm developing under dynamic conditions during one to three months.

Substrate affinity (*Ks*) values of strain A3 for maltose, laminarin, amylopectine, xyloglucan and gelatine were low and ranged from 3.2 to 20.3 g C l^{-1}, whereas *vmax* values varied from 0.210 to 0.328 h^{-1}. A particularly high *vmax/Ks* ratio was obtained for laminarin, indicating the high specific affinity of strain A3 for this biopolymer. The highest *Ks* value and lowest *vmax/Ks* ratio were observed for fructose, the only monosaccharide that can promote growth of strain A3 at g C l^{-1} levels. The specific affinity of strain A3 for this monosaccharide is clearly lower than for the other carbohydrates tested. Thus, strain A3 can rapidly utilize biopolymers at the g C l^{-1} level in water under static conditions. Under dynamic conditions, however, tap water enriched with 10 g amylopectin-C l^{-1} did not enhance biofilm formation compared to the blank. In contrast, biofilm formation was promoted by tap water with 10 g maltose-C l^{-1}. Biofilm formation with 10 g C l^{-1} of both maltose and amylopectin was initially synchronic to biofilm formation with only maltose, but biofilm formation continued with amylopectin when the maximum biomass concentration with maltose had been reached. Caseinate or laminarin at 10 g C l^{-1} in tap water promoted biofilm formation at a significantly lower rate than maltose.

The results of this study demonstrate that: (i) biopolymers are efficiently utilized by planktonic bacteria at concentrations of a few g C l^{-1} (<*Ks*) in oligotrophic aquatic environments under static conditions; (ii) under dynamic conditions, g C l^{-1} levels of biopolymers promote biofilm formation relatively slowly as compared to low-molecular-weight compounds; (iii) both planktonic and biofilm bacteria can utilize biopolymers in oligotrophic aquatic environments.

O058

***Methylacidiphilum fumariolicum* SolV uses the Calvin cycle for carbon assimilation**

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Methane is a potent greenhouse gas, which is emitted to the atmosphere both from natural and anthropogenic sources. Aerobic methanotrophic bacteria utilizing methane are commonly found at the oxic-anoxic interfaces within aquatic sediments. In addition, natural geological sources such as mud volcanoes, may also act as sinks for methane. Until recently, all aerobic methanotrophs could be placed into the Alpha- and Gamma-proteobacteria. In 2007 the isolation of non proteobacterial methanotrophs from geothermal areas was reported. These thermoacidophilic methanotrophs, for which the genus name *Methylacidiphilum* was proposed,[1] represented a distinct new lineage within the bacterial phylum *Verrucomicrobia*. It was the first time that representatives of this widely distributed phylum were coupled to a geochemical cycle. One of the newly discovered methanotrophs, *Methylacidiphilum fumariolicum* strain SolV, was isolated from the very acidic and hot soil of the Solfatara volcano (Naples, Italy) and grows under oxygen limitation on methane as the sole source of energy and dinitrogen gas as sole nitrogen source.[2,3] Based on genome data it is clear that *M. fumariolicum* SolV lacks the key enzymes for both the RuMP and serine pathway, typical for proteobacterial methanotrophs. However a complete set of genes encoding the enzymes of the Calvin Benson Bassham (CBB) cycle are present and suggests that the verrucomicrobial methanotrophs are able to fix CO_2, using methane as energy source. This was further validated by transcriptome and proteome analysis, which showed that the genes required for a functional CBB-cycle were highly expressed and transcribed. Studies with 13C-labeled CH4 and CO_2 in batch and chemostat cultures demonstrated that carbon dioxide is the sole carbon source for growth of strain SolV. In presence of methane, no growth was observed below 0.3% of CO_2 in the headspace and CO_2 concentrations below 1% are growth limiting. No growth was observed with 10% CO_2 in absence of CH4, indicating that methane is the energy source for this microorganism. We also developed a stable isotope method to measure the ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) activity and found a high activity. The presence of RuBisCO was also demonstrated using specific antibodies against the RuBisCO large subunit. Via gel filtration and PAGE the native mass of the RuBisCO complex was determined. Phylogenetic analysis of the strain SolV RuBisCO genes, suggested that the verrucomicrobial methanotrophs possess a new type of form I RuBisCO.

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O059

International collaboration: a necessity in public health and especially in infectious diseases

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The Netherlands is a small country with many connections with other areas of the world through travel and migration of individuals but also through international trade of goods and food. For the prevention and control of infectious diseases we therefore depend heavily on what happens in other countries and how well the control in those countries is organized. Although we are a small country, we have a lot of knowledge about different aspects of infectious diseases in institutes like universities, national institutes, laboratories, health services and the National Institute for infectious Disease Control, part of RIVM. Scientists working in the infectious disease field usually have their own international network. These networks are important for themselves but also because through such networks new knowledge is available before it is published. This is not only relevant for scientists themselves but also for the prevention and control of infectious diseases in our country. Collaboration within the European Union on the control of infectious diseases has been strengthened by the establishment of the European Center for Disease Control (ECDC) in 2005 in Stockholm. ECDC is concentrating on the surveillance of infectious diseases but lacks laboratory knowledge. Improving laboratory networking in the EU is an important goal of ECDC. Although several institutes in the Netherlands have large and small projects on infectious diseases in developing countries, we lack a strong national Institute comparable with eg the London School of Hygiene or the Royal Tropical Institute in Antwerp which hampers structural collaboration with developing countries.

O060

Inappropriate antibiotic use and resistance in Asia and it's global impact; a delicate balance of antibiotic accessibility

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The global problem of antimicrobial resistance is particularly pressing in developing countries, where the infectious disease burden is high and costs constrain the replacement of older antibiotics with newer, more expensive ones. Management of infectious diseases has been critically compromised by the appearance and rapid spread of resistance. In the Asian region where the gram-negative disease burden is high, pan-resistant strains have evolved and also spread to Western countries, as we have seen with NDM-1. The emergence of these resistant strains is largely explained by inappropriate use of antibiotics and lack of good diagnostics and infection control. Asia is currently the economic power house of the world with high population densities and highly connected to the rest of the world. Therefore, the emergence of resistant strains in Asia is a global concern and needs to be adressed accordingly.
Antibiotic drug pressure is one of the most important factors promoting development of drug resistance in bacteria. However, reasons for drug pressure are multi-factorial. Although drug resistance is primarily a medical problem, the causes of resistance are ecological, epidemiological, sociocultural and economic. Patients, physicians, veterinarians, clinics and hospitals, and drug sellers in Asia have little motivation (economic or otherwise) to weigh the negative impact of their use of antibiotics on others, especially those in the future. Standard responses, such as increasing surveillance and launching public information campaigns on the hazards of resistance, while being a necessary part of an overall policy response, may have limited impact on their own. In order to work, policy solutions must alter incentives for patients, physicians, and others in the health care system to act in society's best interests. Evaluating policy solutions involves understanding infectious diseases in populations. Research on evaluating focused, context-specific policy solutions that are likely to have a significant impact on resistance is a first step. Translating these policy solutions to policy action is the second.
This presentation will provide an overview of antibiotic use and resistance in Asia and potential targets for interventions. Antibiotic resistance currently does not top any list of national problems in Asia and elsewhere. It is important that strategies to control antibiotic resistance should not drain resources from more pressing concerns and should preferably be cost neutral or even cost-saving. While improving appropriate use of antibiotics is important, we also need to take care we do not diminish access to these lifesaving drugs; a delicate balance.

O061

'Goulash and boerenkool, an evaluation of the Netherlands-Hungary microbiology exchange program'

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It is always useful to take a look at how your neighbour bakes his bread. With this in mind the Dutch Society for Medical Microbiology (Nederlandse Vereniging voor Medische Microbiologie (NVMM)) organized in 2008 an exchange program with the Hungarian Society. Six Dutch laboratories volunteered to take part in the exchange program and were each linked to an Hungarian counterpart.
Now, several years later, it is time to see if the objectives of the program have been met in any way. So, let's gather the results and draw some conclusions.

O062

Clinical Microbiology from a Dutch and German perspective

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Personal experiences as a clinical microbiologist in both Germany and the Netherlands: A descriptive, anecdotal survey supported by facts, figures and German humor.

O063

Human Enterovirus 71: Europe versus Asia

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Introduction: Since 1997 human enterovirus 71 (EV71) has caused large outbreaks of hand foot and mouth disease with substantial numbers of deaths in several Asian countries. Outside this region the incidence of EV71 infection is relatively low, but in 2007 and once again in 2010 an increase in the number of hospitalized cases of EV71 infection was reported in the Netherlands. These observations triggered us to study; 1. the relation between EV71 epidemiology in the Netherlands and in Asia, 2. whether the increased numbers of reported cases reflect real epidemics or are a result of increased awareness, 3. what drives the potential evolution of viruses capable of causing outbreaks and 4. whether massive outbreaks as reported in Asia can be expected in Europe as well.
Methods: The genetic diversity of EV71 in the Netherlands was studied on the basis of the VP1 encoding genes of 240 EV71 isolates collected as part of surveillance activities in 1963 to 2010. Bayesian evolutionary analysis on the VP1 nucleotide sequences of the Dutch strains and of 556 EV71

isolates collected in China in 1998 to 2010, were performed to study virus population dynamics. To address the third and fourth question genome recombination analyses were performed using strains representing the genetic diversity in the Netherlands in endemic and epidemic years, and cross-antigenicity between subgenogroups circulating in Europe and in Asia was studied by sera neutralisation assays with subgenogroup specific rabbit antisera.

Results: EV71 strains isolated during large outbreaks in the Asian Pacific region belong to other subgenogroups than the ones isolated outside this region. Studies on the virus population dynamics showed that the genetic diversity of EV71 clearly increased in the Netherlands in 2007 and in China in 2007 and 2008 indicating that the increased numbers of reported cases reflect real epidemics. Cross antigenicity was observed between Dutch viruses of endemic and epidemic years. Genome analysis, however, showed evidence for recombination in the 5'UTR of strains isolated during certain outbreaks in Asia and in the Netherlands. Preliminary results of the sera neutralisation assays with Asian EV71 isolates showed that rabbit antisera against viruses circulating in Europe since 1991 could neutralize viruses of most, but not all, subgenogroups circulating in Asia.

Conclusion: The dynamics of EV71 truly seem to be changing and this leaves the question what drives the increased incidence. Detection of recombination in the 5'UTR of strains isolated during outbreaks suggests that genome recombination plays a role in the evolution of strains with increased fitness. The epidemiology of EV71 in the Asian Pacific region seems not to be correlated to the epidemiology observed outside this region. Cross antigenicity between viruses circulating in Europe and most, but not all, subgenogroups circulating in Asia suggests that the European population is protected against these Asian subgenogroups and that in combination with other epidemiologic factors the risk for massive outbreaks is minimal. Introduction of new subgenogroups with an antigenically distinct character, however, should be monitored carefully.

O064

Molecular surveillance of multidrug resistant tuberculosis in the European Union; identification of a major clone and quality of VNTR typing

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Since 2009, the molecular surveillance of multidrug- and extensively drug- resistant tuberculosis (MDR/XDR-TB) in the European Union, is coordinated by the European Centre for Disease Prevention and Control and the National institute for Public Health and the Environment. To detect clusters of international transmission of MDR/XDR-TB in the European Union, 24-locus variable numbers of tandem repeats (VNTR) typing is applied both prospectively and retrospectively.

Although VNTR typing has replaced IS6110 restriction fragment length polymorphism (RFLP) typing as the new gold standard for DNA fingerprinting of *Mycobacterium tuberculosis* isolates, international quality control programs have not yet been developed. Therefore, we have organized a proficiency study within the framework of the European molecular surveillance project. The test panel, containing 30 DNA samples of *M. tuberculosis* complex strains, including 10 duplicate DNA samples, was sent to 37 laboratories, in 30 countries, that routinely perform VNTR typing. The performance of VNTR typing varied significantly per laboratory, indicating problems with standardization and control. Both the intra- and inter-laboratory reproducibility varied from 0 to 100%, with average reproducibilities of 72 and 60%, respectively. Laboratories that used the commercially available kit yielded more reliable VNTR results than laboratories that used in-house methods. However, the kit is very expensive and many laboratories cannot afford it. In conclusion, VNTR typing *of M. tuberculosis* complex strains is not easy to perform and, with the current level of quality, care should be taken when comparing typing results from different laboratories.

Within the framework of the European molecular surveillance project, 1304 VNTR patterns were collected of MDR-XDR-TB strains from 16 countries from the period 2003-2010 in the RIVM database. Extrapolation of the results of the proficiency study to the patterns in the database showed that 76% of patterns were typed by laboratories that had a reproducibility score of 80% or more. One large cluster of identical 24-locus VNTR patterns belonging to the Beijing genotype was detected, comprising 417 MDR/XDR-TB cases isolated from 2003 to 2010, suggesting ongoing transmission. To further investigate the homogeneity of this emerging clone, a subset of outbreak strains which had identical RFLP and VNTR profiles were tested for the distribution of mutations in drug resistance associated genes. The mutations related to rifampicin and isoniazid resistance were all identical. Genes associated with resistance to fluoroquinolones, aminoglycosides and ethambutol revealed a high diversity of mutations. This suggests that in Europe we are dealing with one large emerging clone of MDR/XDR-TB caused by a single strain that is resistant to rifampicin and isoniazid which thereafter acquired additional resistance to second line drugs in different, unrelated events. The diversity of mutations in the genes associated with second line drugs is surprising and requires further investigation. Therefore, a selection of isolates is currently subjected to whole-genome sequencing.

O065

Discriminating Lyme neuroborreliosis from other neuro-inflammatory diseases using levels of CXCL13 in cerebrospinal fluid

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Lyme neuroborreliosis can be a severe but treatable disease. Intrathecal production of chemoattractant CXCL13 has been suggested to be a good biomarker for diagnosing LNB. Our aim was to determine levels of the CXCL13 biomarker in cerebrospinal fluid (CSF) in LNB and several groups of patients with inflammatory neurological diseases, in order to evaluate performance of a CXCL13 ELISA for diagnosing LNB.

Fifty-eight adult and pediatric LNB patients, 36 Lyme non-neuroborreliosis cases, 93 infectious meningitis/encephalitis controls and 74 neurological controls were tested for levels of CXCL13 in CSF.

Levels of CXCL13 were highly elevated in the patients who presented with LNB. Sensitivity using an optimal cut-off of 250pg/ml CSF was 88%. Children (n=24) had lower levels of CXCL13 intrathecally than the adult population (n=35), this difference was not significant (median=932 compared to median 1678; p=0.4).

In the controls elevated levels of CXCL13 in CSF were seen in several groups of patients. Overall specificity was 89%, this was lowest in the HIV positive population where it was 77%.

After treatment there was a rapid decline in CXCL13 levels in CSF of LNB patients. Determining CSF CXCL13 as a marker for follow up after adequate treatment seemed promising.

Determining levels of CXCL13 as a marker for LNB can be useful, but should be interpreted with care especially in the immunocompromised patient and in the patient with an autoimmune disorder. HIV infection should be excluded in individuals with elevated levels of CXCL13 in CSF.

O066

Increased frequency of positive Lyme serology in patients with aspecific skin lesions

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Introduction: Serology for Lyme borreliosis is frequently requested without clinical data being provided to the microbiologist. Therefore, it is not always possible to give a correct interpretation of serologic results. In addition, it has been suggested that immunoblots should always be performed, also after a negative EIA. The present study was undertaken to obtain data regarding clinical indication for Lyme serology, to correlate serologic results with these clinical indications, and to find out whether routine testing in immunoblots would have additional value.

Methods: Clinicians submitting requests for Lyme borreliosis (LB) were contacted by telephonic interview to obtain clinical data regarding 412 patients. Clinical data from 53 additional patients was obtained after a positive screening test. Sera were routinely tested in the C6-EIA. All EIA-positive sera, all EIA-negative sera from patients with possible LB symptoms and 88 sera from patients in whom LB was clinically unlikely were tested in IgG and IgM immunoblot. Control groups of 96 sera from patients with other infectious diseases and of 92 healthy persons were also tested in EIA and immunoblots.

Results: Indication for serology was erythema migrans (EM) for 5.1% of the initially contacted patients, previous EM for 7.4%, atypical skin lesions for 11.3%, other symptoms leading to classification as LB in case of positive serology for 12.5% and other atypical clinical manifestations for 72.1%. The frequency of a positive EIA patients in these groups were 70.0%, 20.7%, 18.2%, 12.5% and 5.7%; the frequency of positive EIAs differed significantly between patients with actual or previous EM or other skin lesions in comparison to patients with aspecific manifestations. In the control groups, positive EIA test were found in 8.3% and 7.6%. A positive confirmatory immunoblot (IgM and/or IgG) was found in similar frequencies, varying between 67 and 83%, in all clinical groups. In EIA-positive control samples, only a positive IgM blot was found in 13%. Three out of 170 EIA-negative clinical samples and four out of 161 EIA-negative control samples were positive in immunoblot only. Two patients had a recent erythema and a positive IgM immunoblot, and were considered as probable LB. One person, a forestry worker, had had a tick bite 5 months earlier, and now arthralgia and numbness at the location of the earlier tick bite, had a positive IgG immunoblot and was classified as possible LB. The four controls had a positive IgM blot.

Conclusions:

1. A high frequency of positive results was found in the subgroup of patients with nonspecific skin symptoms.
2. In 72% of the patients from which serum samples were submitted, only aspecific symptoms were present. In the presence of a positive test, the diagnosis LB remains questionable in this group.
3. Positive serology is equally often found in patients with aspecific symptoms and in control groups. The immunoblot is more often positive in the former group.
4. After a negative C6-peptide EIA, an IgG immunoblot is almost universally negative. IgM immunoblots were positive in 2/78 patients with recent clinical symptoms compatible with LB, but also in 4/161 controls.

O067

Measuring T-cell responses in Q fever using a newly developed ELISPOT assay

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Introduction: Q fever symptoms are not always specific to the disease and accurate diagnosis without appropriate laboratory tests is difficult. In patients with an acute infection, phase II antibody levels are usually higher than phase I antibody levels. In chronic Q fever, antibodies to phase I antigens of *C. burnetii* generally become predominant. In cases of post Q fever fatigue syndrome (QFS) with negative PCR, current serology is not helpfull to explain clinical symptoms. T-cells have a pivotal role in dealing with primary infection and subsequent clearance or control of intracellular pathogens like the *Coxiella burnetii* bacterium. Cellular immunity/pathogen interaction most likely will influence clinical outcome. Very few data are published on Q-fever specific T-cell responses. Aim of our study was to develop a *Coxiella burnetii* enzyme-linked immunospot (ELISPOT) assay to measure Q-fever specific T-cell response.
Methods: Whole blood specimens (lithium heparin) were collected from 7 healthy individuals and 7 patients who were clinically recovered from symptomatic acute Q fever (median time since diagnosis 8 months; range 6-22). Mononuclear cells were isolated using Leucosep tubes (Greiner). *Coxiella burnetii* ELISPOT was performed with phase I and phase II antigens. Phytohaemagglutinin and CEF-pool (a combination of CMV, EBV and Influenza peptides) were used as positive controls.
Results: Mean (SE) spot counts in positive control wells of Q-fever patients and healthy controls were comparable: 271 29 (range 170-350) and 255 48 (range 58-350) spots, respectively (p 0.78). Mean spot counts in CEF controls were 86 45 spots in the healthy control group vs. 121 72 spots in Q-fever patients (p 0.70). Test controls indicate adequate T-cell responses to antigenic stimuli in patients and controls. *Coxiella* specific responses in the control group were marginal: we detected less than 2 spots after stimulation with phase I and phase II antigens. Whereas in Q-fever patients we found 11 5 spots (phase I; p 0.03) and 31 15 spots (phase II, p 0.003). Mean T-cell responses to phase II antigen in recovered Q-fever patients were 3-fold higher than the response to phase I antigen.
Conclusions:
1. We present the first *Coxiella* ELISPOT results reported in literature.
2. The results indicate differential T-cell responses in Q-fever patients and healthy controls, indicating the diagnostic potential of Q-fever ELISPOT.
3. We now have a *Coxiella burnetii* ELISPOT ready for evaluation of various patient categories.
4. Anticipating further research, we postulate that determining T-cell responses to *Coxiella burnetii* antigens by ELISPOT may elucidate current lack of clarity in accurate diagnosis of different Q-fever related clinical outcomes.

O068

Potential value of an ELISPOT interferon gamma release assay as a diagnostic tool in Q fever infection

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Background: Q fever is an emerging zoonosis in the Netherlands with nearly 4000 human cases reported since 2007. Acute disease is followed by clinical resolution in the majority of cases, 10-20% will exhibit the post-Q fever fatigue syndrome (QFS) and 1-5% of patients progresses to chronic disease. Current diagnostic tests measuring humoral immune response to *Coxiella burnetii* infection have considerable limitations in accurately diagnosing these different clinical outcomes. Adverse Q fever outcomes are thought to be the result of a dysfunctional cellular immune response to persistent *Coxiella burnetii* antigens in the host after primary infection (as viable bacteria in case of chronic disease, or remnant *Coxiella burnetii* antigens in QFS). Therefore, measuring *Coxiella burnetii* specific T cell responsiveness might aid the clinician in accurately diagnosing the outcome of a Q fever infection. Moreover, such a test might be useful in guiding therapeutic interventions.
Objective: To determine T cell response to *Coxiella burnetii* specific antigens using an ELISPOT interferon gamma release assay (*Coxiella* ELISPOT) in Q fever patients with different clinical outcomes.
Methods: An in-house developed *Coxiella* ELISPOT interferon gamma release assay, using both phase I and phase II antigens was performed on fresh whole blood samples available from regular blood drawings of Q fever patients followed-up in our centre. Technical aspects of this technique are described elsewhere.
Results: *Coxiella* ELISPOT was performed on blood samples of 9 Q fever patients: patients who reconvalesced after symptomatic acute Q fever (n=7, median time since onset of symptoms 8 months (range 6-22) and 2 chronic Q fever patients (n=1 newly diagnosed, n=1 after completion of treatment). Mean (SE) spot count for patients who reconvalesced was 115 (range 1-42) for phase I and 31 15 (range 1-120) for phase II. Mean spot count ratio (phase II/ phase I) was 3. Of these 7 patients, 1 patient fulfilled the

1994 CDC criteria for chronic fatigue syndrome and was diagnosed with QFS. Interestingly, this patient had the highest spot count in both phase I (42 spots) and phase II (120 spots), indicating a vigorous T cell response, while retaining a predominant phase II antigen reactivity. Of the chronic Q fever patients, one (male, 64 ys) was diagnosed in December 2010. In this case, *Coxiella* ELISPOT showed a predominant responsiveness to phase I antigen: spot count was 209 (phase I) and 177 (phase II), ratio phase II/phase I=0,85. The second chronic Q fever patient (male, 67 ys) had finished a 18 month antibiotic treatment in April 2010 for Q fever endocarditis of a prosthetic aortic valve, resulting in a clinical recovery and a 4-fold decline in IFA phase I antibody titres. *Coxiella* ELISPOT in December 2010 showed a marked T cell unresponsiveness to both phase I (3 spots) and phase II antigens (0 spots).

Conclusion:

1. Different clinical Q fever outcomes are associated with marked differences in *Coxiella* ELISPOT results.
2. The encouraging results in this pilot study will be investigated in a larger Q fever patient cohort.

O069

Low prevalence of *Coxiella burnetii* endocarditis in patients with a history of valve surgery or cardiac valve prosthesis in a Q fever endemic area

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Introduction: Q fever is a zoonosis caused by *Coxiella burnetii*. Following primary infection, which is often asymptomatic, 1-5% of patients develop chronic Q fever, of which endocarditis is the most common manifestation. Q fever endocarditis requires long-term antibiotic treatment and has poor prognosis if left untreated. In a retrospective study the estimated risk of developing Q fever endocarditis after primary infection for patients with pre-existent valvulopathy was 39%, with the highest risk for patients with prosthetic valves. In the Netherlands, there has been a large outbreak of acute Q fever with over 4000 notified cases since 2007, which allows for a more precise risk estimation of chronic Q fever in high-risk groups. We studied the prevalence of chronic Q fever in an endemic area in patients with a history of cardiac valve surgery, including valve prosthesis.

Methods: We selected all patients with a history of cardiac valve surgery from our cardiology outpatient clinic and invited them by letter for microbiological screening. IgG antibodies to phase I and II antigens of *C. burnetii* were tested by immunofluorescence assay. If phase I IgG antibodies were positive, polymerase chain reaction (PCR) on blood for *C. burnetii* DNA was also performed. Chronic Q fever was considered probable if phase I IgG antibody titre was =1024 and definite in case of positive *C. burnetii* PCR in blood.

Results: A total of 663 patients were identified with a history of valve surgery and unknown *C. burnetii* serostatus. As of January 2011, 200 patients had been invited for screening. In total, 175 patients (87.5%) responded and were available for serological screening. Of these, 31/175 patients (17.7%) had phase I and/or phase II IgG antibodies against *C. burnetii*, indicating a previous *C. burnetii* infection. In this group, 2/31 patients (6.5%) had phase I antibodies titres =1024, indicating probable chronic Q fever. *C. burnetii* PCR was negative for both patients.

Conclusion: Despite a seroprevalence of IgG antibodies against *C. burnetii* of 17.7% in patients inan endemic areawith a high risk of developing chronic Q fever, only 6.5% of these patientsmet the criteria ofprobable chronic Q fever. Compared to the previously reported risk of 39% in case of valvulopathy, we found a considerable lower percentage of patients who progressed to chronic Q fever after *C. burnetii* infection. However, as chronic Q fever can develop years after primary infection, further follow-up of seropositive patients is warranted.

O070

QSZB study: a cross-sectional study of Q fever in patients with an aneurysm, vascular and/or heart valve prostheses in an endemic region

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Introduction: In the region of Southeast Brabant, about 500 patients are diagnosed with acute Q fever during the Q fever epidemic. Clinical symptoms only occur in 40% of the patients contaminated with Q fevers pathogen *Coxiella burnetii*. Therefore the actual number of infections will be much higher. Both asymptomatic as symptomatic acute Q fever cases are at risk for chronic infection. This indolent disease can manifest years after primary infection. Morbidity and mortality are related to the time of diagnosis. Known risk factors for developing chronic infection are valvular heart disease, including heart valve prostheses, vascular prostheses and aneurysm of the aorta.

Until September 2010, 16 patients were diagnosed with chronic Q fever in our region: vascular infection (13), endocarditis (2) and hepatitis (1). 4/16 patients died on treatment due to multiple organ failure and arterio-bronchial fistula. 14/16 patients were not known with a previous acute Q fever syndrome. Given the seriousness of this disease in a relatively small population and lack of precise incidence data a screening program was started.

Methods: From September 2010, a cohort of approximately one thousand patients with an abdominal or thoracic aortic aneurysm, heart valve or vascular prosthesis is asked to donate blood for serological testing for *Coxiella burnetii* antibodies. Initially, screening is designed for patients known with these risk factors from 2005. IgG phase I en phase II antibodies are measured using immunofluorescence assay (Focus Diagnostics). Patients are classified as negative (IgG I and II <1:32), past infection (IgG I and/or IgG II=1:32 but <1:1024) or chronic infection (IgG I=1:1024). Acquisition of patients takes place in four hospitals in the region. Patients are selected based on assigned DBC code and/or treatment code and actively approached by clinic visits or by letter.

Results: After 4 months 432 patients were screened (322 surgical and 110 cardiological patients). Antibodies against *C. burnetii* were measured in 40 patients (9.3%). Based on IgG titers, a past infection was found in 34 patients and chronic infection was diagnosed in 6 patients. These were all male with a vascular prosthesis (n=4) or a heart valve prosthesis (n=2). Clinical and biological abnormalities of the infected patients were summarily, although PET-CT showed distinct staining in the four vascular graft infected patients. Patients with past infection are serological monitored three monthly.

Conclusion:

1. These first results show that the seroprevalence in the tested patient population at risk for chronic Q fever in Southeast Brabant is 9.3%. Further serological monitoring should reveal whether patients with an aneurysm, a vascular or heart valve prosthesis are at risk for reactivation after a past infection.
2. All six chronic patients found by screening were almost asymptomatic.
3. Due to timely detection of chronic Q fever infection treatment options are better compared to patients who presented with a clinical overt chronic Q fever infection.

O073

Resistance in gram-negative bacteria: An inconvenient truth

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Antimicrobial resistance is increasing rapidly and there are hardly new antimicrobial agents to be expected in the coming years. Especially the number of patients affected by Extended Spectrum Beta-Lactamases (ESBL) is rising and there are strong indications that this is caused in part by the use of antimicrobial agents in food production animals. In addition, it has been shown that the presence of resistance genes in soil has increased dramatically during the last two decades. There are many arguments against the widespread use of antimicrobial agents in animal husbandry, but this has not resulted in a decreased usage. The current situation is critical and asks for immediate response. In human healthcare the prescription of rescue' antibiotics, e.g. carbapenems has to be restricted and controlled. In animal husbandry the use of antibiotics has to be reduced dramatically and a more sustainable approach to food production has to be supported by government and consumers. Finally, new antibiotics are needed and these have to be used more prudently in humans, animals and agriculture.

O075

How little we know about epidemiology of *E. coli*!

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Escherichia coli is a normal commensal of the gut of most verebrates including man. It has a beneficial function for its host in displacing or preventing – method unknown – unwanted pathogens colonizing the intestines, and participation in the biochemical breakdown of nutrients. Further, it produces important substances utilized by the host such as vitamin K and others. Knowledge is accumulating on how the colonizing ability works, e.g. *E. coli* down-regulates its motility with increasing duration of colonization by various mutational sequences including an insertion element in the pro-motor for the flagellar gene. Certain types of carbohydrate utilization can enhance the colonization ability.

On the other hand, the intestinal colonization increases the risk of spread of *E. coli* to other host sites such as to the vagina via the perineal skin. Such vaginal colonization includes the area around the meatus urethra externae, which further increases the risk of urethral contamination and subsequent ascension into the bladder. If certain host factors allow and the *E. coli* possess relevant virulence factors (e.g. extra-intestinal pathogenic *E. coli* or ExPEC) binding to the bladder epithelium takes place and bladder infection ensues leading to cystitis or urinary tract infection (UTI). Since *E. coli* is the most numerable human pathogen at least in western societies, causing complicated disease and death, the epidemiology of this pathogen is of major importance.

This turnover of *E. coli* in the human intestines stresses the importance of the knowledge, on how *E. coli* is transported into the gut, which by any means must be part of intake via the mouth – food in a broad sense. Person-to-person spread does take place as evidenced by the demonstration of clonal distribution of *E. coli* among families or house holds. Most *E. coli* uptake must however come from food including water as ascertained by the vast amounts of bacteria present in food especially meat products. The proof of spread of *E. coli* via meat products comes in part from infections caused by VTEC producing *E. coli* shown to originate from minced beef as in "Hamburgers".
Recent data have proven, that ExPEC causing UTI in humans are in fact spread via meat products from poultry (chicken) and pork (pigs). So far, however, we do not know which proportions of UTIs are caused by such spread of *E. coli*. Although we can show, that a sizable proportion of *E. coli* from feces of non-diseased pigs and chicken belong to the ExPEC group, we do not know form where these originate; are they spread to the animals from other sources, or do they in fact originate in the intestines of these animals by gene transfer ?
Detailed analysis of antibiotic resistance genes and methodological typing studies do reveal, that resistance genes in human *E. coli* strains can originate from production animals and are here related to antibiotic use in the same animals. Co-existence of virulence factors and antibiotic resistance genes in human *E. coli* pathogens originating from production animals is also proven to take place.
Further research into this area is highly needed in order to increase our awareness of this type of spread which will also enable us to institute preventive measures.

O076

Prevalence of ESBL-producing *Enterobacteriaceae* (ESBL-E) in Raw Vegetables

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Introduction: Recent data show that ESBL-producing bacteria are found in Dutch soil samples (Knapp et al., Environ Sci Technol. 2010), and in food-producing animals such as broiler chickens and pork meat. We wondered whether ESBL-E are also present in raw vegetables. The aim of this study was to evaluate the presence of ESBL-E in raw vegetables in the region of Amsterdam.
Materials & methods: Between October 14 and November 29, 2010, 79 samples of 17 different types of vegetables were obtained from the market, and from organic and conventional stores in the region of Amsterdam. We focused on vegetables that grow on and in the ground. Screening for ESBL-E was performed with a selective enrichment broth and inoculation on a selective screening agar, containing cefotaxim and ceftazidim. ESBL production was confirmed with the double disc synergy test with clavulanic acid. Species identification and further antibiotic susceptibility testing were performed with the Vitek-2 system (bioMrieux). DNA-isolation was performed with the QIAamp DNA mini kit (Qiagen). ESBL genes were characterized by microarray (Check-KPC ESBL Check-Points).
Results: Out of 79 analyzed samples, four yielded ESBL-producing *Enterobacteriaceae* (5%). ESBL-E were found in parsnip, bean sprouts and radish; this means that three (17,6%) of the vegetables types were contaminated with ESBL-E. Of the four positive samples, three were from vegetables of organic origin. The ESBL-producing strains were *Enterobacter cloacae* (in two samples), *Citrobacter braakii* (in one sample) and *Klebsiella pneumoniae* (in one sample). Three strains were positive in the microarray for CTX-M ESBL belonging to the CTX-M-1 family and one for an SHV ESBL.
Conclusion: 1) Our results document the presence of ESBL-producing *Enterobacteriaceae* in some raw vegetables obtained in Amsterdam, the Netherlands, implying that raw vegetables may be a source of resistance genes. 2) The possible impact of our findings on human health highlights the need to further evaluate the presence of ESBL-E in raw vegetables and to explore whether colonization of the human gut from this source does occur.

O077

Prevalence of extended-spectrum beta-lactamase producing *Enterobacteriaceae* in faecal samples of patients in the community

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Introduction: The aim of this study was to determine the prevalence of Extended-spectrum Beta-lactamase producing *Enterobacteriaceae* (ESBL-E) in faecal samples of patients with diarrhoea in the community.
Methods: Faecal samples were obtained in December 2010, from patients who attended a GP with complaints of diarrhoea and from which the GP requested a microbiological analysis of the faeces. All samples were selectively tested for presence of ESBL-E. First, a small portion of faeces (5l) was suspended in a TSB Broth containing cefotaxim (0.25g/ml) and vancomycin (8g/ml). After incubation (37C, 16-18h), 10l of the broth was inoculated on each site of a selective screening agar plate (EbSA, AlphaOmega, 's-Gravenhage, the Netherlands). After

incubation (37C, 16-18h), the agar was inspected for growth. Of all grown oxidase-negative, gram-negative rods, species and resistance pattern were determined using the Vitek2 system (Biomerieux, Marcy l'Etoile, France). Confirmation of presence of ESBL-E was performed according to the Dutch guidelines (NVMM). A genotypic analysis using a microarray is ongoing.

Results: In total 205 samples from 205 unique patients were collected. The mean age of the patients was 38.9 years (SD=24.8). Twenty-four contained one or more ESBL-E (11.7%), two samples contained 2 ESBL-E exhibiting a significantly different resistance pattern. Twenty-two samples contained *E. coli*, and two samples contained *Klebsiella* spp. only. All isolates were sensitive to meropenem, colistin and tigecyclin. Nineteen isolates were resistant to sulphonamide/trimethoprim, eleven were resistant to ciprofloxacin and four were resistant to tobramycin. Six isolates were sensitive to all of the latter three antibiotics, and three isolates were resistant to all three antibiotics.

Conclusion: In total 11.7% of outpatients visiting their GP for abdominal complaints, carry ESBL-E their faeces. The ESBL-E consisted predominantly of *E.coli*. A determination of the resistance genes is ongoing. Further research is warranted to investigate the origin of this high ESBL-E carriage rate.

O079

Of mice and mast: Epidemiology of hantaviruses in North-West Europe

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Hantaviruses, family Bunyaviridae, are the etiologic agents of two distinct human diseases: hemorrhagic fever with renal syndrome (HFRS) in the Old World and hantavirus pulmonary syndrome (HPS) in the New World. HFRS has a complex clinical manifestation characterized by fever, renal dysfunctioning and occasionally acute myopia and haemorrhages. In Europe, three serotypes are known to cause HFRS, Puumala virus (PUUV), Dobrava virus (DOBV) and Saarema virus (SAAV). HFRS has a mortality rate varying from 0.1% to 16% depending on the hantavirus serotype. In northern and western Europe, PUUV is the predominant serotype, which causes nephropathia epidemica (NE), the mildest form of HFRS. A fourth serotype implicated in human disease is Tula virus (TULV) which is widely spread in Europe but its association with human disease has not been established unequivocally. Although circulating in wild rats, Seoul virus (SEOV) is not associated with human disease in Europe other than laboratory-related infections.

Hantaviruses are rodent-borne and each hantavirus appears to have co-evolved with a specific primary rodent species. Virus is shed in saliva, urine and feces of infected rodents and is transmitted to humans by inhalation of aerosolized animal excreta. The geographic distribution of a particular hantavirus is limited by the geographic distribution of its specific rodent host. The primary reservoir host for PUUV is Myodes glareolus (bank vole or 'rosse woelmuis') which is commonly present in Europe. The risk for human hantavirus infection depends on a complex combination of host ecology, virus ecology and human behaviour/condition. Host ecology involves environmental factors related to rodent population densities and structure, viz. land-surface attributes, landscape configuration and climate. Climatic factors control directly the rodent population dynamics by influencing winter-temperature dependent survival rates or indirectly by influencing vegetation growth, snow cover and food supply, f.e. mast production. Virus ecology involves environmental factors related to virus survival outside the rodent host, viz. UV-level exposure, winter temperature, soil pH and moisture. Virus ecology influences the transmission risk to humans directly and indirectly through influencing the virus prevalence in rodents. Some human activities will be associated with a close contact with the rodent host habitats and thus increase the likelihood of human-host contacts or a close contact with areas that support virus survival.

In temperate Europe forest rodent populations are stable with a clear seasonal variation characterized by a short high-density period in autumn and mast (seed crop of oak and beech) driven peaks. The deciduous forests are fragmented and heterogeneous resulting in an inefficient spread of rodents and virus, which can lead to a local patchy occurrence of PUUV and infrequent transmission to human beings (mast-driven increase in human incidence). This is in contrast to northern Europe with predator driven cyclic population dynamics and large continuous forest areas. Eighty percent of the hanta virus cases in the European Union are registered in northern Europe.

In most European countries the incidence of hantavirus infections has increased over the last couple of years. Severe outbreaks were reported for Sweden and Germany in 2007 and 2010. For the Czech Republic, Belgium and the Netherlands an unusual number of cases have been reported early 2008. In Belgium a 3-year epidemic cycle abruptly changed into a 2-year cycle in the year 2000. An extension of hantavirus endemic areas has been observed for Belgium, France, Germany and the Netherlands, including an invasion of urban areas in Germany. Experts predict that with the anticipated climate changes, disease caused by hantaviruses might become highly endemic in Northern and Western Europe.

O080

Only two residues are responsible for the dramatic difference in receptor binding between swine and new pandemic H1 hemagglutinin

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In view of its critical role in influenza A virus (IAV) tropism and pathogenesis we evaluated the receptor binding properties of hemagglutinin (HA) proteins of the closely related swine and new pandemic human IAVs. We generated recombinant soluble trimeric H1 ectodomains of several IAVs and analyzed their sialic acid binding properties using fetuin-binding and glycan array analysis. The results show that closely related swine and new pandemic H1 proteins differ dramatically in their ability to bind these receptors. While new pandemic H1 protein exhibited hardly any binding, swine H1 bound efficiently to a number of a2-6-linked sialyl glycans. The responsible amino acids were identified by analyzing chimeric H1 proteins and by performing systematic site-directed mutagenesis of swine and new pandemic human H1 proteins. The difference was found to map to residues at position 200 and 227. While substitution of either residue significantly affected the binding phenotype, substitution of both was found to act synergistically and reverse the phenotype almost completely. Modeling of the T200A and E227A substitutions into the crystal structure of the new pandemic human H1 protein revealed the loss of potential hydrogen bond formation with Q191, which is part of the 190-loop of the receptor binding site, and with the penultimate galactose, respectively. Thus, a residue not belonging to the receptor binding site may affect the interaction of HA with its receptor. Interestingly, while alanine at position 200 is found in most new pandemic human viruses, the residue at position 227 in these viruses is invariably a glutamic acid.

O081

Immature Dengue virus: A veiled pathogen

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Dengue virus represents a major emerging mosquito-borne pathogen that produces a broad spectrum of clinical responses ranging from asymptomatic infection to severe, sometimes fatal, hemorrhagic manifestations. Humoral response plays a crucial role in controlling DENV infection. The antibodies are not only involved in viral clearance but may be associated with the development of severe disease symptoms by promoting an antibody-mediated enhancement (ADE) of infection. During ADE, antibodies target the bound virions to FcR-bearing cells and thereby expand the number of infected cells and consequently circulating viral titer. The human humoral response to DENV infection is dominated by antibodies against the two DENV glycoproteins, E and prM. The prM protein represents the precursor form of M and is found in noninfectious so-called immature DENV particles, which are produced during a natural infection due to inefficient processing of prM in infected cells.

We have previously demonstrated that in the presence of prM antibodies, immature DENV becomes highly infectious. The enhancing effect of prM antibodies on immature virions has been also corroborated *in vivo*. Antibodies appear to facilitate both the entry of immature DENV into Fc-receptor-bearing cells and the processing of the prM protein by a furin-like protease within the target cell. Importantly, not only monoclonal prM antibodies but also sera from DENV patients promote the infectivity of immature DENV.

Recently, we analyzed the effect of a fusion loop specific, highly cross-reactive and poorly neutralizing antibody, E53, that has been shown to preferentially bind to immature flavivirus particles. We show that E53 is capable of rescuing the infectivity of immature virions in a cell and pH-dependent manner. We demonstrate that the enhancing properties of the antibody depend on efficient cell entry and subsequent virus maturation.

Thus, in addition to prM antibodies, we now have evidence that E53-like antibodies, which dominate the humoral response against E, may unveil 'the pathogenic face' of otherwise, non-infectious, immature DENV.

O082

Emergence and spread of human adaptation markers in avian influenza viruses during an HPAI A(H7N7) virus outbreak

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Introduction: Recent human cases showed that highly pathogenic avian influenza (HPAI) A viruses can directly infect humans and might harbor markers associated with human fatal disease. This raised the concern that an

HPAI virus may acquire all properties of a pandemic virus through adaptive mutations already in the animal host.

Methods: Using full-length HA, NA and PB2 sequences of human and avian viruses collected during the large A(H7N7) HPAI epizootic in the Netherlands in 2003, a maximum Parsimony transmission network was constructed to identify farm-to-farm and farm-to-human transmission events. Virological- and epidemiological data collected during veterinary and medical outbreak control activities were combined to identify possible human adaptation markers supplemented by information on their effect, importance and origin.

Results: The transmission network demonstrated that 35% of the human viruses were identical to poultry farm sequences, while 65% had =1 nucleotide substitution compared to the suspected avian source of infection. Although 59% of these mutations resulted in amino acid substitutions, no known avian-to-human adaptation markers were identified in viruses obtained from 67/89 human A(H7N7) cases except for the PB2 E627K mutation in the virus obtained from the fatal human case. Mapping of virulence and human adaptation markers in poultry sequence data, demonstrated the emergence and spread of a multitude of mutations during the A(H7N7) outbreak in poultry. These include the independent emergence of HA mutants with increased replication kinetics, accumulation of NA mutations facilitating efficient release of virus particles from the host cell and farm-to-farm spread of virus variants harboring a mammalian host determinant in PB2.

Conclusion: The emergence and spread of both virulence and human adaptation markers in HPAI viruses isolated from poultry farms demonstrate that human tropic virus variants can arise in the absence of the human host. This emphasizes the need for global influenza virus surveillance in poultry, to rapidly identify emergence of influenza variants with pandemic potential.

O084

Skin manifestations of parasitic diseases: What you should know, how to diagnose

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Parasitic infections can cause skin manifestations in several ways.

Ectoparasites directly affect the skin and can usually be diagnosed by visual inspection of signs of infestation. The most common infestations are scabies, lice infestation, tungiasis and myiasis. Scabies has a classical presentation but may vary depending on immune status of the patient and duration and localization of the infection. Scabies occurs in travelers; outbreaks may occur in nursing homes. Tungiasis is an infestation of the adult female of Tunga penetrans, a small flea. The characteristic lesion, usually on the feet around the toes, cannot be misdiagnosed. Myiasis is the infestation by larvae of flies (maggots). The African species are usually small and occur on the trunk, under the clothes. The South American species may occur elsewhere, often on the scalp. The lesion resembles a pustule which on close inspection shows movement and two black dots, the respiratory channels of the maggot.

Of the protozoan infections of the skin, leishmaniasis is the most common. Both Old World and New World species can cause cutaneous leishmaniasis. The appearance of the respective infections varies from a rapidly expanding wet necrotic ulcer, for example by *Leishmania guyanensis* to the drier and more crusty lesions of *L. major* and *L. tropica* to the tiny papules by *L. naiffi*. The diagnosis is confirmed by demonstration of parasites in smears, aspirates or biopsies. Culture is gradually being replaced by PCR which is faster, more sensitive, less subject to contamination and has the advantage that it can be combined with techniques for species identification.

Helminthes may cause an eosinophilic syndrome with urticarial rashes during their migration through body tissues. Acute schistosomiasis, fascioliasis and, to a lesser extent, intestinal nematode infections (Loefflers syndrome) are the most common examples. There are several helminthes that migrate through superficial tissues and may cause, sometimes migrating, subcutaneous nodules. Dirofilaria infections, onchocerciasis and gnathostomiasis are examples. The superficial hookworm related larva cutanea migrans and larva currens, the dermal presentation of strongyloidiasis, are diagnosed by clinical inspection. Loa loa infections may cause the so called Kalabar swellings, migrating swellings around the large joints. At this stage microfilaria are usually not yet detectable in blood.

Most of these conditions are characterized by eosinophilia. Serological tests with variable sensitivity and/or specificity are available but not all in the Netherlands. Most of these assays are formatted as IgG ELISA. IgG4 ELISA is more specific but only available at specialized laboratories. There is much cross activity so that filarial antibody tests may be positive with strongyloides infections and vice versa.

Gnathosomiasis may unveal itself when the worm larve emerges from the skin after treatment with albendazole. Surgical excision and histological examination is often how Dirofilaria repens infections and onchocercomas are ‘curatively’ diagnosed. Onchocerciasis may also cause dermatitis. Superficial skinsnips, taken by an experienced person, can confirm onchocerciasis but are often negative. Strongyloidiasis is confirmed by stool analysis or serological testing.

Lastly, swimmer's itch, trichobilharzia, can be contracted almost everywhere in the world. The diagnosis is based on clinical recognition, there is no specific test for this condition.

O085

Photodynamic therapy in cutaneous leishmaniasis

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Cutaneous leishmaniasis (CL) is a parasitic disease of the skin transmitted by female sandflies belonging the *Leishmania* species. Depending on the species of *Leishmania*, the clinical presentation can be that of cutaneous, mucocutaneous or visceral leishmaniasis.
Geographically, CL can be divided in Old World' and New World' leishmaniasis. In most cases, CL is acquired in the Old World, predominantly by *Leishmania major*.
CL is one of the most important tropical' diseases in the Netherlands. This is primarily due to the increasing numbers of CL in travellers and holidaymakers and military units involved with UN and NATO missions overseas.
CL causes single or multiple, painless and often deep ulcers with an erythematous, indurated border. Approximately half of the infected persons develop lesions on visible parts of their head, hands and arms.
Spontaneous healing can occur on average after 6 to 12 months, depending of the exact *Leishmania* species, with disfiguring scars and hypo- and hyperpigmentation.
Although different therapeutic guidelines of leishmaniasis exist, there is no international uniformity in its treatment. A first-line therapy very often used in the treatment of Old world CL is the intralesional injection of sodium stibogluconate (Pentostam). This therapy is painful and cannot prevent the origin of hyperpigmented scars.
Photodynamic therapy (PDT) has been under development since the late 1970s and has seen extensive use in oncology in many specialties including dermatology, surgery, urology, pulmonology, neurology, ENT and ophtomology. For cutaneous lesions, porphyrin precursors such as 5-aminolevulinic acid (ALA) are most often topically applied to the skin or mucosa. ALA is a substrate for the synthesis of haem, and its exogenous delivery leads to the accumulation of protoporphyrin IX (*PpIX*) in atypical cells up to a depth of approximately 2-3 mm.
After a suitable time interval illumination with light of the appropriate wavelength (red or blue wavelengths are commonly used), selective cell necrosis and/or apoptosis takes place due to the activation of *PpIX* and the generation of reactive oxygen species that include singlet oxygen. PDT using porphyrin precursors results in excellent cosmesis; therapy gives no unacceptable scars, fibrosis of the skin or hyposensibilization of the treated area.
However, mostly reversible hypo and hyperpigmentation are regularly seen. Because PDT has been successfully used in different dermatological conditions with no unacceptable scaring, and mostly only local mild burning sensation during illumination, the effectiveness in the treatment of CL needs more attention.
This presentation will summarise clinical studies investigating the use of PDT for the treatment of Old World CL. Advantages and disadvantages of the use of PDT in CL will be discussed. Data are still limited, and PDT cannot at this point be recommended in routine clinical practice. The mechanism of action of this promising therapeutic modality needs to investigated further and additional controlled trials need to be performed.

O086

How do maggots operate?

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Maggot therapy as an ancient method is successfully used for the treatment of acute and chronic wound infections in surgery. The underlying mechanisms of action of maggot therapy are unknown, but could provide information for a novel treatment modality against infection, which is important in these times of increasing antibiotic resistance. Therefore, in this research the effect of living maggots on planktonic cells was investigated. Furthermore, the influence of maggot excretions/secretions (ES) on planktonic cells, on bacterial biofilms and on activation of the human complement system was tested.
Sterile tubes were filled with living maggots in a bacterial suspension and every two hours samples were cultured and compared with controls. A turbidimetric assay was performed to test the susceptibility of six bacterial species to ES. Bacterial biofilms were formed *in vitro* on polyethylene, stainless steel and titanium and ES were added to test their influence. The effect of ES on complement activation was investigated in healthy donor sera and in pre- and postoperatively gained sera from trauma patients. Different immunoassays, that are also clinically used to determine complement deficiencies in patients, were performed in absence or presence of maggot ES.
The results show that living maggots as well as their ES stimulate the bacterial growth of *S. aureus*, *E. faecalis*, CNS, *S. pyogenes* and *K. oxytoca* (all p-values=0.0002). Only *P. aeruginosa* had a decrease of bacterial growth (p=0.002). The strongest biofilms *in vitro* were formed *by S. aureus*, *S. epidermidis* and *P. aeruginosa* in contrast to the weak and inconsistent formed biofilms by *E. faecalis*,

E. cloacae and *K. oxytoca*. For *P. aeruginosa*, stainless steel was the best biomaterial with respect to biofilm formation and for *S. aureus* and *S. epidermidis*, the best biomaterial was titanium. ES were added to the strongest biofilms, named above, and reduced these on all biomaterials. The maximal biofilm inhibition by ES was seen on PE: 82% for *P. aeruginosa* ($p<0.0001$), 61% for *S. aureus* ($p<0.0001$) and 92% for *S. epidermidis* ($p<0.0001$). Furthermore, ES reduced complement activation in human sera from healthy and postoperatively immune-activated human sera up to 99.9% ($p <0.0001$), via all three pathways of complement activation. This study shows that nor living maggots, neither maggot ES have direct antibacterial properties. However, ES do reduce biofilms formed by different bacterial species on commonly used biomaterials. Furthermore, this research shows the first pathway independent complement-inhibitor, that already is successfully used in clinical practice. The biofilm reduction and the immunosuppressive effect of maggot ES may explain part of the improved wound healing caused by maggot therapy. Furthermore, the biofilm- and complement-inhibitor(s) present in maggot ES could provide novel treatment modalities for various diseases, e.g. (chronic) infections in trauma patients, and with respect to complement inhibition also for ischemic-reperfusion injury and Severe Inflammatory Response Syndrome (SIRS). Future research focuses on the identification and isolation of the effective substance(s) in the ES of *the Lucilia sericata larvae*.

O087

Presence of *Pneumocystis jiroveci* colonization in patients with chronic obstructive pulmonary disease

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Introduction: *Pneumocystis jiroveci* belongs to the group of fungi. *P. jiroveci* infections are frequently detected in immunocompromised patients. However, evidence suggests that *P. jiroveci* can also be detected in non-immunocompromised patients. In these cases, the micro-organism does not cause infections but colonizes the airways. Chronic obstructive pulmonary disease (COPD) is characterized by the presence of airflow obstruction and lung destruction. Over the past years, *P. jiroveci* has been linked with COPD. *P. jiroveci* colonization may be associated with the severity of COPD and some evidence suggests a role for *P. jiroveci* in the progression of COPD in smokers. The present study investigates the presence of *P. jiroveci* in sputum samples of patients with COPD, referred for pulmonary rehabilitation.
Material and methods: From March 2009 until September 2010, all sputum samples from COPD patients clinically rehabilitating at the centre of expertise for chronic organ failure (CIRO) were included in the study. Sputum samples were obtained at the beginning and end of the rehabilitation and during an exacerbation. Sputum samples were analyzed for the presence of *P. jiroveci* DNA by a real-time polymerase chain reaction (RT-PCR) assay.
Results: During the study period 509 sputum samples were collected from 218 patients clinically rehabilitating at CIRO Horn. A total of 184 samples (36.1%) were collected during inclusion, 265 (52%) during an exacerbation and 60 (11.9%) at the end of the rehabilitation.
P. jiroveci DNA was detected in 43 (8%) of all sputum samples in 29 patients, with CT-values ranging from 30 to 39 (mean CT 36). A total of 25 positive samples (58%) were detected during an exacerbation and 18 during the stable state (42%). The mean CT-values did not differ between the samples collected during an exacerbation and in the stable state.
Conclusion: In our population, *P. jiroveci* colonization could be detected in 6% of the sputum samples of COPD patients during the stable state and during an exacerbation.

O088

A real-time PCR on the SSU rRNA (18S) gene for cestode detection

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Introduction: Humans can be infected by a range of different cestodes, better known as tapeworms. Adult human tapeworms like *Taenia solium*, *Taenia saginata* and *Diphyllobothrium* can be several metres long and will shed little parts of its body, the proglottids, which are full with eggs. For other cestodes humans act as intermediate hosts. Humans can only harbour the larval stage of *Echinococcus granulosus* and *Echinococcus multilocularis* as cysts. Cysts can be detected by imaging but it is sometimes difficult to distinguish by morphology what kind of cysts is present in a patient and which cestode is the causing agent.
Our laboratory receives different types of patient samples such as cyst fluid, pieces of proglottids or biopsies. Our aim is to detect the presence of cestodes and to type the species. Our standard protocol is to amplify mitochondrial target genes Cox1 and Nad1 and sequence the products when present. Both primer pairs were originally designed for *Echinococcus* typing (Bowles, Blair and McManus. Mol Biochem Parasitol. 1992; Bowles and McManus. Int J Parasitol. 1993). Although the PCR is sensitive and the genes are polymorphic, some species have polymorphisms at the annealing sites of the primers. Frequently, we encounter samples where one of the genes cannot be amplified. *Diphyllobothrium latum*, for instance, cannot be amplified with the Cox1 primers due to various mismatches in the annealing sites. Also we sometimes find weakly amplified products.

Objective: Improve the sensitivity of the detection of cestode DNA.

Method and results: No real-time PCR for detection of all cestodes that infect humans has been published. We developed a real-time PCR on the SSU rRNA (18S) gene, using two primer pairs, combined with a dual labeled probe, that can amplify a wide range of different cestodes with utmost sensitivity. We examined our collection of patient samples and were able to detect all cestodes previously detected using both Cox1 and the Nad1 targets.

O089

Transport of peptidoglycan units across the bacterial membrane

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Bacterial cell growth necessitates synthesis of peptidoglycan. Assembly of this major constituent of the bacterial cell wall is a multistep process starting in the cytoplasm and ending in the exterior cell surface. The intracellular part of the pathway results in the production of the membrane-anchored cell wall precursor, Lipid II. This lipid carries the basic building block of the cell wall (two aminosugars and a pentapaptide). After synthesis this lipid intermediate has to be translocated across the cell membrane to present the basic building block to the penicillin binding proteins that synthesize the cell wall. The translocation (flipping) step of Lipid II was demonstrated to require a specific protein (flippase). We have now identified this protein, and the road to this identification will be presented along with the implications of this identifications for the model of the cell wall synthesis pathway.

O090

Regulation of peptidoglycan synthesis by outer membrane proteins

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Growth of the meshlike sacculus located between the bacterial inner and outer membranes is tightly regulated to ensure cellular integrity, maintain cell shape and orchestrate division. The actine homologue MreB that localizes underneath the cytoplasmic membrane in a helical structure directs placement and activity of peptidoglycan synthetic protein complexes (elongasomes) involved in length growth from inside the cell. The tubulin homologue FtsZ polymerizes in a ring-like structure at mid cell where it directs the synthesis of the new cell poles by protein complexes that together are termed the divisome.[1] However, precise spatiotemporal control over this process is poorly understood. Recently,[2,3] it has been discovered that the peptidoglycan synthases are also controlled from the outer membrane side of the sacculus. Two outer membrane bound lipoproteins, LpoA and LpoB, are essential for the function respectively of PBP1A and PBP1B, the major *Escherichia coli* bifunctional class A peptidoglycan synthases. Each Lpo protein binds specifically to a unique domain of its cognate PBP and stimulates its transpeptidase activity, thereby facilitating attachment of new peptidoglycan to the sacculus. LpoA localizes predominantly in the cylindrical part of the cell as does its cognate PBP and LpoB shows like PBP1B partial septal localization. However, the Lpo localization does not depend on the localization of their cognate PBPs or vice versa. Data suggest that the LpoB-PBP1B complex contributes to OM constriction during cell division. The LpoA/LpoB proteins and their PBP docking domains are not related and are restricted to gamma-proteobacteria, providing models for niche-specific regulation of sacculus growth.

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O091

Positive control of cell division: FtsZ is recruited by SsgB during sporulation of *Streptomyces*

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In bacteria that divide by binary fission, cell division starts with the polymerization of the tubulin homologue FtsZ at mid-cell to form a cell division scaffold (the Z-ring), followed by recruitment of the other divisome components. *Streptomycetes* are filamentous soil bacteria with a complex life cycle, which have a mycelial life style and propagate via sporulation. In these organisms two types of cell division occur; during normal growth cross-walls are formed that compartmentalise the hyphae but do not lead to physical separation, while during sporulation ladders of up to 100 Z-rings are formed in the aerial hyphae, producing uninucleoid spores. Sporulation-specific cell division requires an unparallelled complex coordination of septum-site localization, peptidoglycan synthesis and DNA segregation. We discovered that the SsgA-like proteins

(SALPs), which are only found in sporulating actinomycetes, help orchestrate developmental cell division in streptomycetes.[1]
The textbook view of bacterial cell division control starts from the principle of negative check points, among others involving MinCDE and Noc, that prevent incorrect Z-ring positioning.[2] We recently demonstrated positive control of septum-site localization during sporulation of *Streptomyces*, via the direct recruitment of FtsZ by the membrane-associated divisome component SsgB.[3] In turn, SsgB is controlled by the orthologous cell division activator SsgA. *In vitro* studies demonstrated that SsgB acts similarly to ZipA and promotes the polymerization of FtsZ. The interactions between the various cell division proteins were studied *in vivo* by time-lapse imaging and FRET-FLIM and corroborated via two-hybrid studies. When cell division is initiated, the turn-over of FtsZ protofilaments increases strongly, similar to tubulin turn-over during eukaryotic mitosis. The surprising positive control of Z-ring formation by SsgB implies the evolution of an entirely new way of Z-ring control, which may be explained by the absence of a mid-cell reference point in the long multi-nucleoid hyphae. A model of our current understanding of sporulation-specific cell division in *Streptomyces* is presented.

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O092
Regulation of FtsZ ring formation

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Bacteria replicate by division. This deceptively simple mechanism involves the coordinated action of a set of proteins, conserved in the bacterial kingdom, that allow for division to occur at exactly the right time and place, the middle of the cell. Due to its conserved and unique nature, cell division is seen as one of the most promising targets for the development of new antibacterial drugs. Cell division starts with the formation of a ring composed of protein filaments of FtsZ, just underneath the membrane. This ring organizes all other proteins that play a role in division. Work in my laboratory focuses on a fundamental question: How is the formation of FtsZ filaments regulated by accessory proteins to ensure that the FtsZ ring is formed at the right time and place? Most accessory proteins either stimulate or inhibit the polymerization of FtsZ at different locations or moments in the cell. We use *Bacillus subtilis* as a model organism. *B. subtilis* has at least nine accessory proteins that regulate FtsZ ring formation. We use random mutagenesis of FtsZ, and *in vitro* assays to monitor FtsZ activity in the presence of purified accessory proteins, to probe the molecular details of the interactions between FtsZ and regulatory proteins. I will present data on the interaction between FtsZ and the negative regulator MinC to illustrate our approach, and to highlight the importance of reaction conditions on *in vitro* reconstitution of FtsZ polymerization.

O093
Activity and localization of the pneumococcal Ser/Thr protein kinase StkP is controlled by its PASTA domains

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Many gram-positive bacteria, including the human pathogen *Streptococcus pneumoniae,* contain eukaryotic-type serine/threonine kinases (STKs) with extracellular PASTA-domains. PASTA-domains sense (unlinked) peptidoglycan and it has been postulated that STKs with a PASTA signature are key regulators of the activity of proteins involved in cell wall biosynthesis. Using GFP-fusions in combination with fluorescence microscopy, we show that the STK of *S. pneumoniae*, StkP, is localized to the new division sites. Functionality of the GFP fusions was confirmed by phosphoproteome analysis and we show that GFP-StkP phosphorylates the important cell division protein DivIVA. Interestingly, StkP's cognate phosphatase, PhpP, a predicted cytoplasmic protein, also displays a septal localization pattern which depends on the presence of StkP, but only in growing cells. This suggests that PhpP exclusively interacts with phosphorylated StkP at new cell division sites. Importantly, we show that localisation of StkP to the new cell division site depends on its extracellular PASTA domains and not on its cytoplasmic kinase domain as suggested previously. Furthermore, we show that StkP and PhpP are delocalised in the presence of vancomycin, indicating that unlinked peptidoglycan is the signal for StkP's autokinase activity. Importantly, localisation of DivIVA is severely perturbed in a *stkP* null mutant and cells show elongated and aberrant cell morphologies. Taken together, our data supports a model wherein StkP is localised to new cell division sites by the presence of unlinked peptidoglycan via its PASTA domains where it subsequently regulates the activity of a set of proteins required for accurate cell division and cell wall maturation.

O097

Microbiology outreach – How the mushroom got its spots and other stories

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The idea that scientists have a responsibility to communicate their work to the public is not new. However, talking to the public about science was once an activity conducted by a few committed eccentrics under the radar of the normal academic 'day job'. Today, outreach is a legitimate enterprise supported by a framework of national and international science centres and festivals, and is endorsed by funding bodies and university management alike. The relationship between scientists and the public has also changed. When I first became involved around 15 years ago, few people outside of a lab had heard of DNA and science communication was working largely to a 'deficit model' (with the unfortunate acronym of 'PUS') of increasing public understanding of science by imparting information. The public now has a greatly enhanced scientific vocabulary courtesy of media coverage and the internet, and the focus is on engaging them as active participants through dialogue and discussion. My enthusiasm for microbiology outreach has taken me from primary schools and draughty village halls to the Royal Show and the House of Lords. I will describe some of my activities, including:

- '*Discovering DNA – the Recipe for Life*': a resource for teachers of 9-12 year olds that takes the audience from the concept of a recipe for a cake through to the 'recipe' for a human, using games, simple experiments, puzzles and model-making.
- '*How the Mushroom got its Spots*': a booklet derived from a community –based 'Fungal Village' project. This contains games, puzzles and quizzes for use by anyone wanting to introduce non-experts of any age to the fascinating world of fungi (including the eponymous activity to produce a fly agaric lookalike using a balloon, tissue paper and a good pair of lungs).

Outreach has affected whole aspects of my life. I can no longer cut up an onion without wanting to extract its DNA, nor can I think of body glitter as anything other than a model for the cold virus in mucus. I will recall the fun I have had as one of the early eccentrics and also give a personal perspective on the changing agenda for science communication in the future.

O098

The hypothetical proteases PA0572 of *Pseudomonas aeruginosa* cleaves P-selectin glycoprotein ligand-1

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Introduction: The opportunistic pathogen *Pseudomonas aeruginosa* causes chronic infections in immunocompromised patients. To establish a chronic infection, evasion of the innate immune system is essential. *Pseudomonas aeruginosa* secretes various proteases that degrade proteins of the complement system, cytokines and flagellin that are essential for host defense and bacterial recognition. Another important defense mechanism is neutrophil recruitment to the site of infection. To reach this site neutrophils bind to P-selectin via P-selectin glycoprotein ligand-1 (PSGL-1). We screened for secreted proteases of *P. aeruginosa* that interfere with the PSGL1 - P-selectin interaction.

Materials and methods: Supernatant (overnight culture) of *P. aeruginosa* was screened for the presence of proteases that target PSGL-1 on neutrophils, monocytes and lymphocytes (isolated from healthy volunteers) by a monoclonal antibody inhibition assay. In this assay binding of an antibody against PSGL-1 (N-terminal part) was analyzed by flow cytometry. *P. aeruginosa* supernatant was fractionated with ion exchange and size exclusion chromatography.

Results: Supernatant of *P. aeruginosa* inhibited anti-CD162 binding to neutrophils, monocytes and lymphocytes. After fractionation, the inhibitory protein was identified as PAO572, a hypothetical zinc metalloprotease. Inhibition by PA0572 was more pronounced on lymphocytes in comparison with monocytes and neutrophils and was blocked by EDTA, which inhibits metalloproteases. PA0572 treated cells were recognized by a PSGL-1 antibody (PL2) that recognizes an epitope in the middle of the molecule, in contrast to the antibody that was used for the initial screening (KPL1). This indicates that the cleavage site is located in the N-terminal domain of PSGL-1, which is involved in P-selectin binding. In a static adhesion assay, binding of PA0572-treated neutrophils via PSGL-1 to its ligand P-selectin was impaired.

Conclusion: In this study, we identified PSGL1 as substrate of the hypothetical protease PA0572 of *P. aeruginosa*. This protease impairs the interaction of PSGL1 with P-selectin and may thereby prevents neutrophil rolling and extravasation to the site of infection.

O099

Epstein-Barr virus protein BNLF2a exploits host tail-anchored protein integration machinery for T cell evasion

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Introduction: The human herpesvirus Epstein-Barr virus (EBV) is the causative agent of infectious mononucleosis and is associated with a number of malignancies of both lymphoid and epithelial origin. EBV persists for life in infected hosts in the face of potent antiviral immunity. Especially during the replicative cycle, required for virus production and transmission to other hosts, many viral antigens are expressed. At this stage, immune evasion strategies are likely to be advantageous to avoid elimination of virus-producing cells by the host immune system. Indeed, during productive EBV infection, human leukocyte antigen (HLA) class I-restricted T cell recognition is abolished. In this study, we investigated how EBV interferes with HLA class I-restricted antigen presentation.
Methods: To study immune evasion during productive EBV infection, we employed a dedicated *in vitro* system that allowed separation of latently infected cells from those that have entered the productive phase of infection. A combination of bioinformatics and cytotoxicity assays was employed to identify the EBV-encoded BNLF2a protein as a TAP inhibitor. Cells expressing BNLF2a were used for immunofluorescence, flow cytometry, and biochemical experiments. Membrane insertion of BNLF2a was examined using a cellular expression system and *in vitro* translations. Mutagenesis was performed to identify the domains of BNLF2a involved in immune evasion.
Results: We identified an EBV productive-phase protein, BNLF2a, that mediates escape from cytotoxic T cell recognition. Expression of BNLF2a blocks both ATP and peptide binding to the Transporter associated with Antigen Processing (TAP), thereby inhibiting TAP-mediated peptide transport into the endoplasmic reticulum. As a consequence, cell surface display of HLA class I molecules is downregulated. In virus-producing B cells, early expression of BNLF2a allows for immediate interference with the presentation of viral antigens by HLA class I molecules. BNLF2a is highly expressed early upon EBV reactivation and declines during progression of viral replication.
BNLF2a displays characteristics of a tail-anchored (TA) protein: we find the viral protein to be inserted into membranes post-translationally, with its hydrophobic C-terminal domain functioning as a membrane anchor that retains BNLF2a in the ER. This topology leaves BNLF2a's N-terminal domain exposed in the cytosol, where it inhibits TAP function. BNLF2a interacts with Asna1, a cellular protein involved in membrane insertion of TA proteins. Asna1 facilitates membrane integration of BNLF2a and is required for efficient HLA class I downregulation by the EBV protein. These results illustrate how an EBV-encoded protein exploits a cellular pathway for TA protein biogenesis for evasion of the host immune response.
Conclusion: EBV has acquired an immune evasion molecule that blocks peptide transport via TAP. Interestingly, BNLF2a is unrelated to TAP inhibitors discovered so far in other viruses, both in mechanism and structure. By impairing TAP-mediated peptide transport and thereby HLA class I-restricted antigen presentation, BNLF2a may contribute to creating a window for undetected virus production, thus facilitating the spread of EBV to new hosts.

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O100

Nuclease expression by *Staphylococcus aureus* promotes escape from neutrophil extracellular traps

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Neutrophils are key players in the host innate immune response against bacterial infection. The recent discovery of neutrophil extracellular traps (NETs) has opened a novel dimension in our understanding of how these specialized leukocytes kill pathogens. NETs consist of a nuclear DNA backbone associated with antimicrobial peptides (AMPs), histones, and proteases that provide a matrix to entrap and kill various microbes. Certain leading bacterial pathogens as *Streptococcus pyogenes* or *S. pneumoniae* use secreted nucleases to subvert NET-based host immune clearance. Here, we used targeted mutagenesis to examine a potential role of *S. aureus* nuclease in NET-degradation and virulence in a murine respiratory tract infection model. An isogenic *S. aureus* nuclease-deficient mutant was significantly impaired in its ability to degrade NETs compared to the wild type parent strain USA 300 LAC, as quantified by fluorescence microscopy. Consequently, the nuclease-deficient mutant strain was significantly more susceptible to extracellular killing by activated neutrophils. Moreover, *S. aureus* nuclease production was associated with delayed bacterial clearance in the nose and lung and increased mortality after intranasal infection in mice. In conclusion, this study shows that *S. aureus* nuclease plays an important

role in resisting NET-mediated antimicrobial activity of neutrophils and contributes to disease pathogenesis *in vivo*.

O101

Pneumococcal meningitis: interactions between *Streptococcus pneumoniae* and the blood-brain barrier

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Introduction: *Streptococcus pneumoniae* (the pneumococcus) is a gram-positive human pathogen that causes life-threatening invasive diseases such as pneumonia and bacteremia with high morbidity and mortality throughout the world. Moreover, it is the most common cause of bacterial meningitis, an inflammation of the protective membranes covering the brain and spinal cord, collectively known as the meninges. *S. pneumoniae* is thought to invade the meninges via the bloodstream by crossing the endothelial cell layer of the blood brain barrier. The vascular endothelium is composed of two main heterogenous classes: 1) the microvascular endothelium, the portion of the circulatory system composed of the smallest vessels, such as capillaries, arterioles and venules; and 2) the macrovascular endothelium, comprising the larger vessels including both veins and arteries. Our goal was the visualization, localization and determination of the cerebral vascular endothelium class to which pneumococci bind when they cross the blood-brain barrier and enter the Central Nervous System (CNS).

Methods: A mouse model of early stage of meningitis was developed for this purpose. Balb/c mice were intravenously infected with FITC-labeled (10^8 CFU) of *S. pneumoniae*, serotype 4, strain TIGR4 and were sacrificed at various time points shortly after infection. Immunofluorescence on slides of the mice was performed to detect both the bacteria and the endothelial cells.

Results: Co-localization of *S. pneumoniae* within the vessels of the blood-brain barrier was found to occur at specific anatomical sites within the brain. The type of vessels to which the pneumococci were attached varied depending on the anatomical site.

Conclusions: From these results, it is clear that, to cross the blood-brain barrier and invade the CNS, *S. pneumoniae* adheres to both the macrovascular and microvascular endothelium depending on the anatomical site. This suggests that CNS invasion, the key event for development of meningitis, may occur at numerous sites throughout the brain.

O102

TroA of *Streptococcus suis* is required for efficient manganese acquisition and for full virulence

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Introduction: *S. suis* is primarily a pathogen of pigs and may cause meningitis, sepsis, arthritis and septic shock. Occasionally *S. suis* is able to infect humans as well. Infected humans may show the same symptoms as those seen in pigs. Little is known about the mechanisms *S. suis* uses to acquire nutrients including metal ions.In several other bacterial species efficient acquisition of metal ions is ingeneral mediated by high affinity metal binding lipoproteins. In this study we evaluated the role of a putative high affinity metal binding lipoprotein of *S. suis*, named TroA, and evaluated the role of this protein in growth and virulence.

Methods: An isogenic mutant deficient in the gene expression of TroA was constructed in *S. suis* (Δ*troA* mutant). Growth of mutant and wild type bacteria was evaluated in normal Todd-Hewitt broth (THB), cationic deprived THB and 100% porcine serum. Sensitivity to oxidative stress was evaluated by measuring survival of wild type and Δ*troA* mutant bacteria in THB supplemented with H_2O_2. Virulence of the Δ*troA* mutant bacteria compared to wild type bacteria was evaluated in a CD1 mice model.

Results: The Δ*troA* mutant bacteria were able to grow efficiently in THB, however cationic deprivation resulted in decreased growth of the Δ*troA* mutant bacteria compared to growth of wild type bacteria. Supplementation of cationic deprived media with different metal ions restored growth after supplementation with manganese exclusively. Growth of Δ*troA* mutant bacteria in porcine serum was diminished compared to wild type bacteria as well but could be restored by addition of manganese. Furthermore, the Δ*troA* mutant bacteria were more susceptible to oxidative stress compared to wild type bacteria. In a mice model 60% mortality was observed after infection with wild type bacteria and no mortality was observed after infection with Δ*troA* mutant bacteria.

Conclusions: The high affinity metal binding lipoprotein TroA of *S. suis* is involved in efficient manganese acquisition and is required for full virulence in mice.

O103

Host – pathogen interactions with *Streptococcus pneumoniae* during colonization and infection in an elderly mouse model

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Introduction: *Streptococcus pneumoniae* is a potential pathogen that is able to both asymptomatically colonize the nasopharynx and cause invasive disease. Colonization of the upper respiratory tract (URT) is a necessary first step towards invasive disease. Invasive pneumococcal disease disproportionably affects the very young and the elderly; the factors behind the increased risk for the elderly are poorly understood. The aim of this project was to elucidate potential mechanisms of increased risk of pneumococcal infection in the elderly host using a mouse model of colonization and pneumonia.

Methods: Female C57Bl/6 mice aged 18-23 months (elderly) and 3-4 months (controls) were used for either colonization or pneumonia model experiments. In the colonization model, mice were intranasally colonized with $5x10^6$ CFU of a serotype 6B *S. pneumoniae* strain and either sampled twice a week live (by sneeze sample) or post mortem in order to assess the density of pneumococcal presence in the URT. For the pneumonia model, mice were given aspiration pneumonia by intranasal inoculation with $2x10^7$ CFU of serotype 6B pneumococci under anaesthesia and sacrificed at 2, 3, or 4 days post-infection. Live and post-mortem samples were plated on blood-agar to determine bacterial CFU. Post-mortem nasal lavages were also performed to collect immune cells infiltrating into the nasopharynx. Morphologic determination of these cells was performed by Cyto-spin and Diff-quick staining, and by flow cytometry.

Results: Elderly mice exhibited delayed time to clearance of pneumococcal nasopharyngeal colonization, with 50% of the mice still colonized at the termination of the experiment on day 28. Young-adult mice were 100% clear of colonization by day 21. Furthermore, elderly mice had a statistically significant higher density of colonization, at least one-$\log_{10}$ higher at all time-points tested. We observed increased cell influx into the nasopharynx in elderly mice compared to young mice, with neutrophils being the most predominant infiltrating cell in the elderly at all time-points.

Elderly mice given pneumonia were less effective at clearing the bacteria from the lungs compared to young-adult controls. Also, there was a trend of elderly mice developing more cases of bacteremia following pneumonia infection than young-adult controls.

Conclusion: Elderly mice were not able to clear nasopharyngeal colonization as quickly or effectively as young-adult controls; nor were they able to clear a pneumonia infection as effectively as young-adult control mice when infected with a serotype 6B non-invasive strain. These data suggest a defect in the innate immune system of elderly mice, possibly involving effector cell function. Additional research into the host-pathogen interactions between the immunosenescent host and *S. pneumoniae* will hopefully elucidate a greater understanding of the elderly defects and potential avenues for treatment and/or prevention strategies.

O104

Structural insights on the *Mycobacterium tuberculosis*: the EspB substrate component

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Tuberculosis (Tb) has probably killed more humans than any other disease in history. The causative agent, *Mycobacterium tuberculosis* (Mtb) is a gram-positive bacterium that infects a third of the world's population with an estimated incidence of 9 million cases and 2 million deaths annually. Recently, a novel secretion system designated as T7S system was identified in mycobacteria.[1] This secretion system is indispensable to Mtb, which uses it to secrete substrates that aid its escape from phagosomes once internalized by alveolar macrophages.[2] Strains that lack the locus that encodes for this secretion system are not able to escape from the phagosome and are subsequently processed for presentation on the surface of the macrophage. Thus T7S system is extremely important in the pathogenic life cycle of mycobacteria. We previously characterize the spatial organization of the cell envelope of the M.tb and show the presence of T7S components in the cell envelope extracts.[3] In an effort to unravel the nature of the T7S system by providing a visual proteomic blueprint of this machinery and its interacting partners in a cellular context, we here present the structure of EspB substrate of the secretion machinery. EspB is critical for the functioning of the secretion machinery and essential for intracellular growth of the bacteria. In addition, it is shown that EspB forms a temporary complex by interacting with other substrates that together possibly form a distal component of secretion machinery.[4] We have demonstrated that this 47 kDa protein oligomerizes to form a heptameric structure *in vitro* and that this association represents its true quaternary state *in vivo*. The structure of heptameric EspB determined to 10 Å by cryo-electron microscopy is 100 Å in diameter and 80 Å high, with a 20-30 Å wide

channel. A model for its incorporation within the secretion machinery is presented.

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O105

Metabolites of commensal bacteria modulate the TLR response in epithelial cells

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The dynamics in infection biology relies on host-pathogen interplay. Initial detection of microorganisms by the host occurs via recognition of conserved pathogen associated molecular patterns (PAMPs). Effectors of innate immunity, either professional (dendritic cells, macrophages) or nonprofessional cells (epithelial cells, endothelial cells), sense PAMPs through pathogen recognition receptors (PRRs). Toll-like receptors (TLRs) are a major class of PRRs. Sensing of pathogens by TLRs induces activation of NF-κB and the production of immune mediators such as cytokines and chemokines. In the gastro-intestinal tract, intestinal cells are not exposed to nearby pathogens but simultaneously sense surrounding commensals and their metabolites. Short chain fatty acids (SCFAs) are such metabolites: they are produced by fermentation of dietary fibers. High levels of total SCFA concentrations are present in the colon. The aim of the present study was to determine the potential effect of metabolites produced by commensal bacteria on TLR mediated immune responses in epithelial cells. To this end, various types of cells were transiently transfected with selected human TLRs and a NF-κB luciferase reporter plasmid. Transfected cells were pre-incubated with different concentrations of the short chain fatty acids butyrate and propionate prior to stimulation with TLR agonists. NF-κB activation and expression of cytokine genes was assessed using luciferase reporter gene assays and quantitative RT-PCR (qRT-PCR). Stimulation of the eukaryotic cells expressing TLR5 with bacterial flagellin strongly stimulated NF-κB luciferase gene expression. Exposure of the cells to butyrate and propionate before stimulation enhanced the TLR response. Similar experiments using cells transfected with other types of TLRs yielded comparable results. However, the modulation of the TLR response by SFCA was dependent on time, dose and cell line. TLR5 activation of the cells also induced expression of the pro-inflammatory cytokine IL-8. Again this response was enhanced in the presence of SFCA. Overall our results indicate that: 1) bacterial metabolic products (SCFAs) enhance TLR induced NF-κB activity, 2) the modulation of the TLR response is time-, dose- and cell type dependent, 3) the effect is not restricted to a specific type of TLR. We conclude that SCFAs as derived from ingested food modulate the innate immune system.

O106

Livestock-associated MRSA: The European situation

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Livestock, especially pigs, have recently been shown to constitute a zoonotic reservoir for a few subtypes of methicillin-resistant *S. aureus* (MRSA), mainly belonging to clonal complex (CC)398 by MLST. Livestock-associated (LA-) MRSA was first recognised in the Netherlands in 2004 in pig farmers and their families. Since then, LA-MRSA has been reported from several European countries, Canada, USA and Asia. In addition, to pigs isolates of MRSA CC398 has also been reported from chicken, veal calves, horses and dogs. The natural host of LA-MRSA is not clear, however, it is noteworthy that methicillin-susceptible *S. aureus* CC398 (MSSA CC398) isolates occur naturally in pigs.

LA-MRSA isolates of CC398 is dominated by ST398, t011 but several spatypes including t034 and t108 has been described in addition at least 6 different SCC*mec* cassettes has been described in ST398 isolates.

Nearly all LA-MRSA isolates are resistant to tetracycline, but apart from that a striking diversity in antibiograms has been seen. In addition, carriage of resistance genes against heavy metals within the *SCCmec* cassette is a characteristic feature for the majority of isolates. It is also noteworthy that LA-MRSA isolates do not seem to respect the species barrier as opposed to other *S. aureus* lineages.

In the EU, baseline study performed in 2008, LA-MRSA isolates was found in 16 out of 24 member states. In that study the prevalence was highest in Spain with 50% of the herds carrying LA-MRSA. However, in a Dutch prevalence study as many as 68% of farms were found positive. In this survey, there was a strong correlation to the MRSA status of the supplier of pigs, and a large difference of MRSA carriage was observed between people with no pig contact (2%) and those with intensive pig contact (29%). These data concur with the experience from Denmark. Even so, transmission and smaller outbreaks have been described both from hospitals and nursing homes. This epidemiology

is strikingly different from the epidemiology of "human" MRSA types.
Although MRSA including CC398 has been found in meat, there is no sign of food being a significant factor in the transmission of MRSA CC398 from animals to humans.
Skin and soft tissue infections dominates the clinical picture reflecting that this primarily is seen among otherwise healthy persons, however, serious infections including endocarditis have also been described.
In conclusion LA-MRSA isolates seem to be quite distinct from other human MRSA isolates and LA-MRSA therefore seems to be a separate entity.

O107

Pig MRSA (ST398) - a sheep in wolf skin and the human MSSA (ST398) - a wolf in sheepskin?

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Staphylococcus aureus strain ST398, a clone commonly isolated from pigs, cows and horses, is associated with outbreaks of infections among pig farmers and their families in the Netherlands. These infections have, for the most part, been limited to the farming community, although some invasive nosocomial infections due to these strains have also been reported. In Northern Manhattan a related methicillin susceptible ST398 strain was identified as both a frequent colonizer and pathogen of subjects enrolled in an ongoing study of *S. aureus* transmission in the community. This isolate was subsequently identified in such geographically diverse regions as the Dominican Republic, Martinique, France and China. In contrast with the MRSA ST398, the MSSA ST398 strain appears to be capable of person-to-person transmission with no animal contact. Current studies are underway to examine how this strain is spread as well as its prevalence as a pathogen in different communities.

O108

MRSA carriage and occurrence of disease in swine veterinarians

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Background: Recently, a new clone of MRSA sequence type 398 (ST398) has been identified, which has an extensive reservoir in livestock. Persons who are in direct contact with pigs and veal calves frequently carry this livestock-associated MRSA strain (LA-MRSA). The purposes of this study were to determine 1) the burden of disease associated with carriage of LA-MRSA in veterinarians and 2) the dynamics of carriage in veterinarians and their household members.
Methods: A cohort of swine veterinarians and their household members was prospectively followed for one year between July 2008 and December 2009. Nasal and throat swabs were taken every four months from all participating veterinarians and their household members. At the beginning and the end of the follow-up period, questionnaires were taken with the following variables: age, gender, contact with animals (which kind and how often), presence of animals in the house, hospital contact in the last year, antibiotic use in the last year, underlying disorders (e.g., eczema, psoriasis or other skin diseases, lung diseases (COPD), allergy, recurrent upper respiratory tract infections, malignancies, immunosuppressive drugs, foreign bodies, recurrent furunculosis, abcesses, impetigo), history of MRSA infection and/or colonization, being active in a contact sport, household member working in a health care environment and presence of indwelling catheters and/or open wounds. A total of 137 veterinarians and 389 household members were included in the study. Questionnaires were completed by 133 (97%) veterinarians. All samples were inoculated directly onto chromID MRSA agar and chromID *S. aureus* agar (bioMrieux). Also a broth enrichment containing Muller-Hinton broth with 6.5% salt was inoculated. Subsequently, the overnight broth was inoculated onto chromID MRSA agar and chromID *S. aureus* agar. All agar plates were incubated for 18-24h at 35-37C, and were read independently. When isolates were identified as *S. aureus* the methicillin susceptibility was determined using the cefoxitin disk diffusion test according to CLSI standards. All cefoxitin resistant isolates were confirmed to be MRSA using a duplex PCR for the *mec*A gene and coagulase gene.
Results: Eighty-three veterinarians (61%) were MRSA positive at one or more sampling moments during the one-year follow-up period. The total number of household members that carried MRSA at one or more sampling moments was 41 out of 389 (10.5%). Carriage of MRSA in the household members was significantly associated with the carrier status of the veterinarian. Prevalence of MRSA in household members living with a veterinarians who had persistent MRSA carriage was significantly higher than for the other household members (p=0.001). Persistent MRSA carriers reported significantly more skin and soft tissue infections than the non-carriers (8/41 vs. 2/51; p=0.02). There was a light trend towards an increased risk of having allergies in the persistent carriers of MRSA (14/41) as compared to non-carriers (10/51), which did not reach statistical significance (p=0.15).

Conclusions: Carriage of LA-MRSA is a common feature in veterinarians. Household members had a significantly lower carriage rate and this was associated with the carrier status of the veterinarian. This indicates that human to human transmission of LA-MRSA does occur but carriage is mainly associated with exposure to livestock. Furthermore, in this prospective study covering 133 person-years follow-up, carriage of LA-MRSA was only associated with an increased rate of skin and soft tissue infections.

O109

Methicillin-resistant coagulase-negative *Staphylococci* isolated from pig farms in the Netherlands are a potential reservoir of mecA for *Staphylococcus aureus*

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Introduction: In the Netherlands, the prevalence of MRSA ST398 strains in pigs is high. In livestock animals, besides pigs also veal calves and poultry are found frequently positive. Transmission of MRSA ST398 from animals to human is a public health concern. Coagulase-negative staphylococci (CNS) are considered the reservoir of SCC*mec* for the origin of MRSA strains by horizontal gene transfer. However, little is known about SCC*mec* type in MRSA and CNS isolated from the same niche. In this study we determined if pig farms in the Netherlands contain *mec*A-positive staphylococci that can act as a reservoir for SCC*mec* acquisition.
Methods: 10 farms with an antibiotic usage expressed in DDD (defined daily dose) varying from zero to >30 DDD were selected. Five nasal swabs from pigs and 5 dust samples were taken at each farm. Swabs were pooled in PBS and cultured on Mannitol-Salt Agar for 72h at 30C, and on MRSA selective-Brilliance Staph 24 AGAR for 24h at 37C. Staphylococcal isolates were picked based on typical colony morphology, gram-staining and catalase reaction. Methicillin-resistance was confirmed with a mecA PCR. MALDI-TOF was used for species identification and genetic diversity was detected with GTG-fingerprinting PCR. The SCCmec elements were identified using multiplex PCRs.
Results: 36 *mec*A-positive CNS were recovered from pigs and 10 from dust samples. A relatively higher number of isolates were recovered from 4 farms with antibiotic usage of >30 DDD and 1 farm with 11.2 DDD. Single isolates or no *mec*A-positive staphylococci were isolated from 4 farms with no antibiotics or <13.1 DDD. The species recovered from the nasal samples were: *S. aureus, S. cohnii, S. haemolyticus, S. pasteuri, S. sciuri, S. saprophyticus* and from the dust were *S. aureus, S. haemolyticus, S. epidermidis, S. saprophyticus, S. cohnii,* and *S. sciuri*. SCC*mec* type V was identified in *S. aureus* and *S. haemolyticus*. The SCC*mec* types III, IVc, a type IV variant and nontypeable SCC*mec* were only found in the coagulase-negative staphylococci. SCC*mec* type IVa was primarily detected in *S. aureus*. A trend was visible that on farms with a high antibiotic use a larger number of staphylococcal species was isolated with a higher diversity in SCC*mec* types. GTG-analysis showed that almost all dust isolates clustered together with pig isolates.
Conclusion: Here we report for the first time a cross-sectional study focusing on the presence of a potential *mec*A reservoir in staphylococcal flora recovered from the pig farms in the Netherlands. The presence of SCC*mec* type IVc in different staphylococcal species isolated from different pig farms suggests that it is potentially transferred to or between CNS that may occur on pig farms. Longitudinal surveillance studies are needed to monitor the transfer to *S. aureus* of the novel SCC*mec* subtypes that are currently only found in CNS.

O110

Typing of methicillin-resistant *Staphylococcus aureus* sequence type 398 isolates with a new optical mapping technique

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Introduction: Methicillin-resistant *Staphylococcus aureus* (MRSA) has posed a considerable health threat for decades. In 2003, a new livestock-associated MRSA (LA-MRSA) was identified in the Netherlands and has since then emerged in many other countries. Typing of LA-MRSA isolates is notoriously difficult. Multi-locus sequence typing (MLST) provides hardly any resolution, nor does staphylococcal protein A (*spa*) typing and multiple-locus variable-number of tandem repeat analysis (MLVA) which both yield 2 predominant types. This makes it hard to further differentiate CC398 isolates, especially in transmission events. However, pulsed-field gel electrophoresis (PFGE) can now also be performed using a neoschizomer of SmaI, namely *Cfr*9I. More recently, a high resolution microbial whole genome analysis named optical mapping was introduced. In this study we assessed the capability of optical mapping to better differentiate these LA-MRSA isolates.
Methods: A total of 69 CC398 isolates were typed by optical mapping and PFGE. This collection consisted of multiple isolates obtained from 17 veterinarians and their household members. Prior to this experiment, 6 CC398 isolates were analyzed by optical mapping. These 6 isolates consisted of 3 pairs. Pair 1 comprised isolates from a mother and her

child that most likely was infected by the mother. Pair 2 consisted of isolates from a pig and a pig holder and pair 3 was made up of isolates from a pig holder and one of his family members. All pairs have been typed with *spa*-typing and PFGE using *Cfr*9I.
The cells of the staphylococcal cultures were embedded in low melting point agarose in order to obtain high molecular weight DNA. After lysis and proteinase K treatment, the plugs were melted and the agarose was digested using -agarase to release the DNA. Using the OpGen Argus system, the DNA molecules were stretched and digested by the restriction enzyme *Xba*I in a micro fluids cell. Subsequently, the resulting fragments were sized and assembled to a genomic restriction map by the system.
Results: Both isolates of pair 1 were carrying *spa*-type t108. All isolates of pair 2 and 3 had *spa*-type t011. PFGE performed with *Cfr*9I distinguished the 3 pairs with a 100% similarity between the isolates of a pair. Pairs 1 and 2 showed a high similarity with each other and pair 3 was clearly distinct. Optical mapping of these pairs corroborated the PFGE results and grouped the pairs with a maximum of 0.2% difference between the members of a pair. Pair 1 and 2 were nearly identical and only displayed a 0.4% difference. The difference between pair 3 and pairs and 1 and 2 was 3.7%.
At present, the analysis of the 69 LA-MRSA isolates is not fully completed. The final results of this analysis will be presented at the meeting.
Conclusion: Until now PFGE using *Cfr*9I was the best method to differentiate LA-MRSA isolates. However, this new optical mapping technique proved to be an even better and more robust technique for differentiating LA-MRSA. Furthermore, additional information such as SCC*mec* type, presence of pathogenicity islands and phages can be easily obtained using optical mapping.

O111

Human tumor viruses and rumor viruses

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Approximately 20% of the global human cancer burden is attributable to infectious pathogens, most of them being viruses. The first human oncogenic virus to be identified was Epstein Barr virus in 1964, and of course several important DNA and RNA viruses, such as human T-cell leukemia virus, oncogenic papilloma viruses, Kaposi's sarcoma herpesvirus and Merkel skin cell polyomavirus, have been discovered since that time. However, there have been many claims of association of viruses with cancer and other chronic diseases of humans which have not stood the test of time and these are what I call "rumor viruses". Retroviruses in particular have attracted much interest, although DNA tumor viruses such as SV40 have also gained notoriety as potential human pathogens. One of the difficulties of distinguishing tumor viruses from rumor viruses is the complexity of applying Koch's postulates of causation to long-incubation, multifactorial diseases. Another difficulty is that the extraordinary sensitivity of modern molecular detection methods can lay themselves open to false positive results unless extremely rigorous, forensic style operational procedures are used – standards which are seldom applied in research laboratories.
When high profile announcements are later disproved, it can cause considerable distress among those who suffer from the disease in question and who were relieved to believe that a definitive cause of their disease had been discovered. Because patient websites and rumor mills usually have a longer half-life than unreliable or misinterpreted scientific results, the scientists who challenge or disprove previous claims tend to receive criticism from the members of interest groups who may not fully appreciate the degree of doubt that typically accompanies cutting-edge medical research. Moreover, the high profile journals which published the original reports tend to be averse to publishing subsequent contradictory evidence. And by the time the contrary evidence is published, the original proponents of a rumor virus may well have set up a spin-out company or marketed a commercial test, which compounds the embarrassment of having made an honest scientific error of interpretation. I shall illustrate the phenomenon of rumor viruses with my own experience on what we once called human retrovirus 5 and with SV40, as examples of why we should from the outset have been wary about XMRV.

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O112

Beneficial and detrimental effects of human endogenous retroviruses

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We aim to evaluate the structure and function of Human Endogenous Retroviruses (HERVs) with respect to the benefit they may have for humans or the damage they may cause. Emphasis is laid on their putative roles, if any, in pregnancy, in gene regulation and in cancer. As a basis for this discussion it will first be necessary to briefly describe the structure and function of retroelements, including

HERVs, before addressing their positive or negative effects at the cellular and organismal level, Finally, we will give an outlook in which we will attempt to define priorities for future research.

O113

No XMRV in a well established cohort of CFS patients in the Netherlands

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Objective: The presence of the retrovirus xenotropic murine leukaemia virus-related virus (XMRV) has been reported in peripheral blood mononuclear cells (PBMC's) of patients with chronic fatigue syndrome (CFS). Considering the potentially great medical and social relevance of such a discovery, we investigated whether this finding could be confirmed in an independent European cohort of patients with CFS.

Design: Analysis of a well defined cohort of patients and matched neighbourhood controls by PCR.

Population: Between December 1991 and April 1992, PBMC's were isolated from 76 patients and 69 matched neighbourhood controls. In this study we tested cells from 32 patients and 43 controls from whom original cryopreserved phials were still available.

Main outcome measures: Detection of XMRV in PBMC's by real time PCR assay targeting the XMRV integrase gene and/or a nested polymerase chain reaction assay targeting the XMRV gag gene.

Results: We detected no XMRV sequences in any of the patients or controls in either of the assays, in which relevant positive and negative isolation controls and PCR controls were included. Spiking experiments showed that we were able to detect at least 10 copies of XMRV sequences per 10e5 PBMC's by real time as well as by nested PCR, demonstrating high sensitivity of both assays.

Conclusions: This study failed to show the presence of XMRV in PBMC's of patients with CFS from a Dutch cohort. These data cast doubt on the claim that XMRV is associated with CFS in the majority of patients.

O114

RNAi gene therapy for HIV-1: escape and countermeasures

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We and others have demonstrated a potent antiviral effect of RNA interference (RNAi) approaches that target the viral RNA genome. However, HIV-1 can easily escape from suppression by a single shRNA inhibitor through selection of a point mutation in the target sequence. The viral escape options are clearly restricted when highly conserved viral sequences are targeted as only silent codon changes are selected. We tested two strategies to prevent viral escape. First, we designed secondary shRNAs that specifically target popular viral escape routes. Indeed, the known escape routes are effectively blocked by these secondary shRNAs, but the strategy fails because the virus is able to select novel escape routes. Second, we used a combination of multiple shRNAs to increase the genetic barrier towards resistance. We demonstrated that HIV-1 cannot escape in the presence of 4 potent shRNAs. This strategy is currently being translated in an ex vivo gene therapy. This protocol is based on the HIV-based lentiviral vector system to transduce CD34+ haematopoietic stem cells, and we have set a humanized mouse model for pre-clinical testing. This approach should lead to a single durable treatment of HIV-infected individuals.

O115

HIV-1: old foe repackaged?

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Background: During the course of infection, HIV-1 can rapidly escape from neutralizing humoral immunity, coinciding with changes in the envelope glycoprotein (Env) that include elongation of the variable loops in the gp120 subunit, and an increased number of potential N-linked glycosylation sites. It is unclear whether these changes revert upon transmission or that resistance of Env to antibody neutralization has accumulating over the course of the epidemic.

Methods: The sensitivity of clonal HIV-1 variants isolated from patients presenting with primary HIV-1 infection in Amsterdam either in the period 1985 – 1988 (historical HIV) or 2003 – 2005 (contemporary HIV), to neutralizing antibodies and sera was tested. Moreover, length and glycosylation characteristics, as well as immunogenicity of gp120 of historical and contemporary HIV-1 variants was analyzed.

Results: As compared to historical HIV-1, contemporary HIV-1 variants were more resistant to neutralization which

coincided with an increased length of the variable loops in envelope, in particular the V1 loop, and an increased density of the viral glycan shield. In addition, historical viruses elicited broader NAb responses than more recently circulating viruses.

Conclusions: These finding suggest that over a period of 20 years, HIV-1 has evolved towards a neutralization resistant phenotype by enhancing the masking of epitopes on its envelope. As the increased neutralization resistance of contemporary HIV-1 seems to coincide with a blunted NAb response in recent seroconverters, these findings may be relevant for the choice of envelope in vaccine design.

O117

The role of macrophages in granuloma formation

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Although widely recognised by pathologists from the 19th century on, it is only recently that the cellular and molecular aspects of macrophages in granuloma formation have been studied. Macrophages are a prominent constituent of these organised structures associated with chronic infection (for example, Tuberculosis and Schistosomiasis), foreign bodies and disorders of unknown aetiology (e.g. Crohn's disease, sarcoidosis and Wegener's granulomatosis). The recruitment of monocyte/ macrophages to lesions depends on TNF and CD11b/CD18) (Kindler et al., 1989, Cell;56(5):731-40). Macrophages in the lesions include so-called epithelioid cells, and the hallmark giant cell formation, yet the phenotypic plasticity and function of multinucleated giant cells (MGC) remain elusive.

Recent studies on the plasma membrane molecules that control Interleukin 4/13 induced fusion of macrophages *in vitro* and *in vivo*, have implicated a range of receptors and differentiation antigens, including CD36 (Helming et al. J Cell Sci. 2009;122:453-9.) and DAP-12 (Helming et al. Sci Signal 2008;1(43):ra11). These have been identified by monoclonal antibody screening and by a candidate-based approach. Different stages of the fusion process have been observed, but the in situ functions of such giant cells remain obscure (Reviewed in: Helming and Gordon. Trends Cell Biol. 2009;(10):514-22).

The presentation will summarise microbial and immunobiological aspects of granuloma formation, especially with regard to the nature of the macrophages in this intriguing group of disorders, and the relationship of MGC to osteoclasts, macrophage giant cells with defined function in bone resorption.

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O118

The granuloma in tuberculosis: a host-pathogen collusion

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The pathogenesis of tuberculosis used to be the investigative domain of two relatively separate, albeit interdependent disciplines. Specific survival strategies of *Mycobacterium tuberculosis* (*Mtb*), i.e. the adaptation of metabolic functions, cell wall structure and replication times, were at the center of molecular microbiologists' interest in defining virulence genes and persistence profiles. By contrast, infection immunologists analyzed innate and adaptive host responses necessary for *Mtb* growth containment, often welcoming the pathologist's view on granuloma initiation, maintenance and necrosis. In recent years, a more integrated view of tuberculosis pathogenesis has prevailed, although the concept itself is not new. The granuloma is now viewed as part of the successful life cycle of *Mtb*. This evolutionary perspective takes into account the mutual shaping of the tissue microenvironment, which concurrently allows propagation and transmission of *Mtb,* yet restricts tissue damage to safeguard survival of the host.

In this "holistic" view of the *M. tuberculosis* life cycle, a focal accumulation of mononuclear cells in various states of differentiation, i.e. a granuloma, is not per se protective. Another implication of the integrative view on *Mtb's* life cycle is that, to stop *M. tuberculosis* from multiplying and transmitting, simple imitation or augmentation of the natural host response to infection is likely to fail. Unless T cells can be trained to recognize *M. tuberculosis* as soon as it enters the alveolar macrophage, one of the best vaccination strategies might be to bypass the regulatory networks *M. tuberculosis* itself initiates to establish its niche for replication. If anything, vaccines would have to mitigate TH1 and TH2 responses and altogether blunt regulatory T cell responses to allow more protective immunity while avoiding damaging pathology. This may be impossible to achieve purely by vaccination, leaving ample opportunity for adjunct immunomodulatory measures.

O119

Schistosoma mansoni egg glycoproteins induce type-2 granulomas *in vivo* by a glycan-dependent mechanism

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Schistosomes are blood-dwelling helminths that infect over 200 million people in (sub-) tropical areas around the world. The main pathology of schistosomiasis is initiated by a large number of parasite eggs that instead of being excreted with the feces or urine, get trapped in various organs such as the liver. Here, egg antigens induce a granulomatous inflammatory response, which eventually leads to fibrosis and severe organ damage. Carbohydrate components (glycans) of *Schistosoma mansoni* egg glycoproteins appear to play a central role in the initiation and modulation of granuloma formation.
We have studied experimental hepatic granuloma formation *in vivo* by injection of antigen-coated Sepharose beads as artificial eggs into the caecal vein of mice. Beads that carry egg antigens (SEA) of *S. mansoni* with intact glycans, but not with glycans destroyed by periodate treatment, gave rise to type-2 granulomas comparable to those around schistosome eggs in terms of cellular content, temporal regulation and expression of adhesion and extracellular matrix components. In the same model, synthetic schistosome-related glycoconjugates with terminal GalNAc1-4GlcNAc (LDN) groups were also able to induce these granuloma, but other glycoconjugates including abundant fucosylated elements such as the Lewis X antigen did not.
Mass spectrometry-based glycosylation analysis of the major egg glycoproteins revealed that kappa-5 is the main GalNAc1-4GlcNAc (LDN)-containing glycoprotein of SEA, whereas another set of glycoproteins carries Lewis X antigens. To further investigate if these authentic individual *S. mansoni* egg glycoproteins are granulomogenic and by which molecular principles, we used the mouse lung granuloma model in which formation of granulomas was also observed around SEA-coated beads. In line with the previous findings using synthetic model glycoconjugates, beads coated with kappa-5 were able to induce granuloma formation, while the Lewis X glycoproteins omega-1 and IPSE did not. Moreover, enzymatic removal of its LDN moieties reduced the capacity of kappa-5 to induce granulomas, indicating that the LDN glycan element is critically involved in the granulomogenesis process.
Glycan-mediated recognition of kappa-5 by immune cells would require the involvement of lectin receptors. Macrophages as well as dendritic cells are thought to play a role in schistosome egg granuloma formation. In *in vitro* assays, SEA components have been shown to be internalized by monocyte-derived cells via the C-type lectin receptors DC-SIGN, MR and MGL. Using different cellular as well as biochemical assays we were able to demonstrate that kappa-5 interacts with DC-SIGN and MGL, but via different parts of its glycans. The implications of these data and the possible involvement of these receptors in granuloma formation and associated immune modulation will be discussed.
To conclude, our data suggest that glycans associated with the schistosome egg glycoprotein kappa-5 are involved in the induction of periovular granulomas in schistosomiasis.

O120

Zebrafish embryo screen to identify mycobacterial genes involved in granuloma formation

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The hallmark of tuberculosis is formation of granulomas; organized structures of aggregated infected macrophages surrounded by immune cells. During the last few years it has even become apparent that mycobacteria induce granuloma formation, and that this induction is both essential for long term survival and efficient spreading of the bacteria to new hosts. It is our aim to unravel mechanisms involved in granuloma formation.
We study granuloma formation *in vivo*, by making use of the zebrafish embryo – *Mycobacterium marinum* infection model. *M. marinum*, a close relative of *M. tuberculosis*, is a natural pathogen for zebrafish and causes disease characterized by formation of granulomas. The transparency of zebrafish embryos allows for straightforward monitoring of granuloma formation in real time.
In order to identify mycobacterial genes involved in granuloma formation, we have set up a screen in which 1000 transposon mutants of *M. marinum* expressing mcherry were tested one by one for early granuloma formation in zebrafish embryos. By comparing granuloma formation of the mutant bacteria with the parent strain, we have identified 29 mycobacterial genes important for granuloma formation. Seven of the granuloma mutants had a mutation in the ESX-1 region, encoding a specialized type VII secretion system known to be important for virulence. The currently used BCG vaccine, which induces delayed granuloma formation, also has a deletion in its ESX-1 region. Therefore, the identification of ESX-1 mutants confirms the reliability of our screen. In addition, we have identified mutations in genes encoding mycobacterium specific PE and PPE proteins, the secA2 secretion pathway as well as genes associated with metabolic pathways. Furthermore, we have identified genes involved in the biosynthesis of the mycobacterial cell wall and genes required for the synthesis of molecules that have been implicated in host-pathogen interactions. Finally, we identified several genes with unknown functions that are involved in granuloma formation. By studying the function of these novel identified genes we hope to dissect the process of granuloma formation. Furthermore, we will examine the

phenotype of the granuloma mutants in adult zebrafish, in order to determine their potential to activate the immune system. Our approach might possibly lead to the identification of new drug targets, or to the development of new TB vaccines.

O121

Disruption of *M. marinum* ESX-5 leads to increased granuloma formation and bacterial growth in adult zebrafish

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Mycobacteria use specialized protein secretion systems to transport proteins across their aberrant cell wall. One of these type VII secretion systems, ESX-5, is conserved in pathogenic mycobacterial species and responsible for secretion of PE and PPE proteins. Although their exact function is thus far unknown, these proteins have been implicated in virulence. Using the pathogenic mycobacterial species *Mycobacterium marinum*, ESX-5 has been found to play a role in the induction of macrophage cell death and suppression of pro-inflammatory cytokine release[1].

In this study we made use of an ESX-5 deficient *M. marinum* mutant to studied the effects of ESX-5 protein secretion on survival, bacterial growth and virulence in zebrafish embryos and adult zebrafish. In zebrafish embryos, the ESX-5 mutant strain was slightly attenuated in growth compared to the wild-type strain. Granuloma formation, a hallmark for mycobacterial infection, was not affected by ESX-5. In adult zebrafish however, the ESX-5 mutant strain displayed increased virulence compared to the wild-type strain. Fish infected with the ESX-5 mutant strain showed reduced survival, increased bacterial growth in their organs and early onset of granulomas. Using new zebrafish antibodies, we could show that granulomas were surrounded by a layer of lymphocytes, thereby resembling human granulomas formed during *M. tuberculosis* infection. Mixed infection experiments showed that ESX-5 deficient *M. marinum* outcompeted the wild-type strain in adult zebrafish. This specific advantage was not caused by an increased growth rate, since growth in macrophages, zebrafish embryos and under hypoxic conditions was similar for both strains. There were also no indications for an elevated systemic host inflammatory response.

In contrast to adult zebrafish, embryo's lack an adaptive immune system. We therefore hypothesize that ESX-5 effector proteins modulate the adaptive immune system leading to a moderate and persistant infection. Taken together, our infection studies in zebrafish indicate an important role for ESX-5 and its PE and PPE substrates in the progression of infection in a natural host.

Reference

1. Abdallah et al. J Immunol. 2008;181(10):7166-75.

O122

Lipid signaling regulated by pH: Phosphatidic acid as a pH biosensor

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Lipids play important roles in signaling in all organisms, yet the underlying regulatory mechanisms are poorly understood. We have taken a systems-biology approach in yeast to identify new genes and pathways that regulate lipid signaling. We identify over 200 genes that were not previously known to play roles. In the course we uncovered a fundamental mechanism regulating phosphatidic acid (PA) signaling. Binding of a transcription factor, Opi1, to PA depended on cytosolic pH and the protonation state of the lipid's phosphate head-group. Hence, PA is a lipid pH biosensor and lipid signaling can be regulated directly by pH. We then show that nutrient availability (glucose) regulates phospholipid metabolism to control production of membranes via PA and pH-dependent lipid signaling. Therefore, we identify a physiological context for pH-dependent lipid signaling. We propose that lipid pH biosensors play important roles in metabolic regulation in a wide variety of systems.

O123

Regulation of cellular signaling and cell growth through cytosolic pH

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Regulation of cell growth and proliferation is tightly controlled by nutrient availability, but molecular mechanisms for nutrient sensing are only beginning to emerge. In budding yeast, glucose activates the Ras/PKA pathway, a central regulator of cell growth. Activation of the Ras/PKA pathway depends on a signal derived from glucose metabolism, but the molecular mechanisms of Ras/PKA activation have not been established yet.

Previously, we found that cytosolic pH acts as a cellular signal that regulates PKA pathway activity in response to glucose. Detailed analysis using live cell imaging coupled to a microfluidic setup has demonstrated that cytosolic pH is rapidly and reversibly regulated by glucose metabolism. Changes in cytosolic pH are sensed by the vacuolar ATPase (V-ATPase), a proton pump required for the acidification of vacuoles, which mediates, at least in part, the pH signal to PKA. Thus, these data identify a novel and potentially conserved glucose-sensing pathway.

Interestingly, such a mechanism also readily explains the sensing of alternative carbon sources, such as raffinose or galactose. Reduction of glucose concentration or growth on alternative carbon sources leads to reduced cytosolic pH and a corresponding reduction in cell growth. In contrast, nitrogen limitation does not affect cytosolic pH, while clearly reducing cell growth, suggesting that cytosolic pH might act as a specific signal for cell growth in response to carbon sources. Indeed, we found that increasing cytosolic pH on alternative carbon sources is sufficient to overcome reduced cell growth under these conditions. Taken together, these experiments suggest a novel and unexpected role of cytosolic pH as a cellular signal to promote cell growth in response to carbon sources.

O124

Intracellular pH controls yeast growth

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The pH in a cell or organelle affects the properties of proteins, lipids and metabolites, and therefore influences reaction rates, redox equilibria, protein sorting, and many other processes important for cellular functioning.

We found that both cytosolic and mitochondrial pH vary with changing environmental conditions in a range between well below 5 and up to 7.5, without loss of viability. These changes can be rapid, and are often transient, changing back to neutral when the cells have adapted to the new conditions. To understand how intracellular pH is regulated, we analyzed the cytosolic (pH_c) and mitochondrial (pH_m) pH of almost 5000 haploid yeast deletion strains during exponential growth. This revealed that both cytosolic and mitochondrial pH are robust to genetic changes: The mutant with the lowest cytosolic pH had a pH_c of 6.75, only 0.3 pH units below the wild-type average, while most of the mutants that deviated significantly from wild-type had a pH_c less than 0.1 pH unit more or less than the wild-type average.

Besides the expected mutants functioning in pH regulation and proton pumping, we found mitochondrial function to be important for intracellular pH regulation, both in respiring and in fermenting yeast cells. Additionally, there is a close link between pH_c regulation and lipid biosynthesis.

Lastly, many general growth controlling genes have deviating pH_c. Indeed, also in wildtype there is a remarkably tight correlation between pH_c and growth rate. We will provide evidence that pH_c is causal in this relationship, and that in signals nutritional status to signal growth. Thus, we show that pH_c is a tightly controlled yet highly dynamic signal that integrates nutritional cues to control growth rate.

O125

Weak acid stress in *Bacilli*

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Bacillus species are a spoilage and safety challenge to the food industry due to their extremely resistant endospores. To interfere with (out)growth of spores and vegetative cells, weak organic acids are suitable preservatives. To ensure their continued use while optimally preserving product quality, knowledge of resistance development is important. In *Bacilli* stress responses induced by weak organic acids include intracellular membrane and pH homeostasis and detoxification of reactive oxygen species. Targeted identification of inhibitors and formulation of milder antimicrobial combinations, as desired by consumers, is thereby facilitated. In addition to being food spoilers, probiotic *Bacilli* are known and utilized. Knowledge of weak organic acid stress resistance may be used to develop enhanced strain robustness facilitating their permanence in the gastrointestinal tract.

O126

Prediction of stress induced robustness using molecular biomarkers

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Introduction: Microorganisms are constantly facing changing environmental conditions and have evolved sophisticated stress adaptation mechanisms to be prepared for challenges even before they arise. The stress adaptive response is a crucial survival strategy for a wide spectrum of microorganisms, including food spoilage bacteria, pathogens and organisms used in functional food applications, and can result in increased robustness of microorganisms. Prediction of mild stress induced enhanced robustness will allow to control and/or exploit these stress adaptive traits.

Methods: We designed a framework for identifying molecular biomarkers for mild stress induced microbial robustness towards lethal stresses. Several candidate-biomarkers were selected by comparing the genome-wide transcriptome profiles of our model organism *Bacillus cereus* upon exposure to four mild stress conditions (mild heat, acid, salt and oxidative stress). These candidate-biomarkers – a transcriptional regulator (activating general stress responses), catalases (removing reactive oxygen species), and chaperones and proteases (maintaining protein quality) - were quantitatively determined at transcript, protein and/or activity level upon exposure to mild heat, acid, salt and oxidative stress for various time intervals. Both unstressed and mildly stressed cells were also subsequently exposed to lethal stress conditions (severe heat, acid and oxidative stress) to quantify the robustness advantage provided by mild stress pretreatment. To evaluate whether the candidate-biomarkers could predict the robustness level of mild stress treated cells, their induction upon mild stress treatment was correlated to mild stress induced robustness towards lethal stress and the correlation significance was evaluated using the Pearson correlation coefficient.

Results: Both short- and long-term biomarkers could be identified of which the induction levels upon mild stress treatment were significantly correlated to the induced enhanced robustness towards lethal heat, acid and/or oxidative stress, respectively. The predictive quality of the transcripts differed from that of proteins and activity level, underlining the necessity to measure molecular biomarkers at different functional cell levels (transcript, protein and activity level). The predictive quality of the biomarkers was also stress-dependent, highlighting the significance to evaluate predictive potential of biomarkers for various stress treatments.

Conclusion: The identified molecular biomarkers for mild stress induced enhanced robustness are widely conserved in microorganisms and have indispensable roles in stress responses. Therefore, they might also serve as biomarkers for stress adaptive behaviour in other microorganisms than *B. cereus*. Our study provides a systematic, quantitative approach to search for these biomarkers for adaptive behaviour and to statistically evaluate their predictive potential at different functional cell levels in order to select biomarkers with high predictive quality that can serve to early detect and predict adaptive traits.

O127

How dead is dead? A multiparameter viability toolbox applied to *Listeria monocytogenes*

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Stress profiling on the basis of a multi-parameter viability assay provides insight in the nature and sensitivity of the essential components of the cellular machinery that become affected upon application of a certain stress factor. This is demonstrated here by exposure of the foodborne pathogen *Listeria monocytogenes* to gradients of five different stresses of increasing intensity, typically ranging from moderate to lethal conditions. The stress factors included exposure to heat, acidic pH, a detergent disinfectant, oxidative stress, and hyperosmotic stress. In addition to CFU's and lag time, five different molecular viability parameters were measured by fluorescence-based assays, including membrane integrity, membrane potential, esterase activity, redox activity, and intracellular pH stability. The latter was measured by our recently invented real-time viability assay. Exposure to all stresses resulted in clear dose-response relationships for all viability parameters with the exception of hyperosmotic conditions. A statistical analysis showed strong correlations for (i) the growth parameters plate counts and lag times, (ii) the enzyme-associated functions redox and esterase activity, and (iii) the membrane-associated pH stability and membrane integrity. Results suggested a pronounced difference in the susceptibilities of essential cellular functions depending on the stress factor applied. However, at relatively high stress intensities all of the viability parameters become affected independent of the stress factor. The data presented here were obtained by a novel approach for generic purpose in microbial physiology, forming the basis for an automated, fluorescence-based viability toolbox for simultaneous measurement of multiple stress-related parameters of interest.

O128

Genomic approaches for the characterization of classical genetic mutants and metabolic pathways in filamentous fungi

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Genome sequences are an important tool in accelerating the association of genes with phenotypes. Production of specific secondary metabolites is a phenotype with scientific implications for taxonomy, biochemistry and biotechnology. We have utilized molecular genetic tools available in the industrial workhorse *Aspergillus niger* to associate a polyketide synthase with the production of both DHN melanin and naptha-gamma-pyrones. A wide range of phenotypes are associated with classical genetic mutations in the model ascomycete fungus, *Neurospora crassa*. Using "next generation" sequencing methods it is now experimentally and economically feasible to use a genome sequencing strategy to identify gene mutations associated with phenotypes. Using this strategy we have identified a number of mutations associated with phenotypes in "classical" genetic mutant strains of *N. crassa*. We have sequenced the genomes of several *N. crassa* strains carrying genes whose mutations leads to phenotypes associated morphology, sexual development and pigmentation.

O129

Comparative transcriptome analysis between the opportunistic pathogen *Aspergillus fumigatus* and the rarely pathogenic *Aspergillus nidulans*

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The filamentous fungus *Aspergillus fumigatus* is a significant cause of infection in man and is the leading mould pathogen in leukaemia and transplant patients and is responsible for mortality in a large number of individuals with immunological disorders. Spores of this ubiquitous fungus are present in the atmosphere and it is estimated that several hundred are inhaled everyday. Due to their small size, spores are able to penetrate deep into the alveoli where they encounter lung surfactant which is a phospholipid rich environment (surfactant is composed of >80% phospholipid). We have demonstrated that phospholipid stimulates the surface growth of *A. fumigatus* by altering the branching pattern of the mycelium. This stimulation in surface growth may have important implications in the infection and invasion of the lung and is mediated by phosphorylcholine and choline, breakdown products liberated the action of secreted phospholipases. This effect is not seen in the related rarely pathogenic *A.nidulans*. In order to investigate potential difference in phospholipid metabolism between the two strains, a transcriptomic comparison *A. fumigatus* and *A. nidulans* grown on glucose and lecithin as sole carbon sources under identical conditions in a chemostat was undertaken. While statistically, the GO SLIM lipid metabolism category was significantly affected in both strains on lecithin compared to glucose, there were marked differences in the genes both up and down-regulated despite the fact that both species share a high number of orthologous sequences, suggesting that subtle differences in phospholipid metabolism may play a role in pathogenicity.

O130

Meiotic recombination in sexual progeny of *Aspergillus fumigatus*

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Introduction: Invasive aspergillosis (IA), mainly caused by *Aspergillus fumigatus*, is a rapidly progressive disease in immunocompromised patients, with a mortality rate as high as 85%. For 145 years *A. fumigatus* was only known to reproduce asexually. Recently, however, the sexual cycle was discovered. To gain insight into the genotypic evolution of *A. fumigatus*, we genotyped parental isolates and progeny from different sexual crosses of *A. fumigatus*.

Methods: Sexual crosses were set up using six environmental *A. fumigatus* isolates (AfRB2 x AfIR956, AfIR957 x AfIR928, AfIR964 x AfIR974) and 15 of the resulting progeny were isolated from each cross (O'Gorman et al. Nature. 2009;457:471-4). Analysis of the mating type and RAPD genotypes of the progeny was performed previously. In this work, the cell-surface protein (CSP)-type and genotype of six microsatellite markers was determined.

Results: The progeny appeared to consist of a large variety of genetic types, although all genetic markers examined were derived from the parents. The parents' CSP types were present in either a 40%-60% distribution or a 47%-53% distribution in the progeny. For each of the microsatellite markers separately, the distribution of the

two parental genotypes varied from 27%-73% to 47%-53%. Combining all six microsatellite markers together, between 53%-80% of progeny showed unique genotypes, with 0%-27% of progeny identical to one of the parents. Combining all twelve markers (six microsatellites, CSP, mating type and four RAPD markers) resulted in a unique genotype for 93%-100% of the progeny. There was only one exception, with one of the progeny appearing identical to one of the parents for all of the markers tested.
Conclusion: Our results show that sexual reproduction in *A. fumigatus* generates progeny with a large variety of new genotypes. Although no new genotypes arose for each of the twelve markers separately, through chromosomal segregation and meiotic crossover new combinations of the parental genotypes were created, resulting in a heterogeneous offspring population. This extensive genetic variation clearly demonstrates one of the many advantages of sexual reproduction over asexual reproduction.

O131

Azole-resistance in *Aspergillus fumigatus*: collateral damage of fungicide use?

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Introduction: Since the year 2000 the emerge of multi-azole-resistance in *A. fumigatus* isolates has been reported in clinical isolates containing the TR/L98H mutations in the *cyp*51A gene. Is has been postulated that the TR/L98H mutations have been introduced in *A. fumigatus* in the environment due to the agricultural use of azole fungicides.
Methods: All licensed azole fungicides between 1970 and 2010 were purchased and tested for *in vitro* activity against azole susceptible and azole resistant TR/L98H mutated *A. fumigatus* isolates. Docking studies were performed by using a CYP51A homology model to determine similarities in docking of medical and fungicide azole compounds. Microsatellite typing was used for genotyping the TR/L98H isolates and subsequently used for evolutionary study to determine the origin of the TR/L98H mutations in the *A. fumigatus* population.
Results: Five out of all 30 licensed Dutch azole fungicides show cross-resistance against the TR/L98H isolates, from which four fungicides also showed the highest similarity in docking-studies when compared with the medical azoles. The four agricultural fungicides that show cross-resistance have been introduced between 1990-1996. Analysis of microsatellite data showed that the TR/L98H mutated isolates have been introduced in the Dutch *A. fumigatus* population around the year 1997 (95% CI:1993.7-1999.7).
Conclusion: By providing the link between fungicide use and TR/L98H multi-azole resistance *A. fumigatus* a route of azole resistance development is given which could not be explained by the medically known resistance development within patients. Wind-dispersion of fungal spores and high levels of azole compounds will provide perfect conditions for further spread of TR/L98H azole resistant *A. fumigatus* isolates. As already observed, global spread of azole resistance in *A. fumigatus* can be anticipated.

O132

A cosmopolitan *Burkholderia terrae* equipped as universal migrator along different fungal hyphae

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In 2008, a *Burkholderia terrae* like bacterium, strain BS001, was reported to be consistently found in mycosphere soil i.e. the soil adjacent to the mushroom foot of Laccaria proxima, whereas this organism could not be cultured at all from the bulk soil nearby.
In this study, we prepared bacterial inocula from a range of Dutch soils and introduced these with the exogenously-colonizing fungus Lyophyllum sp strain Karsten into different presterilized soils, allowing the fungus to carry some of the bacteria along its hyphae. By sampling a distant place from the inoculation, we were able to culture different bacteria including many that were simlilar to *Burkholderia terrae* BS001. This yielded a collection of 27 strains akin to the original *Burkholderia terrae* BS001 obtained from a range of soils. This provides a glimpse that this organism could be a cosmopolitan bacterium. We also found these strains to be able to migrate through different soils along different fungal hyphae. Most, but not all, of the strains in the BS001-like group harboured a type-three secretion system which is assumed to contribute greatly to make this organism a cosmopolitan and universal migrator along mycellial network. The occurrence of such organisms in varying soils allows insight in the tight bacterial-fungal interactions in soil in diverse environmental conditions. It was found that BS001 is capable to produce a biofilm around and migrate along with hyphae of different fungi. We also found that strain BS001 assists the fungus in its defense against antagonists. Moreover, by associating with migrating soil fungi, BS001 also helps other bacteria to migrate with, and get benefits from, the fungus.
Whole-genome sequence information was obtained, and this marvelous organism was shown to have an extremely large genome size, with 1-2 copies of the TTSS. Further

study of the genome as well as gene expression will allow us to unravel the mechanisms that make these bacterial-fungal interactions successful in nature. Understanding the behaviour of the BS001-like group will further extend our insight in the role of these soil bacteria in accessing nutrients, degradation processes and the biological control of different diseases.

O133

Daqu – a fermentation starter for Chinese liquor fermentation

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Introduction: Chinese liquor (Baijiu in Chinese) is distilled from alcohol produced from cooked sorghum by solid-state fermentation. For the alcoholic fermentation, a starter '*Daqu*' is used which serves as a source of microbial inoculum, microbial enzymes, and flavour components. *Daqu* exists in several categories, dedicated for specific flavours of liquor. In our joint research, we investigated the eco-physiology of the microbiota in one type of light-flavour *Daqu* named '*Fen – Daqu*'.

Methods: We used a combination of culture-dependent and culture-independent approaches and studied the metabolome by non-targeted ^{1}H-NMR (proton-Nuclear Magnetic Resonance) analysis.

Results: From a library of isolates among 109 bacteria *Brevibacterium* sp., *Bacillus licheniformis*, *Bacillus pumilus*, *Bacillus subtilis*, *Leuconostoc citreum*, *Pediococcus pentosaceus*, *Pseudomonas aeruginosa* and *Lactobacillus plantarum* predominated. Of 81 isolated fungi, the yeasts *Saccharomycopsis fibuligera*, *Pichia anomala*, *Issatchenkia orientalis*, and *Saccharomyces cerevesiae* and the filamentous genera *Absidia*, *Aspergillus*, *Mucor*, *Rhizopus*, *Rhizomucor* and *Penicillium* were encountered. AFLP analysis of the yeast isolates revealed a considerable diversity among the strains of *Pichia anomala* and *Issatchenkia orientalis*. Principal component analysis of ^{1}H-NMR metabolome data of stages representing progressing incubation of *Daqu* revealed clear separation of the samples obtained from different incubation stages. The major compounds that contributed to discrimination were acetate/alanine, arginine, ascorbate, betaine, choline, ethanol, fructose, galactose, glucose, glucitol, glycerate, lactate, maltose, mannitol, phenylalanine, proline, propylene glycol, threonine and tryptophan. These metabolites were regarded as the representative metabolites or biomarkers characteristic for each incubation stage and were related with microbiological changes of importance for quality control in *Fen-Daqu* production.

Conclusions:

1. Different types of *Daqu* could be distinguished by culture-independent DNA-PCR-DGGE (Denaturing Gradient Gel Electrophoresis) profiling, as well as by PCA (Principal Component Analysis) of ^{1}H-NMR data. This is of relevance for the authenticity (AOC: Appelation of Specified Origin) of specific *Daqu* types.
2. *Fen-Daqu* is obtained after a microbial succession in which bacteria, yeasts and filamentous fungi colonize the substrate. Simultaneously, the changes taking place in the metabolome are clearly distinguishable. This will enable further investigation of the impact of specific microbes on targeted metabolic markers which are specific for *Fen-Daqu*.

O134

Profiling of global interactions between human serum proteins and the *Staphylococcus aureus* cell surface

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Introduction: The opportunistic pathogen *Staphylococcus aureus* is a renowned causative agent of severe invasive diseases. Once *S. aureus* has entered the blood stream, it can infect almost every organ system in the human body. This involves direct interactions between bacteria and host proteins, for example for immune evasion and adhesion to tissues. While interactions between staphylococcal cells and a limited number of host proteins have been studied in depth, global analyses to profile staphylococcal-host factor interactions were so far lacking. In the present studies, we explored cell surface shaving' with trypsin and mass spectrometric identification of liberated peptides as a tool to profile the binding of human serum proteins to *S. aureus*.

Methods: Cells of *S. aureus* strains USA300 and Newman were cultured *in vitro*, washed, resuspended in fresh human serum and incubated at room temperature for 15 min. Human serum without bacteria was treated in the same way and used as a control for unspecific binding of serum components to the reaction vessels. Thereafter, the samples were centrifuged to pellet the bacterial cells, washed, and subjected to shaving with immobilized trypsin as previously published (Dreisbach et al. Proteomics. 2010;10(17):3082-96). Peptides obtained from shaving were analyzed by LC-MS/MS.

Results: The shaving of *S. aureus* cells incubated in human serum resulted in the identification of several components

of the complement system, the platelet factor 4, and the isoform 1 of the inter-α-trypsin inhibitor heavy chain H4 on the staphylococcal cell surface. Furthermore, in our negative control experiments, we identified 32 human serum proteins with a high propensity for binding to nave polypropylene.

Conclusions: The present studies provide important proof-of-principle that the surface shaving approach can be applied for the profiling of human serum proteins that bind to the S. aureus cell surface. We are convinced that applications for this approach are not limited to studying the adherence of serum proteins to bacterial surfaces, but that it can also be applied to study bacterial interactions with proteins in other body fluids or samples containing solubilized human cell envelope proteins. The surface shaving technique thus seems a versatile generally applicable tool for monitoring bacteria-host interactions. Likewise, surface shaving can be applied to profile the binding of human proteins to biomaterials as is underscored by our negative controls. It thus seems that the shaving of surface-attached proteins opens up new avenues for studies on the interactions of bacterial and human proteins with each other, and with plastics and other materials that are commonly used in medical implants.

O135

Identification of genes essential for *Moraxella catarrhalis* survival under iron-limiting conditions

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Background: *Moraxella catarrhalis* is an emerging human-restricted respiratory tract pathogen that is a common cause of childhood otitis media and exacerbations of chronic obstructive pulmonary disease in adults. Successful colonization of the human respiratory tract mucosa by *M. catarrhalis* depends on its ability to acquire essential iron. Iron is a key nutrient that is not freely available *in vivo*, where it is complexed with host iron-binding proteins such as lactoferrin and haem. To obtain iron in this iron-limited environment, *M. catarrhalis* expresses specialized iron-acquisition factors that interact with host iron-binding proteins. To extend our knowledge of iron metabolism in *M. catarrhalis*, we used the genomic array footprinting (GAF) technology, a high throughput genome-wide negative selection screen, to identify *M. catarrhalis* genes essential for survival under iron-limiting conditions.

Methods: *M. catarrhalis* RH4 mariner transposon mutant libraries of ~28,000 mutants were either grown in brain heart infusion (BHI) broth pretreated with the iron-chelating agent Desferal (challenge condtion) or grown in untreated BHI (control condition). Chromosomal DNA isolated from both conditions was used to generate mutant-specific DNA probes. Mutants that failed to survive under iron-limiting conditions were subsequently identified by differential hybridization (challenge versus control) of mutant-specific probes to custom designed NimbleGen microarrays. For validation of identified targets, directed gene deletion mutants were generated and tested individually under iron-limiting conditions.

Results: In total, five genes were identified as being essential for survival under iron-limiting conditions (i.e. the corresponding mutants were negatively selection from the population). Mutants of the *yggW* gene, encoding the oxygen-independent coproporphyrinogen-III oxidase predicted to be involved in haem biosynthesis, were most severely attenuated. Further, genes involved in RNA maturation and degradation were identified as being essential for survival under iron-limiting conditions. The genes encoding known specialized *M. catarrhalis* iron-acquisition factors were not among the identified genes, which is most likely due to the high redundancy in iron-transport mechanisms in *M. catarrhalis*. Finally, growth of four directed mutants selected for validation was significantly diminished compared to wild-type under iron-limiting conditions, confirming observed GAF phenotypes.

Conclusions: Our results clearly show the applicability of GAF to identify conditionally essential genes of *M. catarrhalis*, exemplified by the validation experiments using individual gene deletion mutants. Currently, the biological role and importance of identified gene candidates for *M. catarrhalis* iron metabolism are under investigation.

O136

Secretion of virulence factors in pathogenic mycobacteria: type VII substrates are recognized by multiple secretion signals

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Pathogenic mycobacteria such as *Mycobacterium tuberculosis* and the fish pathogen *Mycobacterium marinum* utilize specialized type VII protein secretion systems (T7SSs) to secrete virulence factors. One of the T7SSs, ESX-5, is responsible for secretion of PE and PPE proteins to the bacterial cell surface and culture supernatant. PE and PPE proteins are unique for mycobacteria, and although their precise function is unknown, the particularly high numbers found in pathogenic species suggest that these proteins are associated with mycobacterial pathogenesis.

Since recent evidence support this hypothesis, it is crucial to understand how these proteins are secreted. Therefore, we aim our research at identifying the secretion signal that targets PE and PPE proteins to ESX-5.

In our work we first studied the model substrates PE25 and PPE41. These two proteins are encoded by a single operon and form a dimer of which the structure previously has been determined. Here we show that PE25 and PPE41 are secreted as a heterodimer by ESX-5. By systematically generating small deletions in the N- and C-termini of both PE25 and PPE41, we subsequently showed that a small deletion in the extreme C-terminus of PE25 abolished secretion of the heterodimeric complex. We generated a range of single amino acid substitutions within this secretion signal, and could identify residues crucial for secretion. Subsequently, we showed that these residues are also important for targeting another PE protein, LipY, to ESX-5. Next, we focused on PE/PPE proteins that are not secreted via ESX-5. Recently, our group identified two PE and PPE proteins that are secreted via ESX-1, another T7SS. Deletion of the C-terminus of this PE protein abolished secretion of this protein via ESX-1, indicating that the secretion signal is a general feature of T7SS. To investigate if this signal also determines the secretion system specificity, we exchanged the C-terminal end of the ESX-1 PE with that of the ESX-5 PE protein. To our surprise, exchange of these C-terminal sequences allowed secretion of the ESX-1 PE protein, but did not alter its secretion route.

In conclusion, our data suggests that PE proteins are targeted to different T7SSs via a shared C-terminal secretion signal, but additional signal(s) must be present that determine specificity for the T7SSs. Experiments are underway in our laboratory to determine which domain/residues are responsible for this.

O137

Staphylococcal secreted protease aureolysin mediates immune evasion by cleaving complement C3

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Introduction: Complement is one of the first barriers against bacteria: it effectively opsonizes bacteria with C3b molecules for clearance by phagocytosis and, at the same time, generates C5a for chemotaxis and activation of phagocytes. The human pathogen *S. aureus* successfully evades the complement system by 3 different strategies: i) binding of host molecules thereby covering its surface to prevent recognition, ii) attraction of host immune regulators, which dampens the immune response, iii) secretion of proteins that can target host immune molecules, thereby preventing their action. Of the latter many examples exist, such as for group A streptococci it has been demonstrated that secreted proteases have specific immune evading properties. There are 12 proteolytic enzymes secreted by *S. aureus*. However their influence on the host immune system is still poorly understood. To get more insight into *S. aureus* complement evasion, we investigated the role of these proteases in complement evasion and identified the metalloprotease aureolysin as a potent complement inhibitor that promotes escape of *S. aureus* by the immune system.

Methods: Phagocytosis and killing. Neutrophils were incubated with bacteria, human serum and BSA, rSCIN, and aureolysin. Phagocytic uptake was determined by flow cytometry and killing by counting the CFU. C3b deposition and C5a generation. *S. aureus* was incubated with human serum and BSA, rSCIN, and aureolysin. Bacterial supernatants were used to measure C5a generation by measuring activation of Fluo-4-AM labelled neutrophils by flow cytometry. C3b deposition on bacteria was detected by specific antibodies and flow cytometry. Complement ELISA. ELISA plates were coated with IgM, Mannan, or LPS and incubated with human serum and BSA, rSCIN, and Aureolysin. C3b deposition was detected with specific antibodies. Gel electrophoresis and western blotting. C3 was incubated with aureolysin and subjected to SDS page electrophoresis for analysis by staining or C3-western blot.

Results: Phagocytosis assays showed that aureolysin effectively inhibits bacterial uptake by neutrophils; the level of inhibition was comparable to SCIN. Also, we observed that aureolysin prevents bacterial killing. We investigated the role of aureolysin in complement inhibition by incubating *S. aureus* with serum, which activates the complement system, thereby generating C5a and opsonization of C3b. Aureolysin inhibits the deposition of complement C3b on the bacterial surface and the release of C5a in the supernatant. Complement ELISAs showed that aureolysin blocks all complement pathways indicating C3 as a possible target. Finally, we showed by gel electrophoresis that aureolysin cleaves C3 into C3a and C3b, thereby enabling further degradation by serum components factor I and factor H. In addition we showed that the supernatant of the Aur-KO USA300 strain cannot cleave C3.

Conclusion:

- Aureolysin blocks the phagocytic uptake and killing of *S. aureus*.
- Aureolysin cleaves complement C3 into C3a and C3b thereby depleting C3 which prevents C3b deposition and C5a generation.

O138

Comparison of effectiveness of oral, nasal and subcutaneous infection routes of *Coxiella burnetii* in goats

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Introduction: The 2007 to 2009 Q fever outbreak in humans in the Netherlands is the largest well-documented outbreak of Q fever known. In this outbreak dairy goats and dairy sheep are considered to be the main source of human cases. Two years ahead of the Q fever outbreak in humans, Q fever already caused abortions in dairy goats and dairy sheep. Little is known about the infection route of Q fever in goats. In this study we compare, in an experimental set-up, the oral, nasal and subcutaneous infection routes of *C. burnetii*. For the oral and the nasal infection route two challenge doses were used.

Methods: Healthy pregnant Dutch dairy goats were used in this study. They originate from a farm without an abortion history and they were tested negative for Q fever by PCR and ELISA. The Dutch most predominant goat genotype strain of *C. burnetii* was used for challenge and the mouse infective dose (MID) was measured in a mouse experiment. Groups of two goats each were inoculated on day (D) 90 of gestation orally with 10^4 and 10^6 MID of *C. burnetii*, intranasally with 10^4 and 10^6 MID of *C. burnetii* and subcutaneously with 10^4 MID as positive controls. One negative control group was not inoculated. The goats were screened for abortion and for positivity of placenta and kids for *C. burnetii* by PCR. All animal experiments were approved by the Animal Ethics Committee and were done in BSL 3 facilities.

Results: *C. burnetii* could not be detected in the negative control animals, nor in their offspring. One negative control was euthanized on D 132 of gestation due to severe illness and one negative control kidded after full gestation. One positive control aborted on D 103 of gestation and *C. burnetii* could not be detected. One positive control aborted on D 152 of gestation; placenta and kids were highly positive for *C. burnetii*. One 10^4 oral' goat aborted on D 112 of gestation with placenta and one foetus tested doubtful and two foetuses tested negative. One 10^4 oral' goat was euthanized on D 153 of gestation with placenta and kid tested positive. One 10^6 oral' goat aborted on D 10^4 of gestation and tested negative in the placenta. One 10^6 oral' goat kidded on D 155 of gestation with lively kids which tested negative. One 10^4 nasal' goat aborted on D 139 of gestation with highly positive placenta and kid. In one 10^4 nasal' goat a mummified foetus was found on D 179 after mating and tested doubtful. The two '10^6 nasal' goats aborted on D 144 and D 145 of gestation, all placentas and kids were tested highly positive.

Conclusions:

1. The nasal route is more efficient compared to the oral route of infection with *C. burnetii* and is as efficient as the subcutaneous route
2. Administration of 10^4 or 10^6 MID did not show much difference in the effect of the infection of *C. burnetii*.

O139

Serum lipoproteins neutrolize the virulence of Staphylococcal Phenol-Soluble-Modulins

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Introduction: *Staphylococcus aureus* is a major cause of severe infections ranging from mild skin and wound infections to life-threatening disseminated infections. The virulence of *S. aureus* is mediated by a number of toxins and immune evasion molecules, including the recently discovered phenol soluble modulin (PSM) peptide toxins. Especially, PSM expression is associated with highly pathogenic community associated MRSA strains. Two major functions have been addressed to PSMs, they are cytolytic at micromolar concentrations and chemoattractive at nanomolar concentrations. Hypothesized is that these two functions complement each other, PSMs are actively produced to attract neutrophils to the site of infection and subsequently lyse them. The reproducibility for PSM mediated lyses has lead to some discussion in literature of the biological significance of PSM peptide toxins. We found that the main difference is the addition of serum in the assays for PSM mediated lysis. Therefore this study was conducted to investigate the influence of human serum on the biological functions of PSMs.

Methods: Human neutrophils were isolated from peripheral venous blood of healthy donors using a Ficoll/Histopaque gradient. Plasma lipoproteins (LDL and HDL) were separated by discontinuous density-gradient ultracentrifugation. Intracellular mobilization of calcium was measured by flow cytometry after neutrophil stimulation with PSMs. PSM mediated neutrophil lysis was measured by assaying LDH release of damaged cells.

Results: Addition of serum to our functional assays showed that both neutrophil activation and neutrophil lyses could be diminished by human serum. With size-exclusion chromatography of serum and isolated lipoproteins we showed that FITC-labeled PSMs associate with High density lipoproteins (HDL) and low density lipoproteins (LDL) in human serum. Furthermore with an immobilized PSM pull-down assay, we could isolate ApoA1(the major constituent of HDL) from human serum. In addition we showed that purified HDL and LDL could both inhibit

activation and lysis of neutrophils, whereas lipid free serum lost this inhibitory ability.

Conclusions: We can conclude from these data that PSM mediated virulence is greatly inhibited by lipoproteins from human serum, therefore the *in vivo* relevance of these PSMs should be reconsidered.

O140

Exploring the transcriptome and proteome of *Bordetella pertussis*, the causative agent of whooping cough

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Introduction: Pertussis, or whooping cough, is a highly contagious, acute respiratory disease of humans that is caused by the gram-negative bacterial pathogen *Bordetella pertussis*. In the face of extensive global vaccination, this extremely monomorphic pathogen has persisted and re-emerged, causing approximately 300,000 deaths each year. Using a large number of adhesins, toxins and immune modulating factors, *B. pertussis* is able to create a niche for colonization in the human respiratory tract. During infection, the transcription of nearly all virulence genes is controlled by a two-component regulatory system designated the *Bordetella* master virulence regulatory system (BvgASR). Here, we present a multifaceted approach of transcriptional profiling in combination with label-free semi-quantitative nano-liquid chromatography-mass spectrometry (LC-MS/MS) to identify novel Bvg-regulated genes, which are potential targets for future pertussis vaccines.

Methods: A globally circulating *B. pertussis* strain was grown *in vitro* under Bvg^+, Bvg_i, and Bvg^- conditions, after which RNA and protein was isolated. RNA samples were analyzed on a custom-designed Nimblegen microarray containing eight specific probes for each coding sequence (CDS) and pseudogene, as well as 15-bp overlapping tiling probes that cover both strands of the intergenic regions. The membrane proteins were separated from the cytosolic proteins and both fractions were subjected to in-solution tryptic digestion and subsequent label-free semi-quantitative LC-MS/MS analysis.

Results: Transcriptional profiling showed Bvg-dependent differences in gene expression. By combining high resolution tiling array data of *B. pertussis* intergenic regions with de novo sigma factor binding site predictions we were able to map the transcription start sites (TSS) of the differentially regulated genes. Furthermore, for Bvg-related genes, LC-MS/MS analysis identified the relation between transcript and protein levels. Thus, this combined approach enabled us to comprehensively analyze Bvg-regulated genes at both RNA and protein levels.

Conclusion:

1. By analyzing the transcriptional activity from the CDS and intergenic regions, it is possible to determine the TSS and the BvgA binding sites of the Bvg-regulated genes.
2. Proteomics is a powerful tool for the identification of novel Bvg-regulated genes.

O141

The DNA-binding and -cleavage activities of the *Mycoplasma genitalium* Holliday junction resolvase (RecU) are biochemically uncoupled

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Introduction: Recombination between repetitive DNA elements in the genomes of *Mycoplasma* species appears to lie at the basis of antigenic variation of several highly immunogenic surface proteins. To elucidate the mechanism underlying homologous recombination between these repetitive elements, we have initiated studies to unravel the DNA recombinatorial pathways in these bacteria. Previously, we reported that the *Mycoplasma genitalium* MG352 gene encodes a sequence-specific Holliday junction resolvase (RecU) that has a crucial function in homologous DNA recombination (Sluijter et al. Mol Microbiol. 2010;77:1261-77). The RecU protein was found to (i) bind four-way, four-stranded DNA substrates (or Holliday junctions [HJs]) in a DNA sequence-independent fashion, and (ii) cleave HJs at the sequence 5'-CTAG-3' in the presence of Mn^{2+}. Here, we present a comprehensive structure-function analysis of RecU, and show that the DNA-binding and -cleavage activities of the protein are biochemically uncoupled.

Methods: We generated a series of 16 deletion mutants (9 N- and 7 C-terminal) and 31 point mutants of RecU. The point mutations were introduced at amino acid positions that appeared to be highly conserved among bacterial RecU-like sequences. All mutants were purified to homogeneity, and tested for their ability to bind and cleave HJ substrates.

Results: We found the 5 N-terminal and the 3 C-terminal amino acids of RecU to be dispensable for its catalytic activities. In addition, 11 amino acids were found to be essential for both HJ-binding and -cleavage by RecU. Interestingly, in 10 point mutants these two activities were uncoupled; these proteins displayed similar HJ-binding characteristics as wild-type RecU, but were unable to cleave the HJ substrates. These data indicate that HJ-binding by RecU is necessary but not sufficient for HJ cleavage.

Conclusion: We have identified 11 amino acids within the *M. genitalium* RecU protein that are indispensable for

both the HJ DNA-binding and -cleavage activities of the protein. In addition, 10 other amino acids were found to be essential in the catalysis of HJ DNA cleavage, while they did not appear to affect the DNA-binding activity of RecU. As a consequence, the HJ DNA-binding activity of RecU is biochemically separated from its HJ cleavage activity.

O142

Varicella Zoster Virus and the Central Nervous System

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Varicella zoster virus (VZV) is an exclusively human, neurotropic herpesvirus that infects more than 90% of the world population. Primary infection causes varicella (chickenpox) after which virus establishes latency along the entire neuraxis, including the cranial nerve ganglia, dorsal root ganglia and autonomic ganglia. With a decline in VZV-specific cell-mediated immunity, VZV reactivates from one or more ganglia and travels centrally or peripherally to cause neurological disease. Populations at risk for virus reactivation include elderly, HIV, cancer, transplant and diabetic patients. The most common manifestation of VZV reactivation is herpes zoster (shingles), characterized by a unilateral, vesicular rash and pain restricted to 1-3 dermatomes. Herpes zoster can be complicated by postherpetic neuralgia, defined as persistent dermatomal distribution pain more than 3 months after zoster. VZV reactivation can also result in virus infection of cerebral arteries leading to vascular remodeling and ischemic or hemorrhagic stroke (VZV vasculopathy). While VZV vasculopathy was once considered rare, it has now emerged as a more common and significant complication of VZV reactivation with recent studies demonstrating that zoster increases the risk of stroke by 30% in adults within the following year and by 4.5 fold if zoster is in the ophthalmic distribution. Less frequently, VZV can cause spinal cord disease (myelopathy) through demyelination or infarction, blindness (retinal necrosis), cerebellitis and peripheral vascular disease. Since all the neurological complications of VZV can occur without a preceding rash, it is important to recognize the spectrum of disease caused by VZV reactivation to guide appropriate diagnostic studies and treatment.

O143

Vaccination against herpes zoster, pros and cons

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Herpes zoster (HZ) results from the reactivation of the latent varicella zoster virus (VZV). Clinically significant reactivation occurs when VZV-specific cell-mediated immunity (CMI) declines below a critical threshold. This occurs commonly with aging and diseases or therapies which affect immunity. In contrast to the primary VZV-infection (varicella), which is mainly a childhood disease, HZ predominantly affects older adults. HZ may cause substantial acute and chronic morbidity, the commonest long-term complication being persistent pain. This so-called postherpetic neuralgia (PHN), which can persist for several months to years, may severely impact a patient's quality of life. Because the results of PHN treatment are rather limited, prevention of HZ might be attractive.

Immunization of immunocompetent older adults with a live, attenuated Oka strain VZV vaccine safely increases VZV-CMI responses. A large double-blind, placebo-controlled trial among immunocompetent persons 60 years of age and older showed that a single vaccination markedly reduced the incidence of HZ. The burden of illness due to HZ decreased by 61% and the incidence of PHN by 67%. In a comparison between subjects aged <70 years and those aged >70 years, the vaccine was equally efficacious in reducing the incidence of PHN but was somewhat less effective in reducing the HZ burden of illness and preventing HZ in older subjects. The duration of the vaccines effectiveness, however, is unknown.

The potential effects and cost-effectiveness of programmatic HZ vaccination of elderly in the Netherlands have been assessed according to a framework that was developed to support evidence-based decision making regarding inclusion of new vaccines in the Dutch National Immunization Program. Due to the combination of waning immunity after vaccination and a reduced efficacy of vaccination at high ages, the most optimal cost-effectiveness ratio for HZ vaccination in the Netherlands was found for 70-year olds and just above the socially accepted threshold in the Netherlands of 20000 per QALY. If additional reduction of PHN was included, the cost-effectiveness ratio improved but uncertainty for this scenario is high. Therefore, vaccination against HZ at the age of 70 years seems marginally cost-effective in the Netherlands.

As part of a series of studies on vaccine acceptance, the determinants of compliance of community-dwelling elderly with HZ vaccination in an existing influenza vaccination program were assessed. General practitioners (GPs) sent out a questionnaire to elderly patients and offered them free HZ vaccination simultaneously with the yearly influenza vaccination. In all, 39% were vaccinated against HZ; 76% accepted influenza vaccination. Determinants of non-compliance with HZ vaccination were perceived lack of recommendation by the GP, unwillingness to comply with the doctor's advice, perception of low risk of contracting HZ, perception of short pain duration of HZ, and the opinion that vaccinations weaken one's natural defenses. The

same determinants were associated with non-compliance with both vaccinations, but objections in general towards vaccination, a high education and difficulties to visit GPs were also important. More data on (cost-)effectiveness might encourage GPs to offer HZ vaccination to their patients.

O144

Guideline varicella 2010 - what's new?

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In 2003 the first guideline on varicella was published by a multidisciplinary working group initiated by the Dutch Society of Microbiology (NVMM).

The availability of the varicella vaccine and the more frequent use of antivirals made a revision necessary. This revision was completed in December 2010 and will be published in the first half of 2011.

Compared to the first edition, the indications for serostatus determination of varicella zoster virus (VZV) are described more thoroughly e.g. in pregnancy the doctor or midwife should ask at the first visit to the varicella history and ask for serostatus, if the answer is doubtful or negative.

Furthermore clear indications are given for vaccination: 1) seronegative health care workers employed in Depts. where high risk patients are nursed, 2) siblings of pediatric high risk patients and 3) postexposure prophylaxis for immunocompetent persons after a contact. The committee did not give an advice for universal vaccination in the Netherlands. This because universal vaccination is the competence of the Health Council.

Finally guidelines are given for administration of varicella zoster immunoglobulin and for prescription of antivirals when the deadline of 96 hours is exceeded.

The Varicella Committee is an initiative of the NVMM and consists of representatives of the following Dutch societies:
Koninklijke Nederlandse Organisatie van Verloskundigen
Vereniging voor Infectieziekten
Nederlands Huisartsen Genootschap
Nederlandse Vereniging voor Arbeids- en Bedrijfsgeneeskunde
Nederlandse Vereniging voor Dermatologie en Venereologie
Nederlandse Vereniging voor Kindergeneeskunde
Nederlandse Vereniging voor Obstetrie en Gynaecologie

O145

Diagnosis of Neurological VZV Disease

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The spectrum of neurological disease caused when latent VZV reactivates includes herpes zoster, postherpetic neuralgia, zoster sine herpete, VZV vasculopathy, myelopathy and cerebellitis. Clinical recognition of these diseases, as well as verification by diagnostic tests, is essential for guiding therapy. The diagnosis of herpes zoster is straightforward, with identification of a characteristic unilateral, vesicular rash in a dermatomal distribution and if necessary, the detection of VZV DNA by PCR in vesicular fluid. The diagnosis of postherpetic neuralgia is also based on clinical history with residual pain more than 3 months following zoster in the same dermatome(s). In contrast, the diagnosis of zoster sine herpete (dermatomal distribution pain without history of rash), VZV vasculopathy, myelopathy, and cerebellitits is difficult since these neurological complications can all occur without rash. After clinical history is evaluated and when VZV reactivation is suspected, there are several tests to aid in diagnosis. The most useful test is detection of anti-VZV IgG in the cerebrospinal fluid (CSF) with an increased CSF to serum ratio when compared to total IgG or albumin. Studies of 30 patients with VZV vasculopathy revealed that detection of anti-VZV IgG antibody in the CSF was 96% sensitive in comparison to detection of VZV DNA in the CSF which was only 30% sensitive. However, since there are cases, especially early in infection where CSF does not contain anti-VZV IgG but does contain VZV DNA, we recommend that all patients with suspected neurological complications of VZV have CSF tested for both anti-VZV IgG antibody and VZV DNA.

O146

Compartmentalization of aciclovir-resistant Varicella Zoster Virus: implications for sampling in molecular diagnostics

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Introduction: Aciclovir resistance of varicella zoster virus (VZV) may arise in stem cell transplant (SCT) recipients with VZV disease and is usually a result of mutations in VZV thymidine kinase (TK) which is the target protein of aciclovir. Early detection of such mutations is necessary to enable timely therapy adaptation, e.g. to foscarnet. We aimed to investigate whether TK mutations arise over time, and what sample types might be the most useful for this method.

Methods: Spatially and temporally distinct samples from three SCT recipients with VZV disease unresponsive to aciclovir treatment were retrospectively investigated for the presence of TK mutations by PCR and sequence analysis.

Results: In all three patients, a mutation in the VZV TK coding region was found resulting in an amino acid substitution. TK mutations were not only temporally but

also spatially compartmentalized. In particular, plasma samples frequently showed wild-type TK sequences whereas cerebrospinal fluid or skin vesicle fluid acquired on the same day contained mutant sequences.
Conclusion: This study shows the importance of careful sampling for molecular diagnostics of aciclovir resistance in VZV disease: All affected body sites should be sampled and plasma samples may not be representative for the viral mutation status.

O147

How to keep varicella out of the hospital

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Varicella zoster virus (VZV) is a highly contagious virus. Primary infection with VZV causes chickenpox.
The virus belongs to the herpesvirus family and can cause life threatening infections especially in immunocompromised persons. Therefore this micro-organism should be kept out of the hospital. VZV is mainly introduced in the hospital by the following groups:
1. (other) patients with VZV
2. healthcare workers
3. visitors
4. students and trainees

Ad 1. Admitted patients with VZV may spread infections to other patients by contact and air. These patients need to be strictly isolated when admitted to the hospital. This implies that the patient's room has to fulfill certain requirements in relation to ventilation and air pressure. Furthermore, the department of infection prevention and hygiene should be notified to help reduce the risk of spread. When possible patients with VZV should be discharged from the hospital and elective surgical procedures should be postponed. Seronegative admitted patients with VZV contact need to be isolated from day 8 to day 19 after VZV contact.
It is advised to screen immunocompromised patients for VZV antibodies upon diagnosis. It is important to have this information at hand when unintentional VZV-contact has occurred and protective measures need to be taken.
Ad 2. Seronegative healthcare workers are a potential threat to immunocompromised persons when they contract VZV. Usually they are contagious 2 days before the outbreak of vesicles and in this stage may infect admitted patients for whom they have taken care. This holds especially true for workers on pediatric wards, where seroprevalence of VZV is low, and wards where immunocompromised patients are admitted. To reduce VZV introduction by healthcare-workers seronegative persons should be selected before signing their employment contract. When seronegative, they should be offered VZV vaccination. Upon refusal, they should not be allowed to work on wards where introduction of VZV may be dangerous to admitted patients.
Ad 3. In young visitors, especially children under 5, the seroprevalence is lower than in the adult population. As visitors these young children pose the highest risk on introducing VZV. In many hospitals visitors of young age are sparsely accepted. When sibs contact is needed for psychological reasons, screening for VZV status by asking a history of VZV is sufficient to reduce the risk of VZV introduction. Visitors with chickenpox should urgently be asked not to visit patients with impaired immunity due to their treatment.
Ad 4. The group of students and trainees within the hospital setting is increasing each year. More often, they are students of non-Dutch origin. In this group the seroprevalence is lower than in the Dutch population and the risk of introducing VZV is therefore higher. Furthermore, this group has no access to the hospital's health service (BGD) because the lack an employment contract. Hospitals with students should be aware of this fact and students should be questioned about VZV history and eventually serologically tested.

O148

Improvement of hand hygiene in hospital and health care settings

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Healthcare-associated infection occurs in every healthcare facility, in every country and affects hundreds of millions of patients annually worldwide. This global problem, associated with both sensitive and resistant strains of microorganisms, has received much attention from not only healthcare settings and governments, but also by the public and media.
Since the launch of the WHO First Global Patient Safety Challenge "Clean Care is Safer Care" in 2005, much has been achieved in healthcare settings around the world to improve infection prevention and control, including hand hygiene practices, with the aim to reduce healthcare associated infections. Hand hygiene has been recognised as the single most important measure in these attempts. The main output of the Challenge, the WHO Guidelines on Hand Hygiene in Health Care, includes a suite of tools to implement the recommended multimodal improvement strategy aimed at improving and sustaining hand hygiene. This model was developed within a clinical setting and further validated to ensure applicability to all healthcare settings worldwide, irrespective of resources available.

As part of the Challenge, over 140 countries have pledged their support to implement actions to reduce healthcare-associated infection, corresponding to almost 90% coverage of the world population. At least, 38 countries/regions have reported the existence of formal hand hygiene campaigns and WHO has formed a WHO *CleanHandsNet* to facilitate progress in such countries, as well as to share successes and strategies. In May 2009, WHO Patient Safety launched the *SAVE LIVES: Clean Your Hands* initiative to encourage healthcare workers to be part of a global movement to improve and sustain hand hygiene. By December 2010, over 12,000 healthcare facilities had registered their commitment to the initiative. The major challenge for the next decade will be to maintain the "snowball" effect and to show a significant impact on infection prevention across the world. To truly protect our patients, it will take leadership, commitment, a range of actions, and time. The efforts of WHO, together with countries and facilities, should help bring true ownership to healthcare workers in relation to microorganism transmission and its prevention and, subsequently, long-term patient safety improvement.

O149

Hand hygiene: when, why, how

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Outbreaks involving infected food workers in foodservice settings have resulted in many cases and deaths. Multiple-ingredient foods are most frequently associated with such outbreaks, probably because of frequent hand contact during preparation and serving. Many of the workers are asymptomatic shedders, are in contact with ill family members, diaper changing, handle raw foods of animal origin, or use improper hygienic practices, and their hands can carry billions of enteric bacteria or viruses (up to 10^9). Thus, multiple barriers to limit transfer of pathogens to foods are essential. These include the use of utensils, gloves, and proper hand hygiene. However, gloves have limitations and may become a source of contamination if they are punctured or improperly used. Most importantly, glove use can create a false sense of security resulting in more high risk behavior that can lead to cross-contamination, especially if employees are not adequately trained. The most convenient and efficient way of removing pathogens from hands is through handwashing. Important components of handwashing include potable water for rinsing, and soaps to loosen microbes from the skin, followed by drying. Handwashing should occur after any activity that soils hands and certainly before preparing, serving, or eating food. Antimicrobial soaps are only marginally more effective than plain soaps at any one wash, but constant use with these soaps results in a build-up of the antimicrobial compound on the skin. The time taken to wash hands and the degree of friction generated during lathering are more important elements for removing soil and microorganisms than water temperature. However, excessive washing and scrubbing can cause skin damage and infections. Drying hands with a towel removes pathogens first by friction during rubbing with the drying material, and then by wicking away the moisture into that material. Paper rather than cloth towels should be encouraged, although single use cloth towels are present in the washrooms of higher class hotels and restaurants. Warm-air dryers remove moisture from hands by evaporation and any surface microorganisms loosened by washing and vigorously rubbing the hands together; however, these take too long for efficient use. The newer high-speed air-blades can achieve dryness in 10-15 s without hand rubbing. An effective hand wash should remove most transient and some resident microorganisms and is typically facilitated by the use of soaps, detergents and antimicrobial compounds. However, hands are never sterile and can become recontaminated if they come into contact with a contaminated surface or infected person as soon as the washing process is over. The overall efficacy of hand hygiene depends on many factors including soil types, antimicrobial soap strength or alcohol gel composition. In the food industry, alcohol-based antiseptics should be combined with regular washing of hands and should not replace handwashing and drying, or use of fingernail brushes. Wipes containing alcohol and antimicrobial compounds, as well as moisturizers, wetting compounds, etc., are widely used by the public. These have been shown to be more effective than plain soap and water, and should be considered as a feasible, practical hand hygiene intervention for remote foodservice situations or where water availability is limited. Unfortunately compliance for handwashing in health care settings, food operations and in the general society is not good, and a targeted management plan for hand sanitation in specific food operations needs to be developed and enforced as a part of the corporate culture of the company.

O150

The virucidal efficacy of hand disinfectants

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Human noroviruses (NoVs) are the leading cause of nonbacterial acute gastroenteritis of all age groups. Human NoV is responsible for a number of outbreaks in hospitals,

nursing homes and households. The transmission of virus through person to person contact, food borne and waterborne transmission and indirectly through fomites have been documented. The incidence of health care associated infections, have caused numbers of outbreaks in the hospitals. The control of hospital acquired infection is a major challenge. Nosocomial pathogens are mostly transmitted via the hands of health-care workers and hand hygiene is considered the leading preventive measure to reduce cross-transmission in health-care settings. Soap based and ethanol based hand disinfectant have been shown to be effective against bacteria, fungi and enveloped viruses. The nonenveloped viruses remain the challenge as the virucidal activity against these viruses varies widely. We determined the virucidal efficacy of hand soap and alcohol based hand disinfectants against poliovirus Sabin 1, adenovirus type 5, parechovirus 1 and murine norovirus (surrogate for human norovirus) in a quantitative carrier test. The hand soap tested in-use-concentration showed less than 1log10 infectivity reduction within 1 min of exposure time. The alcohol (60%) hand disinfectant tested showed no significant reduction in infectivity of the tested viruses. The tested hand soap and alcohol hand disinfectants do not posses antiviral activity, therefore physical removal of viruses by hand washing using running water, soap and disposable towels remains the recommendable practice when dealing with infections or outbreaks.

O157

Multivariate approach for detecting interactions between of environmental parameters and composition of microbial communities

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Introduction: To understand the role of the local microflora in pathogenesis of respiratory and invasive diseases, it is crucial to study the effects of environmental and other epidemiological parameters on the composition of this complex microbial community. So far only univariate analysis methods have been applied. However we expect shaping of microflora to depend on multiple factors in parallel. Our aim was to develop a multivariate analysis tool for determinants of microflora profiles.

Methods: We developed a multivariate method in two steps. First, we selected epidemiological explanatory variables that were likely to correlate with the microflora profiles; second, we performed multivariate analysis combined with a linear fitted model to detect joint effects of the selected parameters. The effect per parameter was then expressed in a relative risk ratio.

Results: We tested this method on a complex dataset containing epidemiological parameters and 454-pyrosequencing results of 96 nasopharyngeal samples of 18-months-old children. With the unary Mann-Whitney-U test, we observed a highly significant correlation between season and microflora profiles only. With our multivariate model we observed additional correlations for daycare attendance, sex, and feeding type.

Conclusions: This multivariate method can more accurately detect parameters of microflora profiles and allows for more complex analyses and predictions by including joint effects of multiple determinants.

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O158

Curli fimbriae and cellulose production are influenced by environmental conditions and affect biofilm formation of *Salmonella enterica* subsp. enterica serovar typhimurium

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Salmonella is the causative agent of salmonellosis. *Salmonella* is frequently encountered in food-processing environments, leading to contamination of food products. *Salmonella* is able to adhere to surfaces and subsequently form biofilms. A biofilm is a population of bacterial cells attached to a surface, embedded in an extracellular matrix. Biofilms cause problems in both industrial and medial settings, since they are generally hard to eradicate due to increased resistance against disinfection treatments or antibiotics. It is expected that the extracellular matrix contributes to the increased resistance of biofilms. Earlier studies have shown that curli fimbriae and cellulose are important components of the extracellular matrix of *Salmonella* biofilms. Transcription of the genes involved in curli fimbriae and cellulose production are positively regulated by the regulator CsgD. The expression of *csgD* is influenced by environmental signals. Thus, environmental conditions might influence the production of curli fimbriae and cellulose, and the question arises whether differences in biofilm forming capacity could be linked to differences in the activation of these components, and whether this can be linked to the origin of isolation.

In this study, we analyzed the biofilm forming capacity of 51 *S. Typhimurium* strains from different origin in TSB

and 1/20 TSB at 25 °C and 37 °C, using the CV assay. The strain collection could be divided in three different groups. Group A strains, mainly clinical, outbreak-associated and retail product isolates, produced dense biofilms in both media at 25 °C, and in TSB also at 37 °C. Group B strains, mainly industrial isolates, only formed dense biofilms in 1/20 TSB at 25 °C, and group C strains showed little to no biofilm formation. Combining the results of the CV assay with enumeration of biofilm cells, suggests that biofilm formation as determined by the CV assay is dependent on both the number of biofilm cells and the extracellular matrix. Noticeably, the contribution of both factors is variable between different environmental conditions, with specifically biofilms cultured at 25 °C in 1/20TSB showing distinct composition and morphology. Therefore the contribution of the matrix components curli fimbriae and cellulose to biofilm formation was further assessed. The expression of genes encoding for the transcriptional activator CsgD, the major curli structural subunit CsgA and the post transcriptional regulator of cellulose production AdrA, was analyzed under different culture conditions in planktonic and biofilms cells, using quantitative real-time PCR. Our results showed that *csgA*, *csgD* and *adrA* are particularly induced during biofilm formation at 25 °C in 1/20 TSB. In addition, microscopic images of biofilms grown in 1/20 TSB at 25 °C showed high levels of calcofluor staining, which is an indicator for cellulose production, while only low levels of calcofluor staining was observed in biofilms grown in TSB.

In conclusion, we demonstrated that biofilm forming behavior is affected by environmental conditions and strains origin. In addition, it was shown that curli fimbriae and cellulose contribute specifically to biofilm production under low nutrient conditions at ambient temperatures and that other components are conceivably more important during biofilm formation at 37 °C and/or in nutrient-rich conditions by the group A strains.

O159

Interactions amongst marine archaeal and bacterial nitrifiers and anammox bacteria under oxygen limitation in a lab-scaled model system

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Introduction: Anaerobic ammonia oxidation is a newly discovered process, which may play an important role in the marine nitrogen cycle. In marine oxygen minimum zones (OMZs), the nitrite required for anammox bacteria needs to be provided by either partial nitrification (aerobic ammonia oxidization) or via partial denitrification (nitrate reduction). Since aerobic ammonia-oxidizing archaea (AOA) are highly abundant and co-exist with anammox bacteria in marine OMZs, it is possible that AOA rather than marine ammonia-oxidizing bacteria (AOB) may provide nitrite to marine anammox bacteria. In addition, AOA and AOB may have to compete for their substrates ammonium and oxygen. The present study explored the possibility of cooperation between marine anammox bacteria and nitrifiers as well as the competition between AOA and AOB under oxygen limitation and varying ammonium concentrations in a lab-scale model system.

Methods: A bioreactor containing marine anammox bacteria was supplemented with AOA (Nitrosopumilus maritimus) cells. Oxygen was carefully introduced to ensure growth of both the introduced and indigenous nitrifiers under oxygen limited conditions. A stable culture of AOA, AOB and anammox bacteria was established. Changes in activities of the different functional groups (AOA, AOB and anammox bacteria) were monitored by batch incubations and oxygen respiration measurements. Changes in the community composition were monitored by real-time qPCR of functional genes. Furthermore total RNA was extracted, reverse transcribed and the cDNA sequenced by Illumina technology. The culture was further characterized with respect to lipid content and cellular ultrastructure.

Results: Due to rapid oxygen consumption by AOA and AOB, anammox activity was not inhibited by the introduction of oxygen. Induced expression of ammonium uptake genes was observed for all community members which may be accounted for by the increased competition for ammonium under oxygen limitation. A C18 ladderane fatty acid became highly dominant in the Scalindua anammox bacteria-dominated culture, which was never observed before and may be caused by oxygen exposure. When the residual ammonium concentration was relatively high (more than 300 M), amoA gene copy numbers of indigenous AOB increased and were higher than those of the AOA, but as soon as the residual ammonium concentration decreased (less than 30 M), the amoA copy numbers of AOA increased. The community composition of the culture then remained quite stable with almost equal numbers of AOA and AOB amoA copy numbers. This observation was further confirmed by sequencing mRNA and inhibition activity analyses.

Conclusion: This study is the first direct proof that AOA can provide nitrite to anammox, and this AOA-associated CANON (Completely Autotrophic Nitrogen Remocal Over Nitrite) system might be a new candidate in future treatment of polluted marine waters.

O160

How models can help to understand and improve the production of protein based bio-ingredients by the lactic acid bacterium *Lactococcus lactis*

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Introduction: Both consumers and industry currently show an increased awareness of healthy and tasty foods. In fermented dairy products lactic acid bacteria (LAB) play an important role in production of bio-ingredients that contribute to both aspects. With a focus on protein metabolism knowledge on balanced degradation of e.g. casein, the formation and subsequent breakdown of peptides, is important in order to (i) produce bioactive peptides that have health beneficial effects and (ii) prevent accumulation of peptides that contribute to a bitter and undesirable taste. Moreover, the proteolysis and peptidolysis of proteins is prerequisite to generate free amino acids, which is the main pathway to flavour formation in cheese manufacture. The hydrolysis of -casein by the cell envelope located PrtPI proteinase of *Lactococcus lactis,* however, is a complex process in which the enzyme is able to cleave multiple peptide bonds. A good mathematical description of the degradation of intact protein is a key step in the understanding of the dynamics of this process.
Methods: To study the extracellular hydrolysis of -casein, without the interference of uptake and degradation of released peptides, *Lactococcus lactis* IM17pLP712 was used. This mutant strain lacks the oligopeptide transport system. Purified bovine -casein was hydrolyzed in a buffer system using non-growing cells and two experimental scenarios: (i) constant enzyme concentration and (ii) constant initial enzyme/substrate ratio. Hydrolysis of the intact -casein was monitored by RP-UPLC.
The experimental data were used to estimate the parameters of four candidate kinetic models.
Results and conclusion: The degradation characteristics of -casein demonstrated that the hydrolysis of intact protein becomes slower at higher initial concentrations. At e.g. an initial concentration of 17.6 M the enzyme had degraded 87% of the intact protein after 30 min. whereas at an initial concentration of 29.7 M -casein, only 60% was degraded. Such a relationship may be caused by a decrease in the accessibility (e.g. micelle formation) of the protein and competition for the active sites of the enzyme.
The obtained data were used to estimate the parameters of four candidate kinetic models: First-order kinetics, n^{th}-order kinetics, Michaelis-Menten kinetics, competitive inhibition kinetics. As the hydrolysis rate was affected by the initial protein concentration, the models were modified to take this relationship into account.
Based on the fit to the experimental data, the modified competitive inhibition model was selected as the best one to describe the hydrolysis of intact -casein by *Lactococcus lactis* cells in a broad range of experimental conditions.

O161

Identification of biochemically inert non-fermentative bacteria derived from cystic fibrosis patients by matrix-assisted laser ionization/desorption time-of-flight mass spectrometry (MALDI-TOF MS)

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Introduction: Sputum cultures from patients with cystic fibrosis (CF) frequently contain 'uncommon' bacteria, mostly non-fermentative gram-negatives. Since these bacteria are rather inert, standard biochemical tests are not useful for genus and species identification. Our routine laboratory, situated in a hospital with a large population of CF patients, frequently encounters problems correctly identifying these bacteria to the genus and species level. Identification by our reference laboratory is expensive and time-consuming. It is known that MALDI-TOF MS can correctly identify mucoid non-fermentative bacteria that are biochemically active. It is unknown whether biochemically inert strains can also be identified by this technique. The aim of this study was to compare MALDI-TOF MS with the results from the reference laboratory for these inert non-fermentative gram-negative bacteria.
Methods: A search in the laboratory database was performed. All clinical isolates collected between 01-01-2007 and 01-06-2010 that met the following criteria were included: derived from sputum sample of CF-patient, identification by reference laboratory and biochemically inert. Biochemically inert was defined as at least acetamide, DNAse and lysine negative after 24 hours incubation and no growth on C390/phenatroline agar. From 78 selected isolates, 21 were not available for the study since they were no longer present in the strain collection (11), could not be cultured anymore (4) or duplicate organisms have been cultures from the same sample (4). All remaining 57 isolates were identified in duplicate by MALDI-TOF MS according to manufacturer's instructions. The laboratory technician was blinded to the identification from the reference laboratory based on a combination of biochemical, fatty acid and 16S sequencing analysis.

Results: Compared to the reference laboratory, fifty-two isolates (91.2%) were correctly identified to the genus level and 5 (8.8%) had no identification (*Bordetella* species 2x, *Chryseobacterium* species, *Neisseria animaloris* and *Nocardia farcinica*); there were no incorrect genus identifications. The reference laboratory could identify only 50 isolates to the species level. Of these 50 isolates, 41 (82%) were correctly identified by MALDI-TOF MS, 4 (8.0%) had incorrect species identification, 5 (10.0%) had no species identification. The four incorrect species identifications were: *Pseudomonas monteilli* vs. *P. putida* (3x), *Achromobacter ruhlandii* vs. *A. xylosoxidans*. It should be noted that *P. monteilli* belongs to the *P. putida* group. For *Burkholderia* species, all 11 isolates were correctly identified to *B. cepacia* complex, but within this complex 2 isolates were incorrectly identified: *B. stabilis* vs. *B. cenocepacia* and *B. multivorans* vs. *B. cepacia*.
Conclusion: MALDI-TOF MS can be applied for identification of inert non-fermentative gram-negative bacilli, although some strains can only be identified to *Pseudomonas putida* group and *Burkholderia cepacia* complex.

O162

Quantitative mass spectrometry reveals new therapeutic targets against the human fungal pathogen *Candida albicans*

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The polymorphic fungus *Candida albicans* has a major impact on public health. Its ability to switch from the yeast form to a filamentous morphotype is a major part of its virulence strategy. The cell wall and especially the proteins that are covalently anchored to it are the first site of host-pathogen interaction. Some promising targets for the development of diagnostic biomarkers (e.g. the mannoprotein Mp65) and vaccines have already been identified (the adhesins Als1, Als3, and Hwp1; Hyr1). To identify additional targets we decided to relatively quantify the effect of the yeast-to-hypha transition on the *C. albicans* wall proteome. To this end, we selected three representative methods to induce hyphal formation either by adding the amino sugar GlcNAc (N-acetyl-glucosamine) or 10% Fetal Calf Serum (FCS) to the growth medium YNB-S (Yeast Nitrogen Base supplemented with 2% sucrose as carbon source) or by using the mammalian cell culture medium IMDM-S (Iscove's Modified Dulbecco's Medium). Our relative quantification approach was based on Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FT-ICR-MS) and ^{15}N-metabolic labeling. This method allowed accurate relative quantification of 22 wall proteins of the reference strain SC5314 by comparing ^{14}N/^{15}N peptide pairs per protein. We could identify strongly hypha-associated proteins (Als3, Hwp2, Hyr1, Plb5, Sod5) wall proteins that confer increased adhesion and resistance to host defenses while strongly yeast-associated proteins (Rhd3, Sod4, Ywp1) are more important for host dispersal and immune evasion. We also identified invariant, and therefore morphotype-independent wall proteins that mainly serve housekeeping functions (e.g. the Chitinase Cht2, the Transglycosylases Crh11 and Pga4). We could also show that the quantitative changes in the wall proteome are mainly inducer-independent.

1. Use of IMDM-S resulted in excellent hyphal induction and stable hyphal growth over a period of 18 h and produced the most biomass.
2. This is the first quantitative description of covalently anchored wall proteins and their quantitative dynamics during the yeast-to-hypha transition.
3. Our findings have identified new vaccine candidates (e.g. Ecm33, Plb5, Sap9, Sod5) and will be used for the development of a peptide-based vaccine.
4. In addition, the invariant wall proteins such as Phr1, Crh11 and Ssr1 might prove valuable as diagnostic biomarkers.
5. Our results will also be used to absolutely quantify wall proteins.

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O163

Correlation between bacterial DNA load and severity of disease in *S. aureus* bacteraemia

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Introduction: Bloodstream infection with *Staphylococcus aureus* is a serious infection that requires rapid and adequate treatment. Little is known about the bacterial DNA load (BDL) of *S. aureus* in different types of infection, ranging from less severe uncomplicated phlebitis to severe life threatening endocarditis. Previous studies with other pathogens have shown that level of BDL in infections corresponds to severity of disease. We determined the BDL in blood of patients with *S. aureus* infection and concurrent bacteraemia.
Methods: A cohort of 32 consecutive patients with culture proven *S. aureus* bacteraemia was categorized into superficial tissue infections (e.g. postoperative wound infections and phlebitis), deep-seated infections (e.g. osteomyelitis and arthritis) or endocarditis. Temporarily stored

whole blood samples, which had been drawn at the time of the blood culture, were collected. Quantification of *S. aureus* specific DNA was performed by real time PCR on DNA extracted from 200ul of blood.

Results: Of 32 patients, 18 patients had a soft tissue infection, 10 a deep-seated infections and 4 endocarditis. The *S. aureus* specific BDL was above the detection limit in 19 patients (59%). All endocarditis patients had a positive PCR while there was no significant difference between the percentages of positives in the other 2 groups. The median BDL in the endocarditis group was 422 cfu/ml (range 92-759), significantly higher than the median BDL of deep tissue infections and superficial tissue infections being 12 cfu/ml (range 3-34) and 4 cfu/ml (range 2-74) respectively. For CRP and leucocytes a correlation couldn't be found.

Conclusion: In patients with *S. aureus* bacteraemia, the BDL is higher in patients with a severe infection (endocarditis) compared to patients with other causes of *S. aureus* bacteraemia. Measurement of the BDL could potentially help clinicians recognise patients at risk of having endocarditis. In patients with superficial and deep-seated infections around 50 percent is detected by our PCR and height of BDL does not differ between these groups. This suggests these infections are characterized by lower amount of DNA in the blood stream or intermittent circulation of bacteria.

O164

Period of increased risk for *Clostridium difficile* infection after exposure to antibiotics

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Introduction: *Clostridium difficile* infections (CDI) are common in developed countries and affect more than 25,000 people annually in England and 2700 hospitalized patients in the Netherlands. The most important risk factor for the disease is antibiotic therapy. Development of CDI is associated with certain antibiotic classes, the number of antibiotics used, their dosage and the duration of therapy. However, the exact time-interval of increased risk for CDI after exposure to antibiotics isunknown.

Methods: A case-control study was performed in nine hospitals in the Netherlands. Each hospital participated for a minimum of six months between March 2006 and May 2009. Hospitalized patients with diarrhoea and a positive test for the toxin of *Clostridium difficile* (CDI patients) were matched on hospital, ward and time of diagnosis to patients without diarrhoea (non-diarrhoeal). Besides antibiotic classes, number and quantity of antibiotic therapy as risk factors for CDI, we evaluated the time-interval of increased risk for CDI after exposure to antibiotics by means of a conditional logistic regression.

Results: In total, 337 CDI patients and 337 non-diarrhoeal patients were included in the analysis. All antibiotic classes, except for first generation cephalosporins and macrolides, were associated with CDI. Cephalosporins and carbapenems were the most potent risk factors for CDI. Patients with CDI used a larger amount of antibiotics and more antibiotic classes, compared to non-diarrhoeal patients. At time of diagnosis, CDI patients more frequently used an antibiotic compared to non-diarrhoeal patients (35% vs. 25%). During antibiotic therapy and the first month after cessation of the therapy, patients had a six to ten fold increased risk for CDI. This risk declined in the period between one and three months after the antibiotic was stopped (OR 2.72; 95% CI: 1.20 to 6.15).

Conclusion: Antibiotic use increases the risk for CDI during therapy and in the period of three months after cessation of antibiotic therapy. A six to tenfold higher risk is found in the first month after cessation of the antibiotic.

O165

Male urinary tract infections in Dutch general practices

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Introduction: Most research regarding urinary tract infections (UTI) focuses especially on female patients due to the higher incidence of this disease in women. However, with increasing age the incidence of this bacterial infection also gets common in men. With the acknowledged differences between female and male UTIs, it is equally important to regularly evaluate the diagnostic and therapeutic policy for male UTIs. Therefore, we evaluated the accuracy of dipstick test results, particularly the nitrite test and the leucocyte-esterase (LE) test, and assess whether the prescription behaviour by GPs was in accordance with dipstick test results for male general practice patients in the Netherlands. Besides, we determined the antibiotic prescription rates in the participating general practices, assess the susceptibility of the isolated uropathogenic *E. coli* and provide information on the ESBL-prevalence among isolated *E. coli*.

Methods: During a 18-month period, diagnostic tests were performed and urinary samples were collected from 489 male patients of 18 years and older with symptoms of UTI in 42 general practices in the Netherlands. Uropathogens were identified and the antibiotic susceptibility of *E. coli* was determined. In determining the accuracy of the dipstick test results, the urine culture was chosen as the gold standard. A P-value <0.05 was considered statistically significant.

Results: A high specificity and positive predictive value were observed for the nitrite test (90% and 89% respectively), whereas the sensitivity was the most optimal for the LE test compared to the nitrite test (76% vs 54%, P<0.05). Still 33% (n=46) of the urine cultures were found to be positive, with negative results in both diagnostic tests.
GPs prescribed an antimicrobial agent empirically to 289 male patients (59%). 89% of the patients with a positive nitrite test, irrespective of the LE test, were prescribed an antimicrobial agent compared to 76% of the patients with a positive LE test, irrespective of the nitrite test (P<0.05). Highest prescription rates were observed for the agents co-amoxiclav (27%) and the fluoroquinolones (26%), followed by nitrofurantoin (21%) and co-trimoxazole (16%). 58% (n=286) of the collected urinary samples were considered positive. *E. coli* was the predominant bacterium isolated (49%, n=140), especially in the younger age categories (56% in men 18-70 years vs 44% in patients >70 years, P<0.05). High susceptibility rates were observed to nitrofurantoin, fosfomycin and the fluoroquinolones (100%, 100% and 95% respectively), whereas lower rates were found for amoxicillin, trimethoprim and co-trimoxazole (65%, 77% and 78% respectively). 1 of the isolated *E. coli* was found to be ESBL-positive, representing 0.7% (1/286) of all *E. coli* isolates.
Conclusions:
1) Clinically suspected UTI in men should be evaluated in the lab, especially when dipstick-negative results are observed.
2) GPs primarily relied on the nitrite test when deciding whether or not to prescribe an antimicrobial agent.
3) Dutch GPs prefer to treat male UTIs with co-amoxiclav and fluoroquinolones.
4) Given the relatively high prescription rate, especially the low *E. coli* susceptibility to co-trimoxazole should be monitored carefully in the future.
5) ESBL-prevalence among the isolated *E. coli* was low.

O166

Comparison of two matrix-assisted laser desorption ionisation-time of flight mass spectrometry methods for the identification of clinically relevant anaerobic bacteria

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Introduction: Two commercially available MALDI-TOF MS systems, Bruker MS and Shimadzu MS, were compared for the identification of clinically relevant anaerobic bacteria.
Methods: A selection of 79 clinical isolates, representing 19 different genera, were tested and compared with identification obtained by 16S rRNA gene sequencing.
Results: Correct genus identification was achieved for 71% of isolates by Shimadzu MS and for 61% by Bruker MS. Correct identification at the species level occurred in 61% and 51% respectively (p=0.139). The number of strains that could not be identified because of absence of reference strains in the database was significantly larger for the Bruker system than the Shimadzu system (19% versus 6%, p=0.017).Shimadzu showed pronouncedly better results for identification of gram-positive anaerobic cocci. In contrast, the Bruker system performed better than Shimadzu for the *Bacteroides fragilis* group.When strains not present in the database were excluded from the analyses for each database, both systems performed equally well, with 77% and 75% correct genus identification for Shimadzu and Bruker, respectively (p=0.386). Similarly, when the most recently updated Bruker database was applied, no difference was observed (Shimadzu 71%, Bruker 72% correct genus identification, p=0.372).
Conclusion: We conclude that the composition and quality of the database is crucial for a correct identification. The databases currently available for both systems need to be optimised before MS can be implemented for routine identification of anaerobic bacteria.

O171

Immune modulating properties of mycobacterial type VII secretion systems

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Introduction: During infection of humans and animals, mycobacteria manipulate the host cell causing severe diseases such as tuberculosis and leprosy. To understand the cause of virulence it is of importance to elucidate the mechanism of pathogenicity in host cells. *Mycobacterium tuberculosis* is a major human pathogen that escapes eradication by the immune system by hiding within macrophages and secreting virulence factors that manipulate the host. Five type VII secretion systems, termed ESX-1 through ESX-5, have recently been identified in pathogenic mycobacteria that are specialized is secreting proteins across their thick mycomembrane. ESX-1 secretion system is described to secrete the major virulence factor ESAT-6, while ESX-5 of *M. marinum* is observed to be responsible for the secretion of over 150 PE and PPE proteins. These protein families are specific for mycobacteria and heavily expanded in the pathogenic species. Thus far, little is known about the mechanisms of immune modulation by the ESX secretion systems and the specific contribution therein.

Methods: In this study, ESX-5-dependent secretion was examined in *M. tuberculosis*. Furthermore, *M. marinum* was used as a model for pathogenic mycobacteria. Infection experiments were performed with human macrophages and wild-type *M. marinum* or mutants deficient in secretion of ESX-1 or ESX-5 substrates.
Results: Here, we show that the ESX-5 system of *M. tuberculosis* is indeed responsible for the secretion of PE/PPE proteins, and that its substrates display immune modulating properties. We find that, in contrast to ESX-1, the effector proteins secreted by ESX-5 are not required for the translocation of *M. tuberculosis* or *M. marinum* to the cytosol of macrophages, an important virulence mechanism for mycobacteria. Finally, we demonstrate that not ESX-1, as previously thought, but ESX-5 induces inflammasome activation and IL-1 secretion, depending on potassium efflux, availability of reactive oxygen species, and cathepsin B.
Conclusions: These results reveal distinct roles for two different type VII secretion systems and enlighten how mycobacteria manipulate the host cell in various ways to allow their own replication and propagation.

O172

Regulation of the energy metabolism in *C. jejuni*

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An important adaptation mechanism for bacteria to survive in different environments is their ability to regulate their energy metabolism. To maximize energy generation, bacteria have evolved multiple metabolic pathways regulated through complex regulatory mechanisms. The human gut pathogen *Campylobacter jejuni* changes its energy metabolism in response to oxygen availability, allowing it to thrive in aerobic external environments and under the oxygen-starved conditions present in the host. In the absence of oxygen, less rewarding electron acceptors such as nitrate, fumarate, TMAO or DMSO are used. Although many conserved genes involved in the energy metabolism are present in *C. jejuni*, all known bacterial transcriptional regulators involved in energy metabolism are absence. In this study we found a *C. jejuni* response regulator to play an important role in the energy metabolism in this organism. Micro-array analysis and realtime RT-PCR results allowed us to identify a number of genes regulated by this regulator. Furthermore our results indicate that this response regulator is activated during oxygen limitation in the presence of nitrate and that it is important for colonisation/persistence of the host.

O173

The pervasive effects of a *Citrobacter rodentium* infection on mouse gut microbial diversity

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Environmental conditions and microbial infections can have pervasive effects on the composition of the gut microbiota. Due to infections the microbial profile can change quite rapidly and extensively. This disturbance is often mediated by the host inflammatory response. Host resistance to intestinal pathogens is established by a combination of the host immune system and direct competition of indigenous bacteria with transient pathogens, for space and nutrients. Not surprisingly disruptions of the microbial community translate into alterations of host susceptibility.
We investigated the restoration mechanism of the murine microbiota after a self-limiting intestinal infection with *Citrobacter rodentium*. Results show the dynamics and interaction of specific bacterial phyla (*Firmicutes, Bacteriodetes* and *Deferibacteres*) and even genera *(Lactobacillus, Mucispirillum* and *Enterobacter*) over a time series of 2 moths at different locations of the gut. We used traditional culture based techniques for quantitative measurements of specific bacterial genera and species in addition to pyrosquencing of 16S rRNA gene fragments. Furthermore, we modeled the disturbance and recovery using a novel Bayesian network computational analyses (MC-TIMME). This model provides insight in the network effects that bacteria can have on each other during the disturbance by effectively recovering shared microbial growth trajectories from pyrosequencing time-series data.
Conclusions: *C. rodentium* colonization causes a significant alteration in the composition of microbial communities. The infection is accompanied with a bloom of *Enterobacter hormachei,* and *Deferribacteres* and a diminished abundance the *Lactobacillus reuteri, Lactobacillus johnsonii, Proteus vulgaris* and *Parabacteroides.* Finally Bayesian network analyses show that *C. rodentum* has a direct negative effect on the presence and abundance of *Akkermansia* and *Lactobacillus* in the colon and is positively associated with the presence of *Escherichia/Shigella* sequences. Notably, the microbiota fully recovers and resembles the non infected mice within 2 months after infection.

O174

Identification of immune modulators using a phage display library displaying *S. aureus* secreted proteins

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Introduction: The human immune system plays a crucial role in the defense against invading pathogens and in the pathogenesis of a variety of inflammatory diseases. In order to cause colonization and subsequent infection pathogenic bacteria are equipped with strategies to evade the host immune system using immune modulating proteins. Understanding these mechanisms of immune evasion will teach us more about the pathogenesis of infection and will lead to the discovery of novel targets in anti-inflammatory and anti-infective therapy and vaccine development. Currently used approaches to identify bacterial immune modulating proteins are inefficient and time consuming. To overcome this limitation we use phage display technology to specifically and functionally identify secreted immune modulating proteins present in a bacterial genome.

Methods: A phage library contains a large amount of different phage clones displaying different proteins resulting in a heterogeneous mixture of phages. The phage capsid protein pIII is frequently used to display proteins fused to the C-terminal end of the pIII protein. The pIII protein is encoded on the phage genome preceded by a signal sequence. For effective phage production this signal sequence is essential. The genome of *S. aureus* was randomly shared into DNA fragments with a size ranging from 300 to 2000 bp. These fragments were cloned into a phagemid vector before the gIII. This vector lacks the native pIII signal sequence. Therefore only gene fragments encoding a signal sequence will lead to protein display. Using this strategy we created a phage library expressing 99.9% of all secreted or membrane bound *S. aureus* proteins. This library was used to select phages expressing proteins that interact with different proteins of the human immune system.

Results: We used the secretome phage library to select phages binding to various targets. These targets were IgG, anti-CHIPS antibody, Von Willebrand factor and Complement factor C3b. After 3 selection rounds displayed proteins were identified by sequencing. With IgG as a target we selected phages displaying protein A, a well known IgG binding protein of S.aureus. Screening for interaction with anti-CHIPS antibodies and Von Willebrand factor selected phages displaying CHIPS protein and Von Willebrand Factor binding protein, respectively. Finally screening on C3b led to the simultaneous selection of phages displaying Efb and Sbi, which are both known to bind C3b.

Conclusion: Phage display technology allows for specific expression of secreted proteins and functional selection of immune modulating proteins of both gram-positive and gram-negative bacterial genomes. Once the phage library is created it can be used in consecutive experiments and in high throughput screening. Selection depends on target binding and there is no need for extensive culturing of bacteria so it can also be used for difficult or slow growing bacteria and meta-genome analysis. There is a direct relation between expressed protein and coding gene that allows for rapid identification of selected proteins. Selection efficacy largely depends on the quality of the initial phage library. Secretome phage display is a extremely powerful new technique to rapidly identify bacterial virulence genes and immune modulatory proteins.

O175

Bacterial evasion of Neutrophil Extracellular Traps: *Staphylococcus aureus* inhibits neutrophil elastase and myeloperoxidase

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Neutrophils form an important arm of innate immunity. They can phagocytose invading bacteria to kill them intracellularly with the help of antimicrobial peptides, proteases and reactive oxygen species. Recently it was shown that neutrophils can also kill bacteria extracellularly by the formation of neutrophil extracellular traps (NETs). NETs, composed of excreted neutrophil DNA and antimicrobial components, are able to capture and kill bacteria. Therefore, we hypothesized that pathogenic bacteria would evade NET immunity by inhibiting NET components. In contrast to supernatants of other pathogenic bacteria, *S. aureus* supernatant completely abolished the activity of the important NET proteins elastase and myeloperoxidase (MPO). Two approaches were used to identify the elastase inhibitor(s). First, supernatant was fractionated using different protein-purification techniques (ion exchange and size exclusion chromatography) and recovered fractions were tested in a functional elastase assay. Subsequent mass-spectrometric analysis yielded two protein candidates: immunodominant surface antigen B (IsaB) and extracellular adhesion protein (Eap). Second, we coated magnetic beads with elastase to capture the inhibitors from *S. aureus* supernatant. This led to the identification of gamma hemolysin B (HlgB) and anion binding protein as putative elastase inhibitors. The putative MPO inhibitor was identified by supernatant

fractionation using anion exchange chromatography and was identified as secretory antigen precursor SsaA-like protein. Currently, candidates are purified as recombinant proteins to verify their inhibitory activity. In conclusion, we show that 1) *S. aureus* secretes molecules that inhibit elastase and 2) it secretes molecules that inhibit MPO. Since elastase and MPO are abundantly present in NETs and are also essential for NET formation, these evasion molecules could be important for staphylococcal escape from neutrophil defenses.

O176

Translocation into the cytosol novel pathogenicity factor mycobacteria

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Introduction: *Mycobacterium tuberculosis* has evolved to be among the most important human pathogens. The paradigm of the exclusive phagosomal localization in the human system was recently challenged by the identification of cytosolic *M. tuberculosis* and *M. leprae* (van der Wel, et al. Cell. 2007). We hypothesized that translocation is essential for pathogenicity of mycobacteria. An important determinant of pathogenicity is the ESX secretion system and thus we tested which ESX system is responsible for translocation. The thus far best described mycobacterial secretion system is the ESX-1 secretion system responsible for the delivery of several important virulence factors. The ESX-1 is present in the outermost layer of mycobacteria (Sani et al. PLOS Pathog. 2010) but is absent in the vaccine *M. bovis* BCG. Reintroducing the system in the vaccine strain might reestablish translocation.

Methods: Here we investigated a wide range of patient derived pathogenic and non-pathogenic Mycobacterium species for their ability to translocate to the cytosol of human phagocytic cells. Bacteria of these different mycobacterial species and *M. tuberculosis* and *M. marinum* mutants were cultured and infected THP-1 cells to be processed for cryo-immunogold electron microscopy. In addition, various cryo-EM techniques were applied to analyze and visualize the mycobacterial cell wall containing the ESX-1 secretion system.

Results: We have analyzed the localization of 18 isolates; covering 11 important pathogenic species. Multiple patient derived mycobacteria, translocate in substantial percentages to the cytosol. In contrast, non-pathogenic or opportunistic mycobacteria remain predominantly phagolysosomal. Previously we shown that translocation was abrogated when the ESX-1 secretion system was mutated. Now we demonstrate that reintroduction of the ESX-1 gene cluster into the non-virulent vaccine strain *M. bovis* BCG is sufficient to induce translocation. In addition, we demonstrate that the C-terminal portion of the ESAT-6 protein, secreted by the ESX-1 secretion system, is essential for translocation. In addition, we demonstrate that the ESX5 secretion system has no effect on translocation. This secretion system is involved inflammasome activation and induction of cell death.

Discussion: We thus reveal that the ESX-1 secretion system is the molecular mechanism of translocation and explain increased virulence of the recombinant BCG with the reintroduced ESX-1. Together, these data demonstrate that the ability to translocate is a pathogenicity factor for mycobacteria through an ESX-1 mediated mechanism. In addition, we demonstrate that mycobacterial secretion systems ESX-1 and ESX-5 play distinct roles in cellular localization, inflammasome activation and cell death.

O177

Pathogenesis of JCV reactivation leading to PML

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The JC Virus induced demyelinating disease, progressive multifocal leukoencephalopathy, PML, occurs in individuals with dysfunctional immune systems. The greatest incidence of PML is seen in HIV-1 infection at approximately 1-3% due to not only severe immune suppression but also molecular interactions between the two viruses. Somewhat surprising, the next highest incidence of PML occurs in autoimmune patients, particularly MS patients, treated with immune modulatory biological therapies. Once such treatment for MS patients is the selective adhesion molecule inhibitor, natalizumab, a humanized monoclonal antibody that binds the $\alpha 4$ integrin, $\beta 1$ and 7 epitopes to prevent extravasation of inflammatory cells into the brain and gut. PML occurs in 1 out of 1000 patients treated with natalizumab whose incidence appears to increase with duration of monthly dosing. The mechanisms responsible for this drug's risk for PML seem to center around its action on the immune system. Cells of the bone marrow in natalizumab treated patients mobilize out of the marrow into the peripheral circulation at concentrations 3 to 10 fold greater than controls and persist for months. Studies that we have done link this observation to PML through viral latency

in the CD34+ hematopoietic progenitor cells whose differentiation toward a B lymphocyte pathway increases the ability of JCV to multiply and carry cell associated or free virus into the brain. Once JCV is present in the brain parenchyma, virus lytically infects the microglia cells specifically targeting the myelin producing oligodendrocyte. The clinical end points of this infection are cognitive impairments, motor dysfunction, visual deficits, and other neurological complications that comprise the diagnostic features of PML.
Since the host range of JCV is very dependent on molecular factors inside the nucleus of the infected cell such as DNA binding proteins that act as transcription factors, we have recently identified the protein SpiB that binds the viral promoter and activates infection in neuroglia and immune cells. There are multiple SpiB binding sites in the viral promoter in PML patients' brain tissues. Site directed mutagenesis experiments have found that one site in the TATA less promoter of the viral tandem repeat abrogates infection in glial cells. Natalizumab treated patients over express SpiB in their circulation. The cell compartments in which this occurs are the CD34+ and CD19+ cells that are good environments for JCV growth. Along with blocks to immune surveillance against JCV, the natalizumab risk of PML stems from its physiological effect of mobilizing potentially latently infected cells from the marrow with subsequent increase in SpiB expression toward a pathway to B lymphocytes that make a susceptible cell for viral multiplication. Other immune modulatory therapies such as rituximab, the monoclonal antibody to CD20+ that eliminates these cells from the circulation, may have similar effects on the immune system by biasing the development and release of pre B cells from storage tissues that also may harbor latent infection. The risk for PML in the use of these drugs was never anticipated. However, immune system alterations even if designed for therapeutic benefit should be appreciated for adverse effects that may occur.

O179

Identification and prevalence of a new human polyomavirus associated with trichodysplasia spinulosa

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The Polyomaviridae constitute a family of small DNA viruses infecting a variety of hosts. In humans, polyomaviruses can cause serious disease of the central nervous system, urinary tract, skin, and possibly the respiratory tract. Here we report the identification and prevalence of a new human polyomavirus called trichodysplasia spinulosa-associated polyomavirus (TSV).
TSV was detected in facial spicules of a heart transplant patient with trichodysplasia spinulosa, a rare deforming skin disease seen in immunocompromized patients. Its genome was amplified through rolling-circle amplification and consists of a 5232-nucleotide circular DNA organized similarly to known polyomaviruses. Two putative 'early' (small and large T) and three putative 'late' (VP1, VP2, VP3) genes were identified. The putative TSV large T antigen contains several domains and motifs described for other polyomaviruses with a possible role in cellular transformation. Phylogenetic analysis revealed a close relationship of TSV with an orangutan polyomavirus, and further with the oncogenic human Merkel cell polyomavirus.
The presence of TSV in affected skin was confirmed by quantitative TSV-specific PCR, indicative of a viral load of 10^5 copies per cell. After topical cidofovir treatment, the lesions largely resolved coinciding with a reduction in TSV load. PCR screening demonstrated a 4% prevalence of TSV with a viral load of less than one copy per cell in a group of asymptomatic renal transplant recipients suggestive of latent infection. Analyzing for TSV-VP1 seroreactivity, TSV seroprevalences of 70% and 89% were found in an immunocompetent and an immunosuppressed population, respectively, indicating that TSV infection is common.

O180

The seroprevalence of seven high-risk HPV types in the Dutch population

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Introduction: The human papillomavirus (HPV) is one of the most common sexually transmitted pathogens worldwide. The high-risk HPV types can cause cervical, vaginal, anal and penile cancer. Data about the seroprevalence of high-risk HPV in the Dutch population are scarce.
Methods: In a population-based study (collected in 2006/2007), 5738 sera of 0-79-years-old men and women[1] were tested for HPV16, 18, 31, 33, 45, 52, and 58 antibodies. Sera were determined using a fluorescent microspheres-based multiplex immuno assay with virus-like particles for the seven HPV serotypes coupled to the microspheres (luminex technology).
Results: Overall, HPV16 and HPV45 were the most prevalent HPV types (11.2% and 7.7% respectively). Among

females, peak HPV seroprevalence occurred among those aged 41-50 years for HPV types 16, 18, 45, 52, and 58 (14.3%, 7.7%, 10.6%, 8.9%, and 6.6%, respectively). For HPV types 31 and 33 the peak seroprevalence occurred at a younger age (31-40 years (5.4%) and 21-25 years (9.2%), respectively). Comparable seroprevalence results were found for males but interestingly peak incidences tend to occur at older ages (41-70 years). For all HPV types low seroprevalences were detected in children aged 1-10 years with geometric mean concentrations near the cut-off values. 10.7% of the individuals are positive for more than one HPV type.

Conclusion: Among the Dutch population (males and females) antibodies against seven high-risk HPV types are detected. The definition of the cut-off values, which is not internationally standardized, and a-specific binding in the assay surely would effect the seroprevalence in children for whom contact with HPV is unlikely. Some individuals are multi-positive for HPV indicating cross-reactivity. This study will contribute to gain insight into incidence of high-risk HPV infections in the Dutch population.

Reference

1. van der Klis et al, Neth J Med. 2009;67:301-8.

O181

Detection of Merkel cell polyomavirus in chronic lymphocytic leukemia cells by fluorescent in situ hybridization (FISH)

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Background: Merkel cell polyomavirus (MCPyV) is detected in approximately 80% of Merkel cell carcinomas (MCC). A number of previous studies have shown that MCC patients are at a significantly increased risk to develop chronic lymphocytic leukemia (CLL) and vice versa. Until recently, clonal integration and truncating mutations of the Large T antigen (LTAg) of MCPyV were restricted to MCC. We have recently reported the presence of the MCPyV in highly purified tumor cells of CLL (n=19/70, 27.1%) (Blood. 2010;sep 3). Of these, six revealed a novel 246bp deletion in the helicase gene of the large T antigen (LTAg). The presence of MCPyV was confirmed by immunohistochemistry.

Design: Here we aimed to determine the presence of MCPyV by FISH analysis in CLL cells in order to evaluate whether MCPyV was integrated or episomal. For this purpose we performed FISH analysis as previously described (Int J Cancer. 2005;115(3):419-28) using MCPyV genome as FISH probe. We tested 6 of the previously reported MCPyV positive CLL cases (EDTA decalcified bone marrow trephines) and MCPyV positive MCC (n=3). In addition, we tested MCPyV negative tumors, e.g. colon cancers. All tissues were formaline fixed and paraffine embedded.

Result: Specific MCPyV DNA by FISH analysis was detected in the nuclei of MCPyV-positive MCC (n=3) and CLL (n=4). Although, the CLL specimens derived from EDTA decalcified bone marrow trephines in contrast to the non decalcified specimens of MCCs, MCC and CLL cells revealed comparable dotlike and sometimes granular FISH signals. No signals were obtained by MCPyV FISH in e.g. colon cancer specimens.

Conclusion: The specific detection of MCPyV in CLL cells further supports our previous report of a possible involvement of MCPyV in a significant subset of CLL. The specific dotlike and sometimes granular nuclear FISH signals in MCPyV positive CLL cells point to an integrated as well as episomal presence of MCPyV in CLL cells, which is in line with our previous findings. Currently, we are optimizing the MCPyV FISH protocol including RNAse pretreatments in order to test the granular FISH results and to assess further CLL cases for the presence of MCPyV.

O182

Clinical features of wuv and kiv; pathogens or passengers?

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Introduction: In 2007, two new human Polyomaviruses, KI polyomavirus (KIV) and WU polyomavirus (WUV), were discovered. Genetically these viruses are related to JCV and BKV, but cluster into a separate lineage. In contrast to JCV and BKV, WUV and KIV have predominantly been found in respiratory tract secretions from pediatric patients. Information on their prevalence and disease associations are still ongoing. It has been suggested that these viruses more likely reactivate in immuno-compromised patients and might be responsible for disease.

The objective of our study was to determine the prevalence of WUV and KIV infections in respiratory samples and to collect and analyse the clinical information of patients tested positive within the University Medical Center Groningen. This is a tertiary referral hospital for both

adults and children and has a large solid organ transplant program.

Methods: Respiratory samples collected from September 2007 to August 2009 were tested using RT-PCR methods. In total, 1964 respiratory samples from 1126 patients were tested, regardless of age, underlying disease or immune status. The prevalence, age distribution and seasonal distribution were evaluated. Subsequently, clinical information of the patients tested positive for either WUV or KIV were retrospectively collected.

Results: 60 (3.05%) samples tested positive for KIV and 71 samples (3.62%) tested positive for WUV. No significant seasonal distribution was found for WUV and KIV. Co-infections with other respiratory pathogens were found in 35 (58%) of the KIV and 47 (66.2%) of the WUV positive samples. Three samples were found positive for both WUV and KIV.

In total, 57 patients tested positive for WUV, six of these patients were adults and all adults were co-infected with other respiratory pathogens. WUV mainly infected young children without severe respiratory symptoms. In contrast, 18 of the 40 patients tested positive for WIV were adults. Half of these adults were found without a viral co-infection and 72% of these adults were severely immuno-compromised.

Conclusions: High percentages of co-infections with other respiratory pathogens complicated the interpretation of the clinical features in these patients. However, the majority of adults only positive for KIV, were immuno-compromised patients. These results are suggestive that KIV and to a lesser extent WUV, may reactivate in solid organ transplant and stem-cell transplant patients.

O183

Rapid pneumococcal evolution in response to clinical interventions

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The recombinogenic bacterium *Streptococcus pneumoniae*, also known as the pneumococcus, is a nasophayngeal commensal and respiratory pathogen capable of causing severe infections including pneumonia, bacteraemia and meningitis. *S. pneumoniae* was estimated to be responsible for nearly 15 million disease cases in 2000 including around 800,000 deaths in children under five. Over recent decades, several antibiotic resistant pneumococcal clones have spread rapidly around the world. Vaccines targeting the polysaccharide capsule have inhibited their spread, but some vaccine escape variants have flourished, notably in the USA. Routine epidemiological tools can detect changes in antibiotic resistance profile and polysaccharide capsule type, but lack the resolution necessary to establish the mechanisms of such events, their frequency and the associated evolutionary dynamics. Here, through analysis of nearly 250 whole genome sequences, we show how a major pneumococcal clone has evolved to overcome the anthropogenic selection pressures of vaccination and antibiotic treatment through multiple independent evolutionary events over a quarter of a century. Through high-resolution phylogenomics, we follow the emergence of a multiply resistant genotype in Europe and its intercontinental spread thereafter. Variation accrues primarily through homologous recombination, leading to the rapid diversification of major antigens, including the capsule biosynthesis locus. Multiple instances of antibiotic resistance acquisition through recombination are also observed within the lineage. The data show how this particular pneumococcal lineage has rapidly adapted to multiple classes of antibiotics and vaccines and suggest that future clinical interventions may need to be targeted differently to account for the rapid evolution of some lineages of this pathogen. Many bacterial pathogens exhibit a recombination rate similar to the pneumococcus. The resulting genetic flexibility of pathogen populations presents a major challenge to the design of effective long-term approaches for their control. Monitoring their evolution through whole genome analyses is likely to prove crucial in informing public health management.

O184

Clonal expansions of MRSA

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Infections, local outbreaks, and regional epidemics leave traces in the genomes of pathogenic bacteria. Deciphering these traces may yield detailed information about a pathogen's evolutionary history, its geographic spread, and the demographic development of its population.

In collaboration with international microbiologists and population biologists, we investigate DNA variation in relation to MRSA geographic distribution. By applying a mutation discovery approach on large numbers of genetic loci from a global collection of *Staphylococcus aureus* isolates, we recently discovered that methicillin resistance in this species had emerged more frequently than previously acknowledged. Further, the progeny of resistant strains usually were distributed locally rather than globally, indicating that the long-distance geographical dispersal of MRSA is a rare event compared with the frequency with which the resistance-mediating genetic element SCCmec has been imported into the bacterial chromosome. When we applied a similar approach to another strain of MRSA that causes a currently ongoing epidemic of hospital infections, we found that a drastic demographic bottleneck had preceded the expansion of this clone. We investigated

the subsequent population size development with time and the regional spread of this strain of MRSA based on DNA sequence variation.

Next-generation sequencing technologies enable investigations of representative samples from bacterial populations at the level of whole genomes, providing maximum resolution. Such data is highly informative about the spatiotemporal dynamics of MRSA spread, especially with respect to local and regional scales of infection epidemiology.

O185

Continental scale dynamics

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National epidemics of methicillin-resistant *Staphylococcus aureus* (MRSA) are characterised by the expansion of a relatively small number of successful clones. This seriously undermines the ability of hospital epidemiologists to disclose transmission pathways and gaps in infection control but offers the potential to understand the temporal-spatial spread over larger evolutionary distances. Novel web-based applications pave the way to a better understanding of the epidemiology of emerging and successful clones leading to the appreciation of hospital referral networks that reveal a specific mathematical signature which indicates a deeper principle that governs distributed hierarchical real world networks in nature and culture. The lecture will focus on the advantages of synteny in *S. aureus* and its role as a model organism that improves our conceptional thinking about nosocomial transmission at large.

O186

A quantitative account of genomic island acquisitions in prokaryotes

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Microbial genomes do not merely evolve through the slow accumulation of mutations, but also and often more dramatically, by taking up new DNA in a process called horizontal gene transfer. These quantum leaps in the acquisition of innovative traits can take place via the introgression of single genes, but also through the acquisition of large gene clusters, which are termed Genomic Islands (GIs). Since only a small proportion of all the DNA diversity has been sequenced, it is often impossible to find the appropriate donors for these acquired genes via sequence alignments from databases. In contrast, relative oligonucleotide frequencies represent a remarkably stable genomic signature in prokaryotes, which facilitates compositional comparisons as an alignment-free alternative for phylogenetic relatedness.

In this project, we test whether GIs identified in individual bacterial genomes have a similar nucleotide composition in terms of this genomic signature, and can therefore be expected to originate from a common donor species.

We present a software package that allows the compositional analysis of predicted GIs in prokaryotic genomes by clustering compositionally similar sequences together compared to a set of cut-off values. When multiple GIs are present within a single genome, we find that up to 28% of all tested GIs are compositionally very similar, indicative of numerous recurring acquisitions from the same donor to the same acceptor. Alternatively, these compositionally similar GIs could be relics of intragenomic dispersal events, which still implies a common ancestral donor organism. Simulation of the clustering analysis shows accuracies of ~95% for the least stringent threshold, up to 99.9% for the most stringent threshold. Together, these results imply common origins for many of the horizontally acquired Genomic Islands present in prokaryotic genomes, and this represents the first quantitative assessment of common directional transfer events in prokaryotic evolutionary history.

O187

Characterization of the penA mosaic gene in *Neisseria gonorrhoeae* strains with decreased susceptibility to cephalosporins in Amsterdam, the Netherlands

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Objectives: In 2006 to 2008, an increase in prevalence (7.3%) of multidrug-resistant Neisseria gonorrhoeae (NG) strains with decreased susceptibility to the extended-spectrum cephalosporin (ESC) cefotaxime was observed among visitors of the STI clinic in Amsterdam, the Netherlands. To determine whether this was due to the rapid clonal expansion of a NG strain harbouring a mosaic penA gene, polymorphisms in the penA gene were correlated to the susceptibility to cefotaxime, cefixime, and ceftriaxone. Strain clonality and epidemiological concordance of mosaic penA patterns were assessed by genotyping.

Methods: From 2006 to 2008, 74 NG isolates with a cefotaxime MIC of >0.125 g/ml (group A), 54 with a cefotaxime MIC of 0.125 g/ml (group B), and a control group of 74 with a cefotaxime MIC of <0.125 g/ml (group C), were included in this study. All samples were characterized using

antibiograms, gel electrophoresis of the amplified penA gene, and NG multiple-locus variable-number of tandem repeat analysis (MLVA). Sequencing was used to identify and confirm the mosaic penA positive MLVA types.
Results: The proportion of NG isolates with decreased susceptibility to cefixime and ceftriaxone (MIC≥0.016 g/ml) were higher in group A (68% and 76%, respectively) than group B (11% and 57%), while group C had slightly elevated ceftriaxone MICs only (5%). The mosaic penA gene (n=53) was identified only in group A (64%) and B (11%), and showed a correlation with the higher ESC MICs. Hierarchical cluster analysis of the MLVA data assigned all 53 patients with a mosaic penA positive NG strain to the same large cluster (≥10 patients; n=56). The presence of a mosaic penA gene in this NG strain was confirmed by sequencing and was shown to be identical to a previously published sequence (GU723422). This cluster contained Dutch homosexual men (66%), patients with frequent Chlamydia co-infection (32%), and commercial sex workers (7%). A second large cluster (n=39) was identified that also contained isolates of group A (21%) and B (67%), but no mosaic penA positive NG strains.
Conclusion: A correlation was found between the decreased susceptibility to the ESCs and the introduction of a clonal mosaic penA positive NG strain among visitors of the STI clinic Amsterdam. High-risk sexual behaviour might facilitate the rapid spread of this multidrug-resistant NG strain, posing new public health concerns.

O188

Evaluation of the Dutch surveillance on carbapenemase producing *Enterobacteriaceae*

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Introduction: There is a world-wide concern about the emergence of carbapenemase producing *Enterobacteriaceae*. Local and national surveillance is considered an important component of the strategy to control these strains. Since March 2010 the surveillance on carbapenemase producing *Enterobacteriaceae* (CPE) in the Netherlands consists of three pillars: 1) a (concept) national guideline on the phenotypic detection of CPE in the routine clinical setting, which recommends to confirm a non-wild-type carbapenemase MIC by Etest (IJAA 2010), 2) the national antimicrobial resistance surveillance system (ISIS-AR) providing feedback alerts on isolates carbapenem (intermediate)resistant (I/R) reported by the participating labs but not yet confirmed by Etest or discs, and 3) sending the first screen positive isolate per patient to a reference laboratory for genotypical confirmation. The aim of this study was to evaluate this strategy and to estimate the possible underreporting of CPE.
Methods: All 261.000 *Escherichia coli* (ECO), *Klebsiella pneumoniae* (KPN) and *Enterobacter* spp. (ENT) present in the ISIS-AR database from 1-1-2009 to 11-12-10 were included. These were submitted by 22 labs serving general practitioners, long-term care facilities and 44% of the Dutch hospital beds. Using this database we calculated: 1) the yearly prevalence of I/R ECO, KPN and ENT, 2) the yearly number of screen positive ECO, KPN and ENT isolates. Screen positive was defined as meropenem MIC≥0.5 mg/L or disk diameter ≤23 mm or imipenem MIC≥ 2 or a diameter ≤21 mm, 3) the percentage of screen positive isolates confirmed by Etest or discs, and 4) the number of genotypically confirmed CPE isolates in the reference lab.
Results: In 2009 and 2010 the prevalence of I/R isolates was for ECO 60 (0.05%) and 21 (0.02%), KPN 63 (0.36%) and 87 (0.59%) and for ENT 51 (0.54%) and 16 (0.25%) respectively.
The number of screen positive isolates according the Dutch guideline was in 2009 and 2010 for ECO 190 (0.2%) and 291 (0.3%), KPN 182 (1.0%) and 186 (1.3%) and ENT 213 (2.3%) and 152 (2.3%) resp. Less than 50% of these screen positive isolates was also third-generation cephalosporin resistant making the presence of a carbapenemase less likely.
In 2010 the number of screen positive isolates confirmed by an Etest or disc test was for ECO 9 (3%) (6 confirmed), KPN 11 (6%) (11 confirmed), and ENT 5 (3%) (2 confirmed). In 2010, in total 19 KPN, 5 ENT and 0 ECO isolates were received by the reference laboratory from these laboratories. 8 KPN and 1 ENT (4 laboratories) carried a carbapenemase gene (6 KPC, 2 VIM-1, 1 OXA-48). In two hospitals secondary cases occurred. Among isolates from non-ISIS-AR laboratories 3 KPC and 1 OXA-48 were detected.
Conclusion: Carbapenemase confirmation tests were performed in a small proportion of the screen positive isolates possibly resulting in an underestimation of the CPE prevalence. An increased awareness and compliance to the guideline in the laboratories is required to improve carbapenemase detection in *Enterobacteriaceae* in the Netherlands. In 2011, to support the laboratories ISIS-AR will provide feedback alerts on carbapenemase screen positive isolates instead of the current feedback alerts on carbapenem I/R isolates.

O189

First detection of an Ambler class D OXA-48-type β-lactamase in a *Klebsiella pneumonia* strain in The Netherlands

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Introduction: Infections with carbapenemase producing *Enterobacteriaceae* (CPE) are associated with high mortality as treatment options for these infections are severely limited. This case illustrates the clinical challenges associated with CPE infections.
Case description: A 63-year-old Dutch male patient with interstitial lung disease developed a tension pneumothorax during his holiday in India for which a bullectomy and pleurectomy and hospitalization at the intensive care unit were needed. Lung biopsy showed an usual interstitial pneumonia and corticosteroids were started. Despite antibiotic prophylaxis including ciprofloxacin, piperacillin and tazobactam he developed a catheter related bloodstream infection with multi-resistant Acinetobacter baumanii. Intravenous treatment with imipenem/cilastin and polymyxine was started and after improvement of his clinical situation he was transferred to an ICU in Amsterdam, the Netherlands. At that moment there were no signs of infection and antibiotic treatment was stopped. Surveillance cultures showed a multi-resistant *Acinetobacter baumanii*, similar to the one reported from the hospital in India. However, surveillance cultures of sputa also yielded a *K. pneumoniae*, resistant to broad spectrum cephalosporins, meropenem, ertapenem and imipenem as determined by the Vitek-2 (bioMerieux, Marcy l'Etoile, France), the Phoenix microbiology system (Becton-Dickinson, Erembodegem, Belgium) and E-tests (AB Biodisk, Solna, Sweden) according to EUCAST criteria. In addition the isolate was also resistant to fluoroquinolones, cotrimoxazole, tobramycin and gentamicin, and remained only susceptible to tigecycline and colistin. PCR and sequence analysis on the *K. pneumoniae* isolate, with primers for detection of Ambler class D, class B, and class A -lactamase genes, identified an OXA-48-type -lactamase together with the blaCTX-M-15 genes.
Four weeks after his transfer from New Delhi, he developed a pneumonia, presumably with the carbapenem resistant *K. pneumoniae*, upon which simultaneous treatment with intravenous tigecycline and intravenous colistin was started. The infection recovered soon and the patient was moved to the pulmonary ward where he repeatedly developed pulmonary distress to which he eventually succumbed, 40 days after his ICU admission.
Conclusion: This is the first report of a lethal infection due to a CPE in The Netherlands and the first evidence of an OXA-48-type -lactamase producing *Enterobacteriaceae* in The Netherlands. This case demonstrates the clinical challenges with CPE infections and emphasizes the imperative need to implement guidelines for laboratory detection of CPE and the development of guidelines for treatment of infections with CPE in the Netherlands.

O190

Prevalence of tobramycin-resistant *Enterobacteriaceae* in a Dutch hospital: implications of the harmonised clinical breakpoints of the European Committee on Antimicrobial Susceptibility Testing

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Introduction: In April 2010, the European Committee on Antimicrobial Susceptibility Testing (EUCAST) issued harmonised clinical breakpoints for antimicrobial agents in Europe. The new clinical breakpoints are currently being implemented in the Dutch microbiology laboratories. The new EUCAST breakpoints for tobramycin in *Enterobacteriaceae*, i.e. S≤2 mg/L and R>4 mg/L, are below the previously used breakpoints of S≤4 mg/L and R>8 mg/L set by the Clinical Laboratory Standards Institute (CLSI). Implementation of the EUCAST breakpoints will, therefore, likely result in an increase in the reported prevalence of tobramycin resistance in *Enterobacteriaceae*. This prevalence study aims to provide an estimate of the increase in reported tobramycin resistance in *Enterobacteriaceae*, using the new harmonised EUCAST clinical breakpoints.
Methods: In November 2010, 566 patients that were hospitalised in the Amphia Hospital in Breda/Oosterhout were screened for carriage of tobramycin-resistant *Enterobacteriaceae* using a rectal swab. Rectal swabs were directly inoculated on a MacConkey agarplate containing 6 mg/L tobramycin and 8 mg/L vancomycin (screeningsagar). After incubation at 37C for 18 to 48 hours species identification and susceptibility testing was performed for all oxidase-negative gram-negative rods that grew on the screeningsagar using the automated system VITEK2 (bioMrieux).
Results: In 264 (46,6%) patients there was growth of one or more *Enterobacteriaceae* on the screeningsagar. The following species (n=306) were recovered: *Escherichia coli* (n=267), *Proteus* spp. (n=14), *Klebsiella oxytoca* (n=5), *Serratia marcescens* (n=5), *Morganella morganii* (n=4), *Citrobacter* spp. (n=4), *Klebsiella pneumoniae* (n=3), *Enterobacter* spp. (n=3) and *Pantoea agglomerans* (n=1).
Using the CLSI breakpoints 12 of 566 patients (2,1%) had tobramycin-resistant *Enterobacteriaceae* compared to 79 (14,0%) patients using the EUCAST breakpoints (p<0,001). Co-resistance to ciprofloxacin was observed in five (0,9%) of the tobramycin- resistant strains according to CLSI breakpoints and 13 (2,3%) strains according to EUCAST breakpoints (p=0,013).

Conclusion: The transition from CLSI breakpoints to EUCAST breakpoints in Dutch microbiology laboratories will result in a significant increase in the reporting of tobramycin-resistant *Enterobacteriaceae*. This will not only affect the choice of antimicrobial agents but also increase the number of patients that require isolation measures. It is questionable if the benefits of the approach by EUCAST outweigh the adverse affects.

O191

Eradication of Extended-Spectrum Beta-Lactamases (ESBLs) during Selective Digestive tract Decontamination (SDD)

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Introduction: SDD was beneficial for patient outcome in Dutch ICU patients. SDD consists of topical antibiotics containing tobramycin, colistin and amphotericin B to eradicate potential pathogens, such as *Enterobacteriaceae*, from the intestinal tract. Although most ESBL-producing *Enterobacteriaceae* (ESBL-E) are susceptible to the SDD-regimen, the effect of SDD on eradication of ESBL-E from the intestinal tract is unknown. We quantified eradication rates of non-ESBL and ESBL-E in SDD-patients participating in a 13-center cluster randomized study (NEJM. 2009;360:20).

Methods: All SDD-patients with culture results from >1 rectal sample (with the first sample obtained <2 days in ICU) were included. Rectal carriage with Enterobacteriaceae was determined at admission and twice weekly during ICU-stay. ESBL-E was defined pragmatically as resistance to either ceftazidime, cefotaxim, ceftriaxon or cefuroxim. If colonization with *Enterobacteriaceae* was present at admission, ESBL status and duration of colonization was determined. Kaplan-Meier analysis was performed to test for differences in colonization duration. Patients were censored at ICU-discharge.

Results: 345 (21%) of 1613 patients meeting inclusion criteria were colonized with Enterobacteriaceae on ICU admission: 111 (32%; 6.9% of 1613) with ESBL-E. Median lengths of ICU-stay were 8 (range 2-134, IQR 9) and 10 days (range 2-79, IQR 10) for non-ESBL and ESBL colonized patients, respectively (p=0.13). Median duration of colonization was 4.5 days for non-ESBL patients and 5 days for ESBL patients (p=0.12, logrank test). The most frequent colonizers were *E. coli* (n=257; 31 ESBL-E (12%)), *Enterobacter* spp. (n=43 ; 42 ESBL-E (98%)) and *Klebsiella* spp. (n=37 ; 8 ESBL-E (22%)).

Conclusion: SDD was equally effective in eradicating ESBL and non-ESBL producing *Enterobacteriaceae* present at ICU admission from the intestinal tract.

O192

Dutch patients, retail chicken meat and poultry share the same ESBL genes, plasmids and strains

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Objectives: In the Netherlands, the incidence of infections with ESBL-producing Enterobacteriaceae has increased in recent years despite low levels of antibiotic use and stringent infection control policies. Antibiotic use in the Dutch poultry industry is the highest in Europe and intestinal carriage with ESBL-producing bacteria in food-producing animals has been shown to result in contamination of retail meat. The aim of this study was to quantify the proportion of human *E. coli* isolates with ESBL genes, plasmids and genotypes that correspond to those found in poultry or retail chicken meat.

Methods: The distribution of ESBL genes, plasmids and strain genotypes in *E. coli* obtained from poultry and retail chicken meat in the Netherlands was determined and defined as 'poultry-associated' (PA). Subsequently, the proportion of *E. coli* isolates with PA ESBL genes, plasmids and strains was quantified in a representative sample of clinical isolates. *E. coli* were obtained from 96 non-frozen retail chicken meat samples, a national prevalence survey among poultry, and 516 human clinical samples from 31 laboratories collected during a 3 month period in 2009. Isolates were analyzed using an ESBL-specific microarray, sequencing of ESBL genes, PCR based Replicon Typing of plasmids, plasmid Multi-Locus Sequence Typing (pMLST) and strain genotyping (MLST).

Results: Six ESBL genes were defined as PA (blaCTX-M-1, blaCTX-M-2, blaSHV-2, blaSHV-12, blaTEM-20, blaTEM-52). Of the human *E. coli* isolates, 35% (95% CI 30-39%) contained PA ESBL genes, 86% of which were either blaCTX-M-1 or blaTEM-52. These were also the predominant ESBL genes in poultry (77%) and retail chicken meat (75%). In 19% (95% CI 15-23%) of human *E. coli*, the PA ESBL genes were located on IncI1 plasmids that were genetically indistinguishable from those obtained from poultry (meat). In 11% (95% CI 8-14%) not only the genes and plasmids but also the MLST genotypes were

identical. Of the retail meat samples, 94% contained ESBL producing isolates of which 39% belonged to *E. coli* genotypes also present in human samples.
Conclusion: These findings are suggestive for transmission of ESBL genes, plasmids and *E. coli* isolates from poultry to humans, most likely through the food chain.

O193

Comparison of ESBL contamination in organic and conventional retail chicken meat

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Introduction: Contamination of retail chicken meat by Extended Spectrum Beta-Lactamase (ESBL) producing bacteria may contribute to increased incidence of infections with these bacteria in humans. As producers of organic chicken meat claim restricted use of antibiotics compared to conventionally produced meat, we quantified ESBL contamination in both types of chicken meat.
Methods: In 2010, 98 raw chicken breasts were purchased in 12 stores in Utrecht; 80% were purchased at 9 stores belonging to 5 supermarket chains with a combined Dutch market share of approximately 90% and 20% from organic butcheries. Sixty were sold as conventional and 38 samples as organic (30 'EKO', 8 '1 ster beter leven'). Per sample 25 g was homogenized with 225 ml peptone water in a stomacher. For quantitative cultures, an ESBL selective plate (Oxoid ESBL Brilliance) was inoculated with a 1:1000 dilution of the homogenate. For qualitative culturing, an overnight pre-enrichment was performed with 225 ml of the homogenate. ESBL production was confirmed using Etests, and sequencing of the ESBL gene. Species identification was performed by MALDI-TOF. MICs were determined by broth micro-dilution using EUCAST breakpoints. Strain typing was performed on isolates from 24 meat samples (14 conventional, 10 organic) using MLST.
Results: Of 98 meat samples, 92 (94%) harbored at least one *E. coli* isolate with an ESBL phenotype, yielding 163 isolates (average number of isolates per sample 2; range 1-4). Prevalence of ESBL producing micro-organisms was 100% and 84% on conventional and organic samples, respectively (p<0.001). Median loads of ESBL producing micro-organisms were 80 (range <20-1360) and <20 (range 0-260) CFU/25 gram in conventional and organic samples, respectively (p=0.001). Sequencing of the ESBL genes demonstrated the following distribution: CTX-M-1 (49%), TEM-52 (26%), SHV-12 (16%), CTX-M-2 (4%), SHV-2 (4%) and TEM-20 (1%). SHV-12 was more prevalent in isolates from conventional meat samples than from organic samples (23% versus 3%; P<0.001)), and CTX-M-2, SHV-2 and TEM-20 ESBL genes were exclusively found in conventional samples. In 12 (12%) samples (7 organic, 5 conventional) CMY-2 was detected, an AmpC gene also present in human isolates. Co-resistance rates of ESBL positive isolates were: co-trimoxazole 92%, tetracycline 62%, ciprofloxacin 14%, and tobramycin 2% (no significant differences between organic and conventional isolates). Six of 14 conventional samples harbored 4 MLST types also reported in humans (2 ST10, 2 ST23, ST57, ST117) and 5 of 10 organic samples harbored 3 MLST types also reported in humans (2 ST10, 2 ST23, ST354).
Conclusion: The prevalence of ESBL producing *E. coli* is high (>80%) in both conventional and organic poultry meat, and the median bacterial load per contaminated sample was higher on conventional poultry meat. This supports the concept that restricted antibiotic use in the poultry industry may reduce ESBL contamination of chicken meat.

O194

Characteristics of extended-spectrum cephalosporin-resistant clinical isolates from companion animals and horses

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Introduction: Initially Extended Spectrum Beta-Lactamase (ESBL)-producing organisms were associated with hospitals and institutional care in humans, but they are now increasingly found in the community and in animals, suggesting the transmission of organisms or genes between the different reservoirs. Resistance to extended-spectrum cephalosporins has been studied in detail in gram-negative bacteria isolated from humans and food-producing animals. In the Netherlands, 3rd and 4th generation cephalosporins are authorized for use in horses and companion animals. Data on ESBL and AmpC betalactamase producing *Enterobacteriaceae* in companion animals and horses, however, is limited. The aim of the present study was to investigate ESBL or AmpC-betalactamase production in *Enterobacteriaceae* isolated from clinical infections of companion animals and horses and to further characterize these isolates.
Materials and method: Susceptibility of 10755 clinical isolates to various antimicrobials was routinely tested using agar diffusion method during the study period from 2007-2009 at the Veterinary Microbiological Diagnostic Center of Utrecht University. Gram-negative isolates which had an inhibition zone=25 mm to ceftiofur and/

or cefquinome and/or cefavocin were sent to the Central Veterinary Institute of Wageningen UR, Lelystad, the Netherlands for further analysis. These isolates were tested for ESBL or AmpC-betalactamase production by double disk synergy test. In addition, the isolates were screened for the presence of genes encoding ESBLs, AmpC-betalactamases and other resistance genes of gram-negative bacteria and integrons using a microarray. Antimicrobial susceptibility of the ESBL-positive isolates was determined by microbroth dilution.

Results: Sixty-six isolates from dogs (n=39), cats (n=13), horses (n=12) and a turtle showing an inhibition zone=25 mm to extended-spectrum cephalosporins were identified. Most isolates were *E. coli* (n=50), others were *Enterobacter cloacae* (n=9), *Proteus mirabilis* (n=3), Salmonella species (n=10), *Citrobacter freundii* (n=10) and *Pseudomonas putida* (n=1). Most isolates originated from urinary tract infections (n=31) or wound infections (n=17). Double disk testing showed that 29 isolates had an ESBL-phenotype, 26 isolates an AmpC-type, 4 a mixed ESBL/AmpC type and 6 did no show an ESBL or AmpC type and one isolate was lost before testing. Nucleotide sequence analysis revealed that the isolates carried bla_{CMY-2}, bla_{CMY-39}, $bla_{CTX-M-1}$, $bla_{CTX-M-2}$, $bla_{CTX-M-9}$, $bla_{CTX-M-14/17/18}$, $bla_{CTX-M-15}$, bla_{TEM-1}, bla_{TEM-30}, bla_{TEM-52}, bla_{TEM-80} and bla_{SHV1}. $Bla_{CTX-M-1}$, bla_{CMY-2} and bla_{TEM-1} were most commonly found. All isolates were multidrug-resistant and many isolates showed additional resistances to trimethoprim, sulfonamides, fluoroquinolones, tetracycline, chloramphenicol, macrolides and aminoglycosides.

Conclusion: This study shows the presence of a variety of ESBL or AmpC-genes in ESC resistant bacteria isolated from companion animals. Most of the ESBL and AmpC-genes found in this study are also present in isolates from humans and poultry indicating that there is an exchange of resistance genes between the different reservoirs.

O196

Interactive cases in medical microbiology and infectious diseases

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In this interactive session, cases relevant to anyone working in the field of medical microbiology and infectious diseases will be presented by professor Marc Bonten and Dr. Bert Mulder. Using an interactive voting system, they will challenge all participants to test and develop their knowledge on a wide variety of infectious disease related topics. Exotic and rare 'academic' cases will be balanced by more frequently encountered cases of community acquired infections. Each case will be accompanied by several questions to test your knowledge on:

- the organism's characteristics and laboratory based diagnostics
- pathogenesis of disease and its clinical manifestations
- the history and epidemiology of the disease
- infection prevention
- treatment and resistance

After 90 minutes, the top three scorers will receive a prize of which eternal fame will be the most important component. The remaining 30 minutes will be dedicated to interactive presentations of cases in infectious diseases by residents in training.

We do hope to welcome you all on this session that will be as entertaining as informative.

P001

A proteomics approach to identify novel Tat-related interacting partners and substrates in *Bacillus subtilis*

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Introduction: The movement of molecules across membranes is associated with various cellular functions ranging from metabolism and cell-cell communication to antibiotic secretion and, in the case of pathogens, virulence factors. This implies that the mechanisms and pathways involved in the movement of proteins across membranes are of great interest. The focus of this work was on a particular transportation pathway, the Twin-arginine translocation (Tat) system, which is unique in that it it translocates pre-folded and post-translationally modified proteins. *Bacillus subtilis* is a non-pathogenic gram-positive bacterium with a high capacity for protein secretion, which makes it a relevant model organism to both fundamental and applied science. Although the basic components of the Tat system in *B. subtilis* have been identified, relatively little is known about possible substrates, mechanisms of translocation, pre-protein processing, and quality control.

Methods: To identify novel proteins involved in Tat-dependent membrane protein insertion and protein secretion, a global quantitative proteomics approach was taken. This methodology allows not only the identification but also the quantification of many detectable peptides using metabolically labelled proteins and LC-MS/MS.

Results: Proteins were identified in the cytosolic, membrane and extracellular fractions in various *tat* mutant strains. Comparison between the proteins present in wild

type and *tat* mutants strains were made and a number of novel Tat-affected proteins were identified. One of these, QcrA, appeared to be a Tat-dependent substrate and this was subsequently confirmed using Western blotting. QcrA is the Rieske iron-sulphur protein forming part of the cytochrome *bc* complex. It is the first example where the Tat system in *B. subtilis* is involved in membrane protein insertion.

Conclusions: Taken together two conclusions could be made, firstly a number of novel Tat-interacting proteins were recognised in this work, and secondly a new Tat dependent substrate was indentified. The outcome of this work can be used to further build up the Tat interaction network and expand our understanding of this relevant secretion pathway.

P002

Challenging 'The Great Plate Count Anomaly': Isolation of methanotrophic bacteria

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Introduction: Methane oxidizing bacteria (MOB) are ubiquitous and play a vital role in reducing emissions of the greenhouse gas methane. The isolation of these fastidious organisms into pure culture has proven difficult and laborious. The majority are so far not-yet-cultured. Therefore, next to traditional agar-plating, non-conventional and/or miniaturized high throughput cultivation techniques have been developed recently. This study compares a miniaturized high-throughput extinction culturing approach with plating for the retrieval of novel methanotrophic bacteria.

Methods: Samples were taken from a wastewater treatment plant, cow slurry pit, wetland and biofilter soil. Methane oxidation activity was measured with gas chromatography. MOB were enumerated with the Most Probable Number (MPN) technique using gas-tight vials with methane added to the headspace. From these MPN enrichments, isolation was performed by miniaturized extinction culturing and plating (gellan gum as solidifying agent). Strains were screened via microscopy, gas chromatography and gene sequence analysis.

Results: MOB were present in all four samples. Prolonged incubation of the MPN enrichments, up to 5 weeks at 20 °C, was necessary for the higher dilutions to oxidize methane effectively. No MOB were isolated using the dilution plating technique. In contrast, MOB from all four samples could be isolated from the MPN enrichments using the extinction culturing technique performed in microtiter plates. Moreover, purity could be reached during extinction culturing, making purification steps on plates, often stated as problematic for MOB, redundant. The extinction approach was less laborious and increased capacity, few gas-tight jars required, compared with conventional plate isolation. Preliminary identification showed that several isolates will have to be assigned to novel methanotrophic species.

Conclusion: A miniaturized, high-throughput two-step liquid culturing protocol for rapid isolation of methanotrophic bacteria was successfully validated with three anthropogenic and one natural site. This procedure can be readily applied, in large scale, to other environmental samples.

P003

GyrB sequence analysis and MALDI-TOF MS as identification tools for plant pathogenic Clavibacter

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Introduction: The genus *Clavibacter* includes five subspecies, most of them belong to quarantine or q-alert organisms and cause a variety of plant diseases and serious crop losses. To limit economic losses and avoid dissemination of the pathogen to pathogen-free areas, rapid and reliable identification is required. In the frame project QBOL (Quarantine Barcoding of Life) we focus on the development of an accurate DNA-based identification of *Clavibacter* and close relatives. In addition, quick analysis through matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF MS) was also evaluated.

Methods: A total of 180 strains from 5 *Clavibacter michiganensis* subspecies (*C. michiganensis* subsp. *michiganensis* (Cmm), *C. michiganensis* subsp. *sepedonicus* (Cms), *C. michiganensis* subsp. *nebraskensis* (Cmn), *C. michiganensis* subsp. *insidiosus* (Cmi), *C. michiganensis* subsp. *tesselarius* (Cmt)) and some outgroups were included in the analyses. Firstly, 16S rRNA gene analysis was performed to check the reliability of the collected strains and to separate *Clavibacter* from closely related bacteria. In order to select suitable targets for barcoding several housekeeping genes were preliminary tested: *gyrB*, *dnaK*, Cox1, CTP synthetase, GluRS and *rpoB*. A 500 bp *gyrB* amplicon were selected for further analysis. The gene sequences were analyzed in two ways namely, neighbor-joining tree and character-based approach looking for specific characters within the sequence. The MALDI-TOF MS profiles were generated from cell extract. Subspecies identification within the genus *Clavibacter* was performed based on the presence of unique biomarker ions in the protein profiles.

Results: The *gyrB* sequences showed enough resolution and specificity to identify the subspecies based on sequence

relatedness in a neighbor-joining tree or on specific characters within the sequence. Using MALDI-TOF MS closely related *Clavibacter* subspecies could be differentiated by defining unique biomarker ions. Results from both methods were in concordance and distinguished the five *Clavibacter* subspecies from each other and from closely relatives like *Rathayibacter, Leifsonia* or *Curtobacterium*.

Conclusion: Two quick and reliable identification tools for the plant-pathogen *Clavibacter* were found, a DNA-based barcoding region and specific MALDI-TOF MS biomarkers, able to differentiate between different subspecies of the genus. Our study suggests that the proteomic analysis using MALDI-TOF MS and the *gyrB* sequence barcode are powerful diagnostic tools for reliable identification of the *Clavibacter*.

P004
Identification of nontuberculosis *Mycobacteria* by MALDI-TOF MS fingerprinting

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Introduction: Next to the clinical important *Mycobacterium tuberculosis* complex (MTC) Nontuberculosis mycobacteria (NTM) are pathogens especially in immunocompromised and senior people. Identification of NTM species is important for diagnosis and optimal treatment. The aim of this study is a comparison of genus probe and MALDI based identification results and to investigate the discriminatory power of MALDI-TOF molecular profiling, even for long-time stored samples.

Methods: 19 *Mycobacterium* species from a laboratory collection were characterized conventionally using PCR followed by reverse line blot hybridization of the amplified products to an in-house or commercial (Innolipa, Innogenetics) DNA probe assay. Biomass was harvested and analyzed using the MALDI Biotyper. Mycobacterium samples were heated at 95C for 30 min, washed twice with 500 l of water. The pellet was extracted with 70% formic acid and pure acetonitrile in equal amounts. The supernatant was measured in a MicroflexTM (Bruker Daltonics, Germany) mass spectrometer and spectra analyzed using MALDI Biotyper 2.0 software. Furthermore, identification results of *Mycobacterium* spp. biomass stored for 3 up to 4 years on pyruvate-enriched Loewenstein-Jensen medium at 4 °C and of freshly cultured ones were compared for 11 samples. Samples with divergent identification results between reverse line blot hybridization and MALDI-TOF and 'no reliable identification' MALDI Biotyper samples were sequenced and will be used for database complementation after finalization of analysis.

Results: *Mycobacterium* strains were identified as *M. avium, M. fortuitum, M. gordonae, M. kansasii, M. malmoense* and *M. terrae*. Some strains were assigned to different species by MALDI Biotyper than by the conventional identification methods, some as a different species and few mycobacteria were not identified using the MALDI Biotyper. Adding new species to the database enable their secure identification in future times. Dendrograms were calculated with acquired and database spectra. Diverging identification results were examined by additional methods and discussed.

None of the identification results of the 3 to 4 years old biomass differed from the results of fresh cultivated samples. Therefore, it is suitable to analyze even long-time stored samples by MALDI-TOF MS.

Conclusion: It has been demonstrated that MALDI-TOF MS is a rapid and reliable method for discrimination and identification of NTM species.

P005
Conversion and conservation of light energy in a photosynthetic microbial mat ecosystem

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Here we present, to the best of our knowledge, the first balanced light energy budget for a benthic microbial mat ecosystem, and show how the budget and the spatial distribution of the local photosynthetic efficiencies within the euphotic zone depend on the absorbed irradiance (J_{abs}). Our approach employs microscale measurements of the rates of heat dissipation, gross photosynthesis and light absorption in the system, and a model describing light propagation and conversion in a scattering-absorbing medium. The energy budget was dominated by heat dissipation on the expense of photosynthesis: at light limiting conditions, 95.5% of the absorbed light energy dissipated as heat and 4.5% was channeled into photosynthesis. This energy disproportionation changed in favor of heat dissipation at increasing irradiance, with >99% of the absorbed light energy being dissipated as heat and <1% utilized by photosynthesis at J_{abs} >700 mol photon m^{-2} s^{-1} (>150 J m^{-2} s^{-1}). Maximum photosynthetic efficiencies varied with depth in the euphotic zone between 0.014-0.047 O_2 $photon^{-1}$. Due to steep light gradients, photosynthetic efficiencies varied differently with increasing irradiances at different depths in the euphotic zone; e.g., at J_{abs}>700 mol photon m^{-2} s^{-1}, they reached around 10% of the maximum values at depths 0-0.3 mm and progressively increased towards 100% below 0.3 mm. The present study provides the base for

addressing in much more detail the photobiology of densely populated photosynthetic systems with intense absorption and scattering. Furthermore, our analysis has promising applications in other areas of photosynthesis research such as plant biology and biotechnology.

P006

Closely related *Geobacillus* strains show genotypic and phenotypic differences in denitrification

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Introduction: Little is known about the genetic and phenotypic diversity of gram-positive denitrifying bacteria. However, denitrification activity and/or well-known denitrification genes have been demonstrated in members of the genus *Geobacillus*, such as *Geobacillus thermodenitrificans* (Feng et al., 2007; Manachini et al., 2000; Nazina et al., 2001), *G. stearothermophilus* (Garcia, 1977b; Heylen, 2007), *G. subterraneus* and *G. thermoleovorans* (Nazina et al., 2001).

Materials & methods: We compared the production of gaseous denitrification products (N_2O and N_2) for 20 *Geobacillus* isolates after 1-week incubation under anaerobic conditions. Quantitative nitrate and nitrite reduction analyses and analysis of protein concentration was performed. Screening for denitrification activity was performed by the acetylene inhibition method (AIM). Accumulation of N_2O was measured by gas chromatography. All samples were analyzed in triplicate. 16S rRNA gene sequence analysis and AFLP (Amplification Fragment Length Polymorphism) genomic fingerprinting were performed to assess the genomic variability of the strains. Primers targeting the *nirK* and *norB* genes encoding nitrite reductase and nitric oxide reductase specifically in gram-positive bacteria were designed to confirm the presence of these enzymes.

Results: Preliminary results show that 8 and 16 strains were able to reduce nitrite and nitrate, respectively, to gaseous nitrogen oxides (NOx) with the production of nitrous oxide (N_2O) in all strains. The novel gram-positive *nirK* gene was detected in 14 isolates and only the use of derived amino acid sequences resulted in identification of the correct protein (copper-nitrite reductase). Tentative phylotypic analysis of the 16S rRNA genes and AFLP analysis showed that the strains were closely related, but not identical.

Conclusion: Our research shows the value of studying denitrification in pure cultures to elucidate novel aspects of denitrification, such as novel denitrification genes, among denitrifiers.

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P007

Age-related immune responses following *Neisseria meningitidis* serogroup C conjugate vaccination in the Netherlands: a pre- and post-vaccination survey

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Objective: In 2002 a MenC conjugate (MenCC) vaccination was introduced at the age of 14 months and a mass catch-up campaign was performed targeting individuals aged between 1 and 18 years. We determined age-related immune responses before and after introduction of the MenCC vaccine.

Methods: In two population-based studies, established in pre- and post-vaccination periods, polysaccharide-specific IgG, IgM, IgG subclasses and avidity were determined by a multiplex immunoassay. In addition, in a subset of sera MenC-specific serum bactericidal antibody titers were determined.

Results: Overall SBA seroprevalence was 22% and 45% in the pre- and post-vaccination period, respectively. SBA titers and PS-specific IgG show an age-specific trend, with the highest antibody persistence in the oldest vaccinated age-groups. SBA seroprevalence is not significantly different between the pre- and post-vaccination periods in unvaccinated adult groups, whereas the MenC PS-specific antibodies are. In all immunized age-groups higher levels of IgG1 compared to IgG2 were observed, while naturally derived immunity was mainly restricted to the IgG2 subclass. An age-related increase in IgM levels was observed, correlating with the persistence of IgG antibodies with age. Noteworthy, the increase in IgG2 correlated with a reduced IgG-avidity with age.

Conclusions: MenCC vaccination induced higher IgG levels compared to natural exposure, but only older vaccinated age-groups seem to benefit from antibody persistence. Due to mass vaccination, MenC circulation probably decreased, resulting in lower IgG titers in the unvaccinated older age-groups, posing them at risk if MenC starts re-circulating. The MenCC vaccine response appeared to be a mixture of both T cell dependent and T cell independent responses in terms of humoral immunological characteristics.

P008

Seroprevalence of pertussis in the Netherlands: increased circulation of pertussis among adults

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Background and aims: Despite a high vaccination coverage (~96%), an increase of reported pertussis cases has been observed in the Netherlands in the last decades. In a cross-sectional population-based serosurveillance study in 2006-2007 we estimated the age-specific seroprevalence of pertussis infection, and we compared our findings with a similar serosurvey conducted in 1995-1996.
Methods: IgG-Ptx levels were measured in a multiplex bead-based fluorescent immunoassay and divided into four categories: 0-20, 20 to <62.5, =62.5 (suggestive for infection in the preceding 12 months) and =125 EU/ml (suggestive for infection in the last 6 months). Weighted seroprevalence rates were calculated adjusted for age, sex, urbanization degree and ethnicity. To exclude high levels of IgG-Ptx induced by vaccination with acellular pertussis vaccine, only individuals who were not eligible for acellular vaccination (born before 1998) were included.
Results: In 2006-07, 9.3% (95%CI 8.5-10.1) of the population above 9 years of age had an IgG-Ptx concentration above 62.5 EU/ml, which was more than double, compared to 1995-96 (4.0%; 95%CI 3.3-4.7).
Conclusion: Alongside an increase in reported symptomatic pertussis, the seroprevalence of *B. pertussis* has significantly increased 2.3-fold in adolescents and adults in the past ten years. This increase in presumptive pertussis infections is of the same magnitude as the increase in the reported incidence, indicating that the latter is not due to improved reporting rate but to a real increase in the disease burden.
Although changes in the vaccination program have reduced pertussis morbidity in childhood, they have not affected the increased infection rate in adolescent and adult pertussis. Since parents are an important source of infection in infants this emphasizes the need for new vaccination strategies to protect unvaccinated infants who are at highest risk for severe pertussis. In the long term, however, improved pertussis vaccines should be developed which induce long lasting immunity to reduce the pertussis disease burden.

P009

Screening for antagonistic and plant growth promoting properties in bacteria isolated from the Central Andean Highlands

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The potato plant, Solanum tuberosum, is an important crop because of its nutritional and economic value. The demand is growing and production has become worldwide, increasing over the last 10 years. Its origin is situated in the Central Andean Highlands of Peru, Bolivia and Ecuador. Due to thousands of years of mutualism between the plant and bacteria in this region, it is likely that these bacteria have developed plant beneficial properties specific for potatoes. After all, soil microorganisms are known to closely interact with plants and some have been shown to exert plant beneficial properties. Therefore, these bacteria may have interesting biofertilizing, rhizoremediating, phytostimulating, stress controlling or disease suppressing properties.
Potato rhizosphere samples were taken from 8 fields in the Central Andean Highlands of Peru and Bolivia, varying in altitude, soil composition, climatic conditions and land use. Hence, a large variety of micro-organisms was expected. A total of 555 bacteria were obtained using tenfold diluted trypticase soy agar medium and a medium containing ε-caprolactone, a compound structurally related to N-acylhomoserine lactones, as the sole carbon source. Isolates were screened for antagonistic activity against the pathogens Phytophthora infestans and Rhizoctonia solani on plate assays and, if positive, they were further tested for phosphate solubilisation, ACC deaminase activity and IAA production; the latter two activities were also quantified.
Approximately 10 percent of the isolates (58 isolates) seemed to be effective pathogen suppressors and were subjected to further testing. Results showed that a number of isolates were able to produce the plant growth factor IAA and over half the isolates had ACC deaminase activity. Phosphate solubilisation activity ranged from no activity to high levels being observed in the isolates. Subsequent identification of the isolates was performed in two steps: (i) dereplication with MALDI-TOF MS and (ii) partial 16S rRNA gene sequencing. Mainly members

of the genera *Pseudomonas* were found, but the genera *Curtobacterium*, *Bacillus*, *Paenibacillus* and *Flavobacterium* were also represented.
To our knowledge this research is the first to screen plant beneficial properties from a large number of isolates that were obtained from fields where the potato plant has its origin. The study aims at benefiting the plant-food-consumer chain and could help to reduce excessive use of agrochemicals.

P010

Evolution of a high-prevalent clone of *Pseudomonas aeruginosa* in a cystic fibrosis patient during chronic infection

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Introduction: Adaptation of *Pseudomonas aeruginosa* (PA) during chronic lung infection in cystic fibrosis (CF) patients includes conversion to a mucoid phenotype, increased antibiotic resistance and reduced virulence.[1] We reported previously on the existence of a high prevalent PA clone, ST406, among Dutch CF patients.[2] To investigate adaptation of this clone to the CF lung during chronic infection two ST406 isolates, recovered from the same patient three years apart, were analysed.
Methods: The first ST406 strain was obtained one month after first PA colonization (2004) and the second isolate three years later. Both had identical MLST, MLVA and SNP typing (AT biochip) and were sequenced using 454 pyrosequencing, and annotated using RAST. Gene content differences were confirmed by PCR and evaluated in a set of 30 different ST406 isolates and 30 non-ST406 isolates. Differences in gene expression were assessed using a PA Affymetrix microarray.
Results: Whole genome sequencing revealed a highly conserved gene content in both isolates. The only observed difference was the insert of a gene cluster of five genes in the late isolate next to a tRNA gene. The genes putatively encode a bacteriophage protein, an intergrase and three hypothetical proteins. This gene cluster was found in 12 of 30 ST406 isolates and in none of 30 non-ST406 isolates.
Transcriptomics revealed that in the late isolate 76 genes were at least 2-fold up-regulated and 34 genes 2-fold down-regulated compared to the early isolate. The down-regulated genes include known virulence genes like those encoding the type III secretion apparatus, exoS and iron transport proteins.
Conclusion: Whole genome sequencing revealed high conservation of the gene content in an early and late isolate recovered from a CF patient, after one month and three years of colonization, respectively. Yet, it is not clear if or how the acquired gene cluster facilitates adaptation during chronic infection. Several of previously described virulence factors were down-regulated in the late isolate corroborating previous findings of reduced virulence of chronically infecting PA strains.

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P011

Role of the IL-23 receptor for IL-17 responses in human Lyme disease

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Introduction: Interleukin (IL)-23 is known to play a crucial role in the development and maintenance of T helper 17 cells. It has been previously demonstrated that IL-17 is involved in the pathogenesis of experimental Lyme arthritis. However, the precise role of IL-23 and the IL-23 receptor pathway for the Borrelia-induced IL-17 responses or Lyme disease has not been yet elucidated.
Methods: IL-23R SNP rs11209026 was genotyped in 309 individuals suspected of Lyme disease using Taqman assay. Functional studies were performed using peripheral blood mononuclear cells, cytokines were measured using enzyme-linked immunosorbent assay.
Results: Live B. burgdorferi induces production of IL-1, IL-6, IL-23 as well as IL-17 in a dose-dependent manner. Interestingly, when IL-23 bioactivity was inhibited by a specific antibody against the p19 subunit, IL-17 production was significantly downregulated after Borrelia stimulation. In contrast, the production of IFN-α was not affected after blockade by the anti-IL-23 antibody. Moreover, individuals bearing a missense single nucleotide polymorphism in the IL-23R gene (Arg381Gln) produced significantly less IL-17 after stimulation of PBMCs with Borrelia spirochetes, compared with individuals homozygous for the wild-type allele. Despite of this effect, the IL-23R polymorphism did not influence the development of chronic Lyme disease in a cohort of patients.
Conclusions: This study demonstrates that IL-23R signaling is needed for Borrelia-induced IL-17 production and that a IL-23R SNP leads to impaired IL-17 production. However, the IL-23R Arg381Gln polymorphism is not crucial for the pathogenesis of chronic Lyme disease.

P012

Antimicrobial resistance of *Helicobacter pylori* to clarithromycin and metronidazole in a tertiary hospital in the Netherlands

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Introduction: Antimicrobial resistance of *Helicobacter pylori* (*H. pylori*) is increasing world-wide and is one of the main reasons for eradication failure. Data about the resistance of *H. pylori* to clarithromycin and metronidazole in adults in the period between 1993 and 2003 in the Netherlands are known. Since 2003 no further data on resistance in adults have been published, and there is no known resistance data for infections in children. As failure of first eradication treatment diminishes eradication success in the future, we investigated the resistance prevalence of *H. pylori* in children and adults to the most commonly used antibiotic components of the triple therapy regimen: clarithromycin and metronidazole. Our objective was to compare the results with the data known from literature.

Methods: A single centre retrospective database study from 1 January 2000 to 31 December 2009 in the Leiden University Medical Centre (LUMC). All patients undergoing an upper endoscopy with a *H. pylori* positive culture from the antral and/or corpal biopsy confirmed with gram stain and a positive oxidase, catalase and urease test were included. Antimicrobial susceptibility of the *H. pylori* positive strains was determined by E-test with cut-off values for clarithromycin=0.25 mg/L=sensitive and >0.25 mg/L=resistant and for metronidazole=8=sensitive, >8 and=16 mg/L=intermediate and >16 mg/L=resistant. Results were compared to data from literature on Dutch adults before 2003 and to data from a multicentre study of children in Europe.

Results: 1144 cultures from 1092 adults and 78 cultures from 77 children were included. Resistance prevalence of *H. pylori* to clarithromycin in adults was 10.1% and in children 7.2%. Resistance to metronidazole in adults was 22.2% and in children 11.8%.

In earlier studies in the Netherlands the prevalence of resistance in adults to clarithromycin was=5% and to metronidazole 7-33%. Resistance prevalences of *H. pylori* in children in our centre were compared with data from the European study of Koletzko et al. in 2006. Overall clarithromycin and metronidazole resistance prevalences in their multicentre study were 24% and 25% respectively.

Conclusion: Comparing our resistance data of *H. pylori* in adults with the data from earlier Dutch studies, clarithromycin resistance in the LUMC is higher, while metronidazole resistance is equivalent. Resistance prevalences of *H. pylori* to clarithromycin and metronidazole in Dutch children are lower when compared to European data. In our study clarithromycin resistance in adults is higher than in children. Based on these results we conclude that surveillance of local *H. pylori* resistance is recommended in order to increase the success of the empirical eradication treatment.

P013

Identification of regulatory genes involved in *Enterococcus faecium* biofilm formation

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Biofilm (BF) studies have become important tools in understanding the role of putative virulence factors. BF consists of a population of bacteria, encased in a matrix of exopolymeric substance, attached to various biotic and abiotic surfaces, usually at a solid-liquid interface. BF formation involves a complex process divided into distinct phases: attachment and immobilization on a surface, cell-to-cell interaction, formation of microcolonies, development of three dimensional BF structures, and dispersal of seeding cells. The nosocomial pathogen *Enterococcus faecium* (Efm) is the third most frequent pathogen causing nosocomial bacteremias. This pathogen is intrinsically resistant to many antibiotics and has been very successful in acquiring additional resistance traits. BF formation is a critical step in many types of difficult-to-treat infections, including Efm infections, and interference in this process by targeted drugs will be needed to combat such pathogens in the future. As hardly anything is known about BF formation in Efm, the aim of this work is to elucidate this process, specifically at the level of gene regulation. Using a BF semi-static model with shear forces, transcriptional profiles of planktonic cells and biofilm grown for four hours of clinical bloodstream isolate E1162 were compared by microarray analysis. Based on differentially expressed regulatory genes, single-crossover mutants were constructed to investigate the role in biofilm formation. The effect of gene deletion on cell metabolism was estimated, by growth under various conditions, and the effect on BF formation was analyzed in polystyrene assays and the semi-static model. Three-dimensional images of BF in the latter model were acquired by a confocal scanning laser microscope and quantified by Matlab/Comstat software. 133 genes were differentially regulated (30 up and 101 down); of which six genes were putative regulator genes. Three genes, marR, ctsR, abrB, family regulator, were selected for gene deletion. Growth kinetics of deletion mutants weren't affected. However, BF formation was significantly reduced in the polystyrene assay and in three days BF in the semistatic model compared to the wild

type strain. BF formation was not completely abolished. In conclusion marR, ctsR and abrB contribute to BF formation in Efm.

P014

Group A *Streptococcus* virulence gene expression is enhanced during co-culture with *Moraxella catarrhalis*

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Introduction: *Moraxella catarrhalis* is an important colonizer of the respiratory tract, and the recent recognition of *M. catarrhalis* as a pathogen has resulted in an increased interest in the pathogenesis of *M. catarrhalis*. Although the literature has tended to report on *M. catarrhalis*, *Streptococcus pneumoniae* and *Haemophilus influenzae* as being the predominant bacteria causing otitis media (OM), studies have shown that group A *Streptococcus* (GAS) may also be included alongside these 3 bacterial species as one of the most frequent causative agents of OM. In fact, the incidence of GAS related OM infections is actually increasing in certain countries of the European Union, approaching 12% in Hungary between the years 2006-2008. The majority of knowledge gathered to date regarding OM infections has been derived from work involving single bacterial species, though recent evidence suggests that OM infection may be significantly associated with the involvement of multiple bacterial species. In this context, the present study was set up to test the hypothesis that co-culture of GAS with *M. catarrhalis* facilitates changes in GAS transcriptome expression such that the pathogenic potential of GAS isolates is increased.

Methods: A serotype M3 GAS strain and a clinical OM *M. catarrhalis* strain were cultured in Todd-Hewitt broth supplemented with 0.2% yeast extract. A co-culture experiment was set up in order to study the gene expression profile of GAS upon co-cultivation with *M. catarrhalis*. Bacterial cells were co-cultured at 37C for 2.5 hours, harvested, and total RNA isolated. This RNA was converted into cDNA, end-labeled and added to a GAS-specific custom Affymetrix microarray. Controls comprised RNA extracted from GAS grown in pure culture under identical conditions as co-culture experiments.

Results: Principal component analysis indicated that the data were of high quality and that the two different growth conditions (co-culture versus single culture) generated distinct GAS gene transcription profiles, with GAS genes being differentially expressed upon co-culture with *M. catarrhalis*. Briefly, when using a gene expression cutoff of value >4, gene expression in co-culture resulted in significant differences in several GAS two-component systems, response regulatory systems and virulence factors, compared to the results observed for GAS pure culture alone.

Conclusion: In a previous study, the author has shown that co-colonization of *M. catarrhalis* and *H. influenzae* is significantly more likely than single species colonization in a child cohort population. Further, evidence has indicated that quorum sensing signaling occurs during *M. Catarrhalis* and *H. influenzae* co-culture, which influences the indirect pathogenicity of these bacteria in polymicrobial infections. Many pathogenic bacterial species use quorum sensing systems to coordinate their gene expression, for example during the formation of biofilms. Biofilm formation has become a 'hot' topic of interest in the study of GAS pathogenicity, as it has been found that many of the GAS genes required for biofilm formation are also important virulence factors. This study provides the first indication that polymicrobial infections involving GAS and *M. catarrhalis* may positively affect the expression of GAS related virulence and biofilm genes, possibly leading to increased pathogenicity during polymicrobial infections.

P015

Horizontal spread of highly resistant gram-negative rods (HR-GNR) - Evaluation of the Diversilab® typing method

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Objectives: The worldwide prevalence of highly resistant gram-negative rods (HR-GNR) is increasing. Typing methods are needed in case of an outbreak or to monitor the endemic situation. In this study we investigated the performance of the DiversiLab typing method in comparison with the AFLP typing method.

Methods: A collection consisting of 653 HR-GNR, that were obtained during a 6 months prospective survey in 18 Dutch hospitals, was typed by AFLP and DiversiLab. Results were compared directly to each other. Subsequently, the sensitivity and specificity of DiversiLab were calculated, using AFLP as the reference method. Furthermore, results were compared by means of epidemiological linkage and Cohen's kappa for agreement was calculated.

Results: DiversiLab considered significantly more isolates (275) to belong to a cluster than AFLP (198). The sensitivity was 83.8%, and the specificity was 78.6%. When epidemiological linkage was included in the analysis, DiversiLab considered 9 isolates as secondary cases which were considered unique in AFLP. Only 2 secondary cases

according to AFLP were missed by DiversiLab. This results in a Kappa for agreement of 0.983.

Conclusion: In daily practice a typing method has to be used in combination with epidemiological information. When this was done, DiversiLab showed to be a highly reliable method for the typing of HR-GNR. This in combination with the ease of use and the speed, makes DiversiLab an appropriate screening in routine clinical practice. When a cluster is suspected and the consequences of these findings are substantial, a confirmatory analysis should be performed.

P016

Identification of *Arcanobacterium (Trueperella) abortisuis*, a novel species of veterinary importance, by Matrix-Assisted Laser Desorption Ionization-Time of Flight (MALDI-TOF) mass spectrometry

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The species description of *A. (T.) abortisuis* was based initially on a single strain isolated from a placenta of an aborted sow in Japan. However, according to Yassin et al. (2010) *Arcanobacterium (A.) abortisuis* together with *A. bernardiae*, *A. bonasi*, *A. bialowiezense* and *A. pyogenes* should be reclassified in the newly described genus *Trueperella* (T.) and genus *Arcanobacterium* should be restricted to *A. haemolyticum*, *A. phocae* and *A. pluranimalium* and to *A. hippocoleae*, a phylogenetic neighbor of this group. In the present study 23 *A. (T.) abortisuis* isolated from pigs and bovines during routine microbiological diagnosis in a period of 12 years could be reliably identified by determination of CAMP-like synergistic hemolytic activities, biochemically, genotypically by polymerase chain reaction with the help of *A. (T.) abortisuis* 16S-23S rDNA intergenic spacer region specific oligonucleotide primer and by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS). The latter appeared to be a promising tool for future identification of this species and might help to elucidate the role *A. (T.) abortisuis* plays in infections of pigs, bovines, possibly other animals or humans.

P017

Evaluation of MALDI TOF mass spectrometry for identification of microorganisms of veterinary origin

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The identification of microorganisms of veterinary origin is usually achieved using cultural and biochemical tests or by semi automated methods. These methods are time consuming, expensive, and technically demanding. In future rapid and reliable, full automated and not expensive methods for identification and differentiation of microorganisms of veterinary origin will become increasingly important. In this study we evaluated the MALDI Biotyper system (Bruker Daltonik) for identification and differentiation of 81 different field isolates obtained from routine veterinary diagnostic and 53 reference strains. The field isolates were previously identified with morphological and biochemical tests or with the API test system. The bacteria used in this study included isolates of genera *Enterococcus*, *Corynebacterium*, *Nocardia*, *Yersinia*, *Actinobacillus*, *Pasturella*, *Bordetella*, *Moraxella*, *Campylobacter*, *Brachyspira*, *Streptococcus*, *Staphylococcus*, and species of the family *Enterobacteriaceae*. In addition, the species *Arcanobacterium pyogenes*, *Actinobaculum suis*, *Ornithobacterium rhinotracheale*, *Mannheimia haemolytica*, *Riemerella anatipestifer*, *Histophilus somnii*, *Avibacterium paragallinarum*, *Taylorella equigenitalis*, *Erysipelothrix rhusiopathiae*, *Janthinobacterium lividum* and *Clostridium sordellii* were included. A total of 115 (85.8%) isolates were identified to species level and 12 (9.0%) isolates to genus level. Seven (5.2%) isolates were not reliably identified. For further improvement of the system bacterial strains from genera *Brachyspira*, *Corynebacterium* and *Nocardia* and strains from the species *A. paragallinarum*, *S. equi subsp. equi* and *S. equi subsp. zooepidepidemicus* have to be added to the database. The present results showed that MALDI-TOF MS is a fast and reliable method for identification of most of the species of veterinary origin used in this study.

P018

Haemophilus influenzae and *Haemophilus haemolyticus* identification and serotyping by molecular methods

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Isolates of the non-pathogenic *Haemophilus haemolyticus* from respiratory samples are often misclassified as non-typeable *H. influenzae* because bacteriological techniques are not sufficient to distinguish these two closely related species. The presence of *H. haemolyticus* among the *H. influenzae* isolates hampers studies on the pharyncheal flora and limits the use of Slide Agglutination SeroTyping (SAST), since *H. haemolyticus* may give false positive results. In this report, we describe a novel protocol for the distinction of these two *Haemophilus* species and the subsequent serotyping of *H. influenzae* isolates by molecular methods. The protocol consists of two parts: a PCR to distinguish between *H. influenzae* and *H. haemolyticus* using the 7F3 epitope of the ompP6 gene, followed by PCRs on the bexA and capsular genes for serotyping. Among 386 isolates from vaccinated children, we found 18% H. haemolyticus isolates and 82% *H. influenzae*. The latter species was confirmed by the presence of the iga gene, a marker for *H. influenzae*. By SAST we found that all the *H. haemolyticus* isolates reacted falsely with the serotype a antiserum, while as expected the bexA and capsular genes were absent in these isolates. We conclude that the distinction of the two *Haemophilus* species by the ompP6 PCR and the subsequent serotyping of *H. influenzae* gives unequivocal results that will aid studies on the pharyncheal flora.

P019

In vitro derived small RNAs in *Staphylococcus aureus*

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Background: Survival and pathogenesis of microbial pathogens in different host environments requires the ability to respond to stress by altering gene expression in a coordinated manner. Small RNAs (sRNAs) have been shown to play a major role in tightly regulating gene expression. We are interested in finding and characterizing sRNAs regulating virulence genes in *Staphylococcus aureus*.
Methods: A microarray containing 60-mer probes located 20 nt from each other on both strands was developed, thereby covering the complete genome of 14 sequenced *S. aureus* strains. Highly reproducible growth experiments were performed for MSSA476 under standard growth conditions and for 7 MRSA strains with diverse genetic backgrounds under infection related conditions e.g. human serum and CO2. In addition, survival of MSSA476 and MW2 in whole blood was characterized. RNA transcripts were identified by correlating expression patterns of adjacent probes.
Results: Probes in the intergenic regions and opposite of coding regions with a correlation coefficient of =8.0 were considered for the presence of either untranslated regions of mRNAs, new genes or sRNAs. For this we looked at the coding sequence, conservation, expression patterns, location, comparison to known sRNAs, the Rfam database, ORFfinder, etc. Among the ~70 potential sRNAs we have identified, 36 were described previously. Target mRNA was predicted with IntaRNA (http://rna.informatik.uni-freiburg.de:8080/IntaRNA.jsp) using 19 virulence related mRNA sequences. High binding capacities were found for several sRNAs. The 5'- and 3'- ends of these sRNAs were determined and gel mobility shift assays were performed to confirm an *in vitro* interaction between sRNA and virulence related target mRNA. Three sRNAs are under research in over expression and knock-out studies to characterize the *in vivo* function of the sRNAs.
Conclusion: RNA transcripts can be relatively easily identified by correlating expression patterns of adjacent probes in highly reproducible growth experiments, with an accuracy of >80%. We have been able to show an *in vitro* interaction between three sRNAs and virulence related mRNA targets. *In vivo* relevance of this interaction has still to be determined.

P020

Molecular detection of bacterial bloodstream infections: the SepsitestTM assay

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Introduction: Implementation of rapid molecular diagnostics in bloodstream infections could significantly improve speed of diagnosis, and thereby outcome. Current molecular tests directly on whole blood samples have suboptimal sensitivity, due to the use of small volumes. Larger volumes show an inhibitory effect of human DNA. The SepsitestTM-assay (Molzym, Germany) incorporates a pre-test enrichment method, which selectively eliminates human cells. This may lead to an increase in input volume and subsequent diagnostic sensitivity. We investigated the use of this assay in a cohort of patient with sepsis on the ICU.
Methods: We analysed 55 septicaemic patients on the ICU in whom blood cultures (BC) were taken. Together with the BC (n=90), an additional blood sample (EDTA) was taken from the same sample site, for analysis with the SepsitestTM-assay (ST). The assay consists of a pre-test-enrichment on 1 ml EDTA-sample, followed by bacterial lysis and DNA-isolation, and subsequent amplification using 16S-based universal primers for gram-negative and gram-positive bacteria. Amplicons are detected by

agar-based gel-electrophoresis. In accordance with instructions of the manufacturer, EDTA-samples were analysed in duplicate, and a sample was considered positive if at least one of the duplicates was positive.

Results: Of the 90 BC, 5 (6%) yielded positive results; *S. aureus* (n=1), *E. faecalis* (n=3), and *CNS* with *E. faecalis* (n=1). Bacteraemia was diagnosed in 3/55 (5%) of the patients. ST was positive in 11/90 (12%) of the samples. Compared to BC, sensitivity was 80% (4/5 positive BC). In total 2/3 patients (66%) with positive BC was positive with ST. Seven patients had positive ST and negative BC. In all of these patients, the clinical suspicion of bacterial infection was high, and 3 of them showed positive BC during septicaemia, but at another time point when no EDTA-samples had been taken for this study. These results might therefore represent additional yield of the SepsitestTM-assay. Sequencing of amp icons is currently being performed, to provide species-specific results and control for false-positive results.

Conclusions:

- The SepsitestTM-assay can be used in clinical practice for molecular analysis directly on blood samples.
- Implementation of the assay may provide additional detection of bacteraemic patients, but results have to be evaluated with sequence results.
- Gel-based analysis is applicable, but may be troublesome to implement in the routine workflow.

P021

Molecular survey of beta-lactamases conferring third generation cephalosporin resistance in *Enterobacteriaceae* from the Netherlands

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Objectives: Despite increasing incidence of infections caused by pathogens harbouring beta-lactamases (BLs) conferring resistance to third-generation cephalosporins (3gCeph) few large scale European surveys on the molecular epidemiology of Extended-Spectrum BLs (ESBLs) and AmpC BLs have been published. The aim of the study was to describe the molecular epidemiology of BL genes causing 3gCeph resistance in *Enterobacteriaceae* in the Netherlands.

Methods: From February 1st to May 1st 2009, 31 laboratories submitted all *E. coli, K. pneumoniae, K. oxytoca, P. mirabilis* and *Enterobacter* spp. isolates with a positive ESBL screen test (ceftazidime/ceftriaxone MIC>1 mg). The first 25 non-repeat isolates (if available) per lab, were analysed using an ESBL array (Check-Points, Wageningen, NL) detecting TEM, SHV and CTX-M genes. A random selection of 50% was sequenced. Isolates negative in these analyses were investigated by multiplex PCRs, detecting class A BLs, plasmid-borne AmpC and OXA BLs. In addition, the presence of a plasmid-borne AmpC (pAmpC) was tested in all isolates with a cefoxitin MIC=16 mg/L, and if pAmpC negative, the promotor of *E. coli* chromosomal AmpC was sequenced to detect mutations associated with derepression. Genetic relatedness of isolates (except *P. mirabilis*) was determined with DiversiLab (bioMerieux, Marcy l'Etoile, France). 51 ESBL-positive *E. coli* were genotyped by MLST.

Results: 558 (88%) of 633 isolates (75% *E. coli*) included carried a plasmid-borne BL: 526 (83%) at least one ESBL, 52 (8%) a plasmid-borne AmpC. The most prevalent ESBLs were CTX-M-15/28 (44%), CTX-M-1 (18%), SHV-12 (8%), CTX-M-14 (8%), and TEM-52 (6%). pAmpC's belonged to the CMY-2 group (52%; all CMY-2/55), the ACT/MIR group (46%), and DHA-1 (2%). Among the 75 (12%) isolates without a plasmid-borne BL, 3gCeph resistance of 29 (5%) *E. coli* was caused by AmpC promotor mutations, of 5 (1%) K. oxytoca by OXY hyperproduction and for 22 (4%) *E. cloacae* likely by chromosomal AmpC. For 19 (3%) isolates no resistance mechanism was detected. DiversiLab showed no evidence of dissemination of a single strain, nationally or locally. However, 22 *E. coli* isolates belonged to ST131.

Conclusions: The most prevalent BL genes causing 3gCeph resistance in *Enterobacteriaceae* in the Netherlands were CTX-M ESBLs, especially CTX-M-15. In comparison with other European surveys a relative high prevalence of CTX-M-1 and TEM-52 genes was observed. CMY-2/-55 was the most prevalent AmpC.

P022

Evaluation of the Check-KPC ESBL microarray as a confirmation test for the presence of extended-spectrum beta-lactamases in the routine clinical setting

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Objectives: For rapid detection of ESBL of the CTX-M, TEM and SHV families we have co-developed an ESBL microarray. The diagnostic characteristics of this microarray have not been determined as a confirmation test for the routine clinical setting. The aim of this study was to determine the accuracy of the ESBL microarray as a confirmation test of ESBLs in the routine laboratory setting, i.e. on randomly selected clinical isolates with a positive ESBL screen test.

Methods: The microarray (Check-KPC, Check-Points, Wageningen) was evaluated on a random selection of 344 clinical isolates with a positive ESBL screen test (MIC >1 mg/L for cefotaxime or ceftazidime or an ESBL alarm from the Phoenix or Vitek-2 expert system) collected from 31 clinical microbiology laboratories in the Netherlands in 2009, using sequencing as the reference method.

Results: Of the 344 isolates, 75% were *E. coli* (n=257), 10% *K. pneumoniae* (n=35), 10% *E. cloacae* (n=33), 3% *P. mirabilis* (n=10) and 3% *K. oxytoca* (n=9). Based on PCR and sequencing, 245 isolates were ESBL positive and 99 ESBL negative. Among the 245 ESBL-positive isolates in total 251 ESBL genes were identified: 208 CTX-M, 25 SHV, 16 TEM, 1 GES and 1 PER. The sensitivity of the microarray was 97% (237/245), the specificity 98% (97/99), the positive predictive value 99% (237/239) and the negative predictive value 92% (97/105). In 6 isolates, a CTX-M-1 group ESBL gene was not detected even after repeating the test and 2 isolates contained an ESBL gene not included in the design of the array (PER and GES). A false-positive result was obtained in 2 isolates containing a TEM-1 gene. However, a TEM-17 and a TEM-19 group ESBL gene were identified by the array and both isolates had an ESBL-positive phenotype as determined by Etest ESBL. These false-positive results may be explained by the limitation of using an unselective PCR and sequencing as the reference test, which may fail to detect TEM ESBL genes in the presence of non-ESBL TEM genes, whereas the microarray system uses a selective amplification approach that detects these TEM-ESBL genes accurately.

Conclusion: This study shows that the ESBL microarray is an accurate confirmation test for the presence of ESBL genes in the routine clinical setting.

P023

Performance of the Dutch national phenotypic ESBL detection guideline in clinical setting

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Objectives: In 2008, the ESBL working party of the Dutch Society for Medical Microbiology (NVMM) formulated and implemented a guideline for phenotypic screening and confirmation of ESBLs in *Enterobacteriaceae*. This guideline recommends to confirm ESBL production with ESBL Etest or combination disc (CD) in *Enterobacteriaceae* with a ceftazidime/ceftriaxone MIC>1 mg/L or an ESBL alarm by Phoenix or Vitek-2. The objectives of this study were to determine the accuracy of phenotypic ESBL detection in Dutch clinical laboratories using this guideline and to compare the performance of Etest and CD as ESBL confirmation tests in the clinical setting.

Methods: From February 1, 2009 - May 1, 2009, 20 laboratories submitted all *E. coli*, *K. pneumoniae*, *K. oxytoca*, *P. mirabilis* and *Enterobacter* spp. isolates with a positive ESBL screen test to a reference laboratory. The first 25 non-repeat isolates per laboratory were included. Genotypic detection of ESBLs using microarray analysis and sequencing was used as reference test. Phenotypic confirmation tests were centrally repeated in case of a discrepant result of the phenotype reported by the participating laboratory and the ESBL genotype.

Results: Of 440 included isolates (74% *E. coli*, 12% *E. cloacae*, 8% *K. pneumoniae*, 3% *P. mirabilis* and 2% *K. oxytoca*), 313 (71%) isolates harboured an ESBL gene. The sensitivity of the phenotypic ESBL confirmation tests as performed in the participating laboratories was 95%, the specificity 72%, the positive predictive value (PPV) 93%, and the negative predictive value (NPV) 89%. When the phenotypic confirmation was repeated centrally, the phenotype of 6/11 (55%) false negative and 14/21 (67%) false positive isolates was in agreement with the genotype. ESBL confirmation was performed with Etest on 279 isolates, with CD on 135 isolates and with both on 26 isolates. Sensitivities of Etest and CD were not statistically different (96% and 92%, resp). The CD was more specific than Etest (91% vs 61%, resp; $p<0.001$). However, Etest and CD did not have significantly different PPV (94% vs 96%, resp) and NPV (94% vs 83%, resp).

Conclusion: Implementation of a national guideline has resulted in adequate phenotypic detection of ESBL-positive isolates, and generation of reliable surveillance data in the Netherlands. Although the sensitivity, PPV and NPV of CD and Etest as ESBL confirmation tests were comparable, Etests were less specific due to non-determinable and false positive results.

P024

Antibiotic resistance levels in commensal *E. coli* on pig farms in the Netherlands – a pilot study

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Introduction: Antibiotic resistance in bacteria is an increasing problem in both human and veterinary healthcare. Usage of antibiotics results in selection and spreading of antibiotic resistance determinants. The resistance of commensal microorganisms in the intestines (like *E. coli*), reflects the resistance selective pressure caused by antibiotic usage.

The objective of this pilot study was to evaluate the usage of pooled samples for the determination of the resistance level on a farm and to determine the resistance level for five selected antibiotics on 12 pig farms.

Materials and methods: From 12 randomly selected pig farms (4 piglet producers, 4 finisher farms, 4 farms with both weaned and finisher pigs) ten individual faecal samples were collected from weaned (average age: 60,5 days) and/or finisher (average age: 168 days) pigs.

The samples from each farm were pooled (pools of 10 and 5 samples). Each sample (individual and pools) were serial diluted and plated out onto MacConkey agar plates. After overnight incubation from each sample, 90 *E. coli* colonies were suspended each in a well of a 96-well plate filled with Cation Adjusted Mueller-Hinton Bouillon (CAMHB). Using a 96-pin replicator these suspensions were transferred onto Mueller-Hinton agar plates with the following antibiotics: amoxicillin 25 mg/l, tetracycline 25 mg/l, cefotaxime 0.5 mg/l, ciprofloxacin 0.125 mg/l, trimethoprim/sulfamethoxazole 8/152 mg/l and neomycin 8 mg/l. After incubation all grown colonies were counted and the percentage of resistant *E. coli* colonies was calculated.

Results: The average resistance level of commensal *E. coli* in this pilot study, using the results of individual samples, was highest for tetracycline (64,2%) in weaned piglets. Furthermore, a high level was determined for trimethoprim/sulfamethoxazole (54.4% in finisher pigs, 50.4% in weaned piglets), for tetracycline in finisher pigs (46.6%) and for amoxicilline (41.8% weaned piglets, 48.1% finisher pigs). Low level of resistance were detected for neomycin, cefotaxime and ciprofloxacin in weaned piglets (3.4%; 1.4% and 1.3%, respectively), and 1.7% for neomycin in finisher pigs. No resistance for cefotaxime and ciprofloxacin were detected in faecal samples from finisher pigs. Analysis of pooled samples (pools of 5 or 10 samples) generates comparable resistance level for most of the antibiotics.

Conclusions:

1. The resistance level in commensal *E. coli* is highest for tetracycline in weaned piglets, and high for trimethoprim/sulfamethoxazole, amoxicilline for weaned and finisher pigs and for tetracycline in finisher pigs.
2. For neomycin, cefotaxime and ciprofloxacin low levels of resistance were detected (if detected at all) in this pilot study.
3. According to this pilot study, pooled faecal samples can be used for the determination of the antibiotic resistance level in pig farms.

P025

Possible transmission of *Bartonella henselae* after a tick bite?

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Bartonella henselae causes cat-scratch disease (CSD) in humans. The cat is the main reservoir of this bacteria, and is the only known source of transmission to humans. However, *Bartonella* species are also detected in ticks, and *B. henselae* has been reported in 1.4% to 3.7% of *Ixodes ricinus* ticks in Europe. CSD is frequently diagnosed in patients who report no contact with cats, so tick bites might be an additional source of *Bartonella* transmission. We investigated this risk by analyzing the blood samples of tick bite and erythema migrans (EM) patients for *Bartonella*-specific IgG.

Sera were collected from tick bite patients at the time of the bite (n= 298) and from EM patients at the time of onset (n= 102 EM), and from both groups 12 weeks thereafter. The sera were then analyzed for the presence of *Bartonella henselae* IgG antibodies using an in-house ELISA.

No significant differences were observed between the serological outcomes (age and gender distribution) of EM and tick bite patients. *Bartonella*-specific IgG was present in the first blood sample from 46% and 49% of the tick bite and EM patients respectively, indicating a high seroprevalence in the general population. Two (0.7%) seroconversions were observed after a tick bite, and none were observed in EM patients. A clear increase in IgG level was seen in 5 (1.7%) and 2 (2.0%) tick bite and EM patients respectively. In contrast, a considerable decrease was also observed in 2 (0.7%) and 1 (1.0%) of the tick and EM patients respectively.

The two observed seroconversions in the tick bite group may indicate the possible transmission of *Bartonella* by ticks, but this cannot be concluded based on serology results alone. The ticks from these patients should also be examined for the presence of *Bartonella* species and the duration of attachment. Any reported clinical symptoms that may have been reported by patients with seroconversion or clear increases in antibody levels are also of interest. It should be stated that the 12 week duration between sample collections may not have been optimal for studying the dynamics of Bartonella antibodies – especially for EM patients, in whom *Bartonella*-specific IgG might already have been present in the first sample (in the case of a recent transmission).

Our results show that the transmission of *Bartonella* by ticks is unlikely to play a major role in the transmission of CSD, but cannot be excluded.

P026

The role of immature virus particles in dengue pathogenesis

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Dengue virus (DENV) is a major emerging pathogen which causes disease symptoms ranging from febrile illness to devastating hemorrhagic manifestations. Increased disease severity is associated with pre-existing DENV antibodies and high circulating virus titers, suggesting that antibodies directly influence the infectious properties of the virus. The molecular mechanism by which antibodies enhance DENV infection however remains elusive.
Cells infected with DENV release a high proportion of prM-containing, immature virions. It is generally believed that immature particles are irrelevant by-products of infected cells since numerous functional studies have demonstrated that fully immature particles lack the ability to infect cells. On the other hand, dengue-positive patients secrete substantial levels of prM antibodies, which may suggest that immature particles are involved in disease pathogenesis.
In an attempt to unravel these contrasting paradigms, we investigated the infectious properties of immature DENV, opsonized with anti-prM antibody, in various FcR-expressing cell lines and also in human primary PBMC (peripheral blood mononuclear cells). We found that immature DENV particles become virtually as infectious as wild-type virus in the presence of antibodies. We demonstrate that prM antibodies facilitate efficient binding and cell entry of immature particles into Fc-receptor expressing cells. In addition, we observed that enzymatic activity of furin is critical to render the internalized immature virus infectious. Furthermore, we found that multiple E antibodies interact with immature particles and our current analysis suggests that some of these antibodies stimulate the infectious properties of the virus. Not only monoclonal antibodies, but also DENV immune sera were observed to trigger the infectivity of immature DENV.
Taken together, our data suggest that in presence of antibodies, immature DENV has the potential to become highly infectious. Hence, immature DENV may well play an important role in DENV pathogenesis, in particular during secondary infections which may involve the development of severe disease manifestations such as Dengue Hemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS).

P027

Optimization of transformation of the mushroom *Agaricus bisporus*

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Introduction: The white button mushroom *Agaricus bisporus* is commercially the most important edible mushroom. It is extensively cultivated throughout the world and contributes to about 40% of the total world production of mushrooms. Despite its commercial importance, molecular tools for *A. bisporus* have been poorly developed. For instance, there are no reported cases of genes that have been inactivated by homologous recombination. It is our aim to improve transformation of *A. bisporus* and to set up a gene inactivation system based on homologous integration.
Methods: To improve transformation we systematically optimized protoplast formation and protoplast regeneration. We assessed which enzymes and starting material to use for protoplasting. Regeneration was quantified in different media.
Results: Hardly any protoplasts were obtained when vegetative mycelium was used as starting material for protoplasting. In contrast, 1108 protoplasts/ml were obtained from basidia of young mushrooms. For this, we used 0.75mg/ml of lysing enzyme of *Trichoderma harzianum*. Malt extract, compost, potato dextrose broth and carotene had a positive effect on regeneration. An optimal regeneration medium was obtained using DOE (design of experiment) methodology.

P028

The use of a microsphere-based fluorescent multiplex immunoassay for the quantitation of serum antibodies in immune surveillance studies and vaccine trials

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Introduction: To improve multiple testing of humoral immunity against vaccine components of the national immunisation program (NIP), rapid and simple microsphere-based fluorescent multiplex imunoassays (MIA, Luminex xMAP technology) were either developed or adopted and optimized. The vaccine components included the *Bordetella pertussis* antigens: pertussis toxin (PT), filamentous hemagglutinin (FHA), pertactin (Prn), fimbriae (Fim2/3) and diphtheria- and tetanus toxin as a hexaplex assay (DTaP4), mumps, measles, rubella and varicella viruses as a quattroplex assay (MMRV),

Haemophilus influenzae type b and *Neisseria meningitidis* serogroup A, C, Y, W-135 polysaccharide as a pentaplex assay and for the 13 vaccine *Streptococcus pneumoniae* serotypes as a 13-valent assay. The various MIAs were succesfully applied for the quantitation of IgG serum antibodies directed against vaccines or vaccine antigens in immune surveillance studies and vaccine trials.

Methods: The various purified vaccine antigens were covalently coupled to activated carboxylated microspheres (beads) with a two-step carbodiimide reaction. Before coupling capsular polysaccharides were first conjugated to poly-L-lysine. Development, validation and optimization of the MIA was assessed serologically with different serum panels and if possible compared to internationally standardized ELISAs.

Results: Reproducibility of bead conjugation was high for all MIAs (R ranging from 0.97-0.99) and conjugated beads could be stored at 4 C for 12 upto 24 months without quality reduction. No evidence for bead interference was found between monoplex and multiplex assays. The specificity of the methods was shown by a low percentage of heterologous inhibition and a high percentage of homologous inhibition. Compared to the capsular polysaccharides the specificity of the MIAs using coupled protein antigen (DTaP4 and MMRV) was higher. The MIA appeared much more sensitive than ELISA demonstrated by a decrease in lower limit of quantitation ranging from 100-fold (all capsular polysaccharide MIAs) upto 10,000-fold (protein antigen MIAs). With a minimum of one or two (DTaP4 and MMRV MIA) single serum dilutions over 90% of the total antibody concentration range could be measured using as little as two microliter of sample.

Assay reproducibility was high for all individual MIAs with low intra- (<10%) and inter-assay variability (<20%). Most importantly, the correlation of the MIA with ELISA was good to excellent (R ranging from 0.91 upto 0.99) with the best correlations found for the protein antigen MIAs.

Conclusion: Serum (and plasma) IgG antibodies against various vaccine components of the NIP can be measured easy, specific, reproducible and rapid using a multiplex immunoassay. Strong additional advantages of the MIA over ELISA are increased sensitivity and sample throughput as well as small sample volumes and antigen required. The good to excellent correlation with the classic ELISA and successful application in a large national survey and vaccine trials demonstrated that the MIA is a fast alternative method for the detection of antibodies directed against vaccines or vaccine antigens.

P029

Putative role for desmoplakin in RSV budding and syncytia formation

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Respiratory Syncytial virus (RSV) encodes two envelope glycoproteins, which are pivotal for virus entry into host cells and are capable of inducing neutralizing antibodies. The attachment protein (RSV-G) is responsible for initial binding to host cells and the fusion protein (RSV-F) mediates the fusion of viral envelopes with cellular plasma membranes. RSV-F determines species specificity and is essential for virus infectivity. RSV infected cells fuse with other cells via RSV-F as well, which is characterized by syncytia formation. The detailed mechanisms by which RSV entry, budding and syncytia formation occur and the host membrane proteins involved in these processes are still largely unknown though latter molecules potentially all interact with RSV-F.

We aimed to identify RSV-F interacting host membrane proteins using different RSV-F fishing tools. Purified virions, as well as recombinant RSV-F from a stable HEK-293 transfectant were used as bait in both immunoprecipitation and EDC cross-linking reactions with cell extracts from RSV permissive cell lines. Complexes containing RSV-F and host membrane proteins were subsequently identified by Western blotting and identified by mass spectrometry.

Recently, we identified desmoplakin, the prominent subunit of desmosomal complexes, as one of the proteins interacting with RSV-F. Experiments with an anti-desmoplakin antibody, but not the isotype control, showed inhibition of RSV replication. The inhibitory effect was observed late during infection, suggesting that the blocking antibody does not inhibit initial binding, in stead blocking the release of newly assembled RSV particles. We therefore hypothesize that desmoplakin might play a role during RSV budding and syncytia formation.

Identification of specific RSV-F interacting host membrane proteins will expand the current knowledge on RSV entry, budding and syncytia formation and will offer valuable targets for the development of effective prophylactic and/or therapeutic treatment.

P030

Evaluation of one-sample testing of SVS and FCU separately and in combination for the detection of *Chlamydia trachomatis* by two amplified DNA assays

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Objective: *Chlamydia trachomatis* (CT) is the most common bacterial sexually transmitted infection. Diagnosis is made by CT detection by nucleic acid amplification tests (NAAT). Testing both a self obtained vaginal swabs (SVS) and first-catch urine (FCU) in two separate assays, results in the highest sensitivity. In most laboratories however, one-sample testing is performed for reasons of cost efficiency. To further improve one-sample testing, we assessed the laboratory performance of three different testing approaches to find the most sensitive one-sample test procedure: SVS versus FCU versus a combined specimen of FCU/SVS.
Methods: All women visiting a STD clinic above the age of 16 were asked to participate in the study. Each client was asked to take a FCU and a SVS with a dual swab. One swab was used to create the combination sample. The FCU, SVS and FCU/SVS combination were tested for CT by Strand Displacement Amplification assay (SDA) by Becton Dickinson (ProbeTec ET system, Maryland, USA) or Polymerase Chain Reaction (PCR) by Roche Diagnostics Inc. (Cobas Amplicor system, California, USA). Clients with at least one out of three sample types (SVS, FCU, SVS/FCU combination) tested positive for CT by NAAT, were regarded as CT positive (comparison standard).
Results: In total 791 females were included and CT prevalence was 12% (96/791). The CT detection rate for SVS, FCU and SVS/FCU combination were 94%, 90% and 94%, respectively, if results of NAAT by SDA and by PCR were analyzed together. The detection rate was not significantly different between any of the sample types, when tested solely. Discordance in NAAT results between the different sample types was found in 16 out of 96 CT positive results. Changing the comparison standard to two out of three' samples tested positive by NAAT, did not change the aforementioned results significantly.
Conclusion: Our results show that the detection rate of SVS/FCU combination is equal to that of FCU or SVS alone. SVS is an acceptable and feasible specimen for females. Moreover, SVS is the most cost-effective sample type for a STD clinic population We can therefore conclude that SVS is the specimen of choice to detect CT in females.

P031

Genetic diversity within capsular loci of *Streptococcus pneumoniae* serogroup 6 and 19 isolates

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Introduction: The main virulence factor of *Streptococcus pneumoniae* is the capsule. The polysaccharides comprising this capsule are encoded by approximately 15 genes in the capsular locus which are different for each serotype. The aim of this study was to investigate the genetic diversity of the capsular genes within the serotypes belonging to serogroup 6 (serotypes 6A, 6B, 6C) and serogroup 19 (serotypes 19A and 19F).
Methods: The complete capsular gene locus was sequenced for 25 serogroup 6 isolates and 20 serogroup 19 isolates. If a gene varied in more than 10 base pairs from the reference sequence, the capsular locus was designated as a variant gene (allele). Allele-specific PCRs and sequencing of a number of highly variable capsular genes were performed on all isolates within our pneumococcal collection to identify capsular subtypes. In total 164 serogroup 6 isolates and 195 serogroup 19 isolates were screened using these specific PCRs and sequencing.
Results: Sequencing the whole capsular locus revealed 6 capsular subtypes within the serotype 6A isolates, 3 serotype 6B subtypes and a single serotype 6C subtype. The isolates belonging to serotype 19A and 19F revealed 3 and 4 capsular subtypes, respectively. The screening of the pneumococcal collection with allele-specific PCRs and sequencing added another 6 subtypes within serotype 6A, 3 within serotype 6B and also 3 within serotype 6C. For serogroup 6, the genetic background, as determined by Multiple Locus Variable number of tandem repeat Analysis (MLVA), seemed to be closely related to the capsular subtypes. The relation between MLVA type and capsular subtype was less pronounced for serogroup 19 isolates, however, there were still MLVA complexes comprising of predominantly a single subtype. Comparison of serotype 19A isolates obtained before and after the introduction of pneumococcal vaccination suggests a switch towards another predominant capsular subtype in the post-vaccination era.
Conclusion: There is considerable DNA sequence variation of the capsular genes within pneumococcal serotypes belonging to serogroup 6 and serogroup 19. It is still unclear what the consequences of these genetic variations are for the antigenic properties of the polysaccharides. However, certain variants may be less sensitive for the vaccine induced immunity. We have isolated the polysaccharides of these serogroup 6 and serogroup 19 variants and we are currently investigating the reactivity of vaccine induced and of naturally induced antibodies with these polysaccharides. Preliminary results suggest that considerable differences may exist.

P032

Presence of ESBL genes and plasmids in *Escherichia coli* from the community

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Introduction: In a previous study we determined the prevalence of carriage of ESBL-producing *Enterobacteriaceae* (ESBL-E) in patients presenting to their GP with gastrointestinal discomfort in the region of Amsterdam. Fifty of 471 (10.6%) samples yielded ESBL-E. This high prevalence of ESBL-E in Dutch outpatients was unexpected. The isolates comprised 49 *Escherichia coli* strains and 1 *Shigella sonnei* strain. The most prevalent ESBL gene was CTX-M-15. The aim of the present study was to further characterize the ESBL-positive *E. coli* strains by molecular typing to gain understanding of the epidemiology of this emerging resistance in the Dutch outpatient population and to determine which plasmids are involved.

Methods: The 50 ESBL-positive strains were analyzed for genetic relatedness by Amplified Fragment Length Polymorphism (AFLP). Identification of plasmids was done by PCR-based replicon typing (Carattoli et al. J Microbiol Meth. 2005).

Results: We found no indication for clonal spread of particular strains; in a few cases, two identical strains were identified, but we found no indication for an epidemiological relation between the patients (they lived in different geographic areas). The following plasmids were identified: ColE (20/50), Frep (8/50), R (6/50), FIA (4/50), IncI1 (4/50), B/O (2/50), ColEtp (2/50) and P (1/50). In our previous study we identified CTXM-15 in 24/49 ESBL-positive strains. Of these, 37,5% were ColE positive.

Conclusion: 1) Strain typing showed no evidence for clonal spread of particular ESBL-positive strains in the community. 2) ColE was the most prevalent plasmid; this plasmid has been found previously in strains of *Salmonella* and *Klebsiella* isolated from humans. The significance of the frequent occurrence of ColE plasmids in *E. coli* in Dutch outpatients needs to be explored further.

P033

Two major medicinal honeys have different mechanisms of bactericidal activity

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Honey is increasingly valued for its antibacterial activity, but knowledge regarding the mechanism of action is still incomplete. We assessed the bactericidal activity and mechanism of action of Revamil source (RS) honey and Manuka honey, the sources of two major medical-grade honeys. RS honey killed *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* within 2 hours, whereas Manuka honey had such rapid activity only against *B. subtilis*. After 24 hours of incubation, both honeys killed all tested bacteria, including methicillin-resistant *Staphylococcus aureus*, but Manuka honey retained activity up to higher dilutions than RS honey. Bee defensin-1 and H_2O_2 were the major factors involved in rapid bactericidal activity of RS honey. These factors were absent in Manuka honey, but this honey contained 44-fold higher concentrations of methylglyoxal than RS honey. Methylglyoxal was a major bactericidal factor in Manuka honey, but after neutralization of this compound Manuka honey retained bactericidal activity due to several unknown factors. RS and Manuka honey have highly distinct compositions of bactericidal factors, resulting in large differences in bactericidal activity.

P034

High concordance of test results in *Chlamydia trachomatis* detection and genotyping with the rapid and easy Ct-DT assay compared to the Cobas Amplicor CT/NG test

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Introduction: Improving diagnostic methods for the detection of *Chlamydia trachomatis* (CT), including genotyping, can contribute to control of CT by acquiring knowledge on epidemiology, transmission, sexual networks and pathogenicity. In the present study, we have compared the performance of the *Chlamydia trachomatis* detection and genotyping (Ct-DT) kit (Labo Bio-medical Products BV, Rijswijk, the Netherlands) with the COBAS Amplicor CT/NG (Roche Diagnostics Systems, Basel, Switzerland) in a well described female population consulting a sexually transmitted infection (STI) clinic.

Methods: Self obtained vaginal swabs (SVS) were collected from females visiting a STI clinic. The presence of *Chlamydia trachomatis* DNA was determined by the COBAS Amplicor CT/NG. In agreement with the manufacturer, 200 L of COBAS Amplicor CT/NG medium was used for DNA extraction, using the Qiagen DNA mini kit (Qiagen GmbH, Hilden, Germany). DNA was eluted in 100 L, of which 10 L was used for the Ct-DT PCR-DEIA. Ct genotyping was performed using the Ct-DT RHA on all samples showing a DEIA optical density (OD) of at least 0.75 times the OD of the borderline DEIA control. Discrepant samples were retested using COBAS TaqMan CT Test v2.0 (Roche Diagnostics Systems, Basel, Switzerland). Furthermore, Ct load was determined by

using an in-house TaqMan assay. A sample was considered Ct positive (comparison standard) if the COBAS Amplicor CT/NG and Ct-DT DEIA result were both positive or if one these assays and the COBAS TaqMan CT Test v2.0 were positive.
Results: In all, 772 clients were included in the original study. COBAS medium was available from 71 CT positive clients and 179 CT negative samples were randomly selected. With the Ct-DT kit, 68 out of 71 CT positive samples (97%) tested positive and 1 borderline, leaving 2 discrepant results. Retesting of the latter two samples using the COBAS TaqMan assay resulted in two positive tests. All COBAS Amplicor Ct negative samples were also negative with the Ct-DT. The agreement between the Ct-DT and COBAS Amplicor CT/NG was very good: 99.2%, κ=0.98. The Ct load on average in the discrepant samples were 576 and 324 copies/SVS, respectively and these loads are low compared to other SVS tested in the original study. Genotyping results are presented. In 96% of the DEIA positive samples, the serovar could be determined. Of the three non-typable samples, one was serogroup C, one B and in one case no serogroup could be determined. The Ct load was only available in the sample from serogroup C (average 2197 c/SVS). Two clients were infected with two different serovars. (I/F plus C/? and B/E plus C/K). Serovars D/Da, E and F were most prevalent. The serovar distribution is comparable to previously published Dutch data.
Conclusion: In conclusion, we have found a very good agreement between the Ct-DT and COBAS Amplicor CT/NG and were able to determine the serovar in 96% of the Ct positive samples. Due to its excellent performance, we believe this rapid and easy to perform assay can play a major role in future epidemiological studies.

P035
Towards detection of (extinct) life on Mars
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Mars has long inspired scientists as another habitat for life, and it is believed that the conditions on early Mars were similar to those of the early Earth. ESA's ExoMars mission, currently scheduled to be launched in 2018, aims to search for signs of past and present life on Mars. One of the analytical systems that will be installed aboard the ExoMars rover to look for signs of life is the Life Marker Chip (LMC). The LMC is an antibody microarray platform designed for robust detection of markers of life': molecules that are unambiguously associated with life, but that are stable enough to withstand harsh Martian conditions. We aim to develop a set of robust and sensitive aptamers that could potentially replace antibodies currently used in the LMC. Nucleic acid based affinity tools like aptamers could be useful for application in the LMC; due to their sequence and shape aptamers can recognize and bind molecular targets ranging from whole cells and proteins to small organic molecules. Specificity and selectivity of aptamers is often comparable to those of antibodies, however, aptamers are more stable than antibodies. High stability is obviously desired for an assay performed in an extraterrestrial setting.
Streptavidin was used as a proof-of-principle target for the development of an aptamer selection procedure in our laboratory. Aptamers binding streptavidin were finally enriched from a large pool of randomized DNA. Sequence analysis of a clone library (91 clones) prepared from the enriched pool revealed that 5 aptamer sequences are present in different relative amounts. Subsequently binding kinetics were measured using Surface Plasmon Resonance (SPR), showing that similar aptamers have very different binding kinetics.
In conclusion, a successful aptamer selection procedure was established, as well as a method to determine the binding kinetics and affinity of an aptamer. Streptavidin itself is not very suitable as a marker of life, but the developed procedures will be used for the selection and characterization of aptamers specific for markers of life; current efforts focus on ectoine and a-aminoisobutyric acid.

P036
Molecular host-pathogen interactions of a highly virulent complemented *Streptococcus suis* isolate
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Introduction: *Streptococcus suis* causes infections in piglets like meningitis, arthritis and pericarditis. 33 serotypes have been described for *S. suis* of which serotype 2 is most frequently isolated worldwide. Within serotype 2 pathogenic, weakly pathogenic, and non-pathogenic strains can be found. In the past a genomic library of a pathogenic strain (10) was introduced into a weakly pathogenic strain (S735). After infection of piglets with the library one specific transformant containing a 3-kb fragment of the pathogenic strain was dominantly enriched in diseased pigs. The selected fragment increased virulence of the weakly pathogenic strain tremendously, resulting in a more virulent strain than donor strain 10. The fragment contained two open reading frames (ORF) in an operon-structure. One ORF showed similarity to folylpolyglutamate synthetase (*folC*), whereas *orf2* did not have homology in the database. The same fragment from the weakly pathogenic strain did not increase the virulence.
Methods: Piglets were infected intravenously with 10^6 CFU of different *S. suis* transformants to determine which ORF

was responsible for the increased virulence. Transformants were analysed *in vitro* using routine molecular tools like quantitative PCR (qPCR), SDS-PAGE and immunoblotting. Innate immune response of porcine blood mononuclear cells (PBMCs) were measured after interaction with *S. suis* transformants using qPCR analysis.

Results: Experimental infection of piglets with S735-pCOM-folC^{10} and S735-pCOM-orf2^{10} demonstrated that orf2^{10} was responsible for the increased virulence. The innate immune response of PMBCs was studied to determine whether S735-pCOM-orf2^{10} induced a cytokine storm. PBMCs were infected with *S. suis* transformants in a 1:1 ratio. All strains showed an induction of pro-inflammatory cytokines that peaked at 4 hr. post-infection (p.i.). No differences were detected between the strains that could explain the difference in virulence.

Sequence analysis revealed differences between the *folC-orf2* operon of S735 and strain 10. One point mutations was located in the putative promoter sequence of the operon. Expression levels of *folC* and *orf2* differed between *S. suis* strains S735 and 10, suggesting the promoter sequence influences expression levels. Two transformants were included in the expression analysis: S735-pCOM-orf2^{10} and S735-pCOM-orf2[S735]. In those transformants there was a 5 fold difference in expression, S735-pCOM-orf2^{10} having the highest expression level. Currently, the promoter sequence of S735 is mutated into the promoter sequence of 10.

Conclusion: We show that overexpression of orf2^{10} substantially increases the virulence of the weakly pathogenic strain S735. *In vitro* studies suggest that, despite the septic shock like symptoms observed, this increased virulence is not due to a cytokine storm induced by the transformant. Promoter studies suggest that differences in expression level of *orf2* might be responsible for the differences in virulence. However, the influence of other sequence differences present in the sequences of *orf2* needs to be examined by mutagenesis studies. Further research into the promoter of orf2, as well as studies into the function of ORF2 *in vivo* are required to fully explain the increased virulence.

P037

Towards the characterization of the Spo0A regulon of *C. difficile*

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Clostridium difficile is a gram-positive, anaerobic bacterium that can form highly resistant endospores. The bacterium is the causative agent of *C. difficile* infection (CDI), for which the symptoms can range from a mild diarrhea to potentially fatal pseudomembranous colitis. Recent years have seen an increase in nosocomial and community acquired CDI due to hypervirulent types. These types are characterized by more frequent and severe disease, potentially linked to increased toxin production and more robust sporulation.

Endospore formation in Firmicutes, including *C. difficile*, is governed by the key regulator for sporulation, Spo0A. In other organisms, this transcription factor is also directly or indirectly involved in processes such as competence for genetic transformation, biofilm formation, resistance to and production of antimicrobial compounds and DNA replication.

We seek to characterize the regulon of Spo0A in *C. difficile*, through an integrated approach of bioinformatics, genetics and biochemistry. We find that *C. difficile* Spo0A shows a high degree of similarity to the well characterized B. subtilis protein, and report that our laboratory strain 630Derm contains an 18bp-duplication near the DNA-binding domain. We have purified both full length Spo0A and its DNA binding domain in order to conduct *in vitro* experiments and are performing chromatin immunoprecipitation analyses using polyclonal anti-Spo0A antibodies to determine *in vivo* targets. The results from these analyses will be presented.

P038

The effects of fluconazole on hyphal growth, wall integrity, the wall proteome and the secretome of the clinical fungus *Candida albicans*

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The pathogenic fungus *Candida albicans* is found in various niches of the human body and frequently causes mucosal infections or even systemic infections when the immune system is compromised. To treat these fungal infections azoles, especially fluconazole (FCZ), are widely used. Azoles block the ergosterol synthesis by inhibiting sterol 14a demethylase, which leads to ergosterol depletion and the accumulation of toxic methylated sterols. As a consequence, membrane fluidity increases, and, hence, the activity of cell wall polymerases and cell wall integrity are affected. In this study we used two experimental set-ups (agarose-grown and planktonic cultures) to investigate the effects of FCZ on the wall proteome and secretome of *Candida albicans*. Whereas agarose-grown cultures better mimick mucosal infections, planktonic cultures are experimentally less demanding. Interestingly, under both culture conditions *C. albicans* responded similarly to FCZ at 0.5 mg/L, showing moderately reduced biomass formation, severely reduced

ergosterol levels, and almost complete inhibition of hyphal growth. By qualitative LC-tandem mass spectrometry analysis of the secretome and relative quantification of the wall proteome using Fourier transform ion cyclotron resonance mass spectrometry (FTMS) we could identify a severe impact of the azole on the abundance of numerous cell surface or excreted proteins. Interestingly, agarose-grown and planktonic cultures showed similar changes in the wall proteome. The composition of the secretome was strongly affected and included seventeen fluconazole-specific secretory proteins. Importantly, several secreted proteins were unaffected, including a set of proteins that seemed consistently and abundantly present under all conditions tested both in this study and in earlier studies, suggesting that they are interesting candidates for clinical marker development. Relative quantification using FTMS of 14N-query walls and a reference mixture of 15N-labeled yeast and hyphal walls revealed considerable changes in relative abundance of nineteen out of twenty-two covalently linked cell wall proteins (the fluconazole-specific Orf19.7104, called Fwp1; higher levels of Als4, Crh11, Pga4, Phr1, Phr2, Pir1, Sap9, Sod4, and Sod5, and of Rbt5 by immunological means; lower levels of Als3, Cht2, Mp65, Rbt1, Rhd3, Plb5, and Ywp1, and of Hwp1 by immunological means). The fluconazole-associated wall protein profile correlated with the known transcriptional data of the corresponding wall protein-encoding genes observed upon cell wall stress. Fluconazole-treated cells also displayed increased sensitivity to compounds that exacerbate cell wall defects. 1. We conclude that planktonic cultures can be used to study mucosal infections. 2. Our data show further that FCZ affects the integrity of the fungal wall and furthermore strongly influences the wall proteome and the secretome. 3. These findings provide valuable information for understanding the mode of action of azole drugs as well as for alternative therapeutic strategies and for early infection markers.

P039

Protective human antibodies against multi-drug resistant *Staphylococcus aureus*

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Introduction: *S. aureus* is an opportunistic community- and hospital-acquired pathogen that employs a great variety of cell wall-associated and secreted virulence factors to subvert its human host. With the ultimate aim to provide new strategies to combat life-threatening staphylococcal infections, it is the objective of the Top Institute Pharma project 'AntiStaph' to develop protective human antibodies for antimicrobial therapy against multi-drug resistant and highly virulent *S. aureus* strains, including MRSA.

Methods: A combination of proteomics, genomics, bioinformatics and immunological approaches was applied to identify conserved immunogenic determinants of relevant *S. aureus* isolates. Purified conserved proteins of *S. aureus* were used for the identification and isolation of antibody-producing B cells from peripheral blood of individuals, who are persistent *S. aureus* carriers. Monoclonal human antibodies were cloned and tested for specificity.

Results: Surface-exposed protein domains (the 'surfacome') of clinically relevant *S. aureus* strains have been identified by gel-free proteomics. Using peptide arrays, a detailed overview has been obtained of potentially relevant immunogenic epitopes in *S. aureus* surface proteins that are recognized by human antibodies. First human monoclonal antibodies, with high affinity for one of the conserved *S. aureus* surface proteins, have been identified.

Conclusions: Good progress has been made in the development of human monoclonal antibodies against identified immunogenic proteins and peptides of *S. aureus*. The prophylactic and therapeutic efficacy and the safety of these human monoclonal antibodies now needs to be determined in preclinical animal models.

P040

Staphylococcal enterotoxins: potential immune evasion molecules

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Introduction: Various bacteria secrete superantigens. These proteins are characterized by their capacity to activate T-cells non-specifically. This ultimately can lead to shock and death of the host. Besides qualities as superantigens, staphylococcal enterotoxins (SEs) confer gastrointestinal toxic properties. SEs are known as the causative agent of classical staphylococcal food poisoning. Over the last years, several subtypes of SEs have been identified. SEs show a remarkable structural homology to staphylococcal superantigen-like proteins (SSLs). These SSLs do not possess superantigenic properties, but were shown to have a role in modulation of innate immune responses. Our hypothesis is that SEs might be involved in immune evasion as well. In this study, we present the results of the first assays exploring this proposition.

Methods: Several SEs were cloned from defined *S. aureus* strains into an expression vector containing a poly-histidine tag, and subsequently transformed into *E. coli* for protein expression. The histidine-tagged proteins were purified

using nickel affinity chromatography. Human leukocytes were isolated from peripheral venous blood of healthy donors. Binding of the histidine-tagged proteins to cells was detected using flow cytometry. Binding of histidine-tagged proteins to immunoglobulin (Ig), coated on a multiwell plate, was examined by measuring optical density.

Results: The following recombinant SEs were tested: SEA, SED, SEG, SEH, SEI, SEM, SEN, SEO, SEP, SER, SEU and SEV. Both SEI and SEM were observed to bind to monocytes and the human acute monocytic leukaemia cell line THP-1 in a concentration dependent manner, with an optimum at 3 ug/mL. Specific binding to all IgG subclasses and to IgM was detected for SEN.

Conclusion and discussion: Based on the binding of SEI and SEM to monocytes and on the binding of SEN to immunoglobulin, these staphylococcal enterotoxins were selected as potential immune modulating molecules. The genes of all three SEs are encoded by the enterotoxin gene cluster (*egc*). This operon structure is present in the majority of *S. aureus* isolates. *Egc* encoded SEs differ from other SEs in that they are expressed in the exponential phase of growth, and that neutralizing antibodies are absent in clinical sera. This makes SEI, SEM and SEN interesting candidates to test in future functional assays.

P041

Differences in the J1 region of SCCmec type V [5C2(&5)] subtypes of *Staphylococcus aureus* ST398 lineage might be host related

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Introduction: *Staphylococcus aureus* is a major human and animal pathogen, which is difficult to combat due to its resistance against multiple antibiotics. Methicillin resistance is associated with the presence of the mobile genetic element Staphylococcal Chromosomal Cassette mec (SCCmec). In *S. aureus* eight types of SCCmec elements have been described so far. Each cassette contains a mec gene complex with the mecA gene for methicillin resistance, and a ccr gene complex with genes for recombinases. These complexes are flanked and separated by so-called 'joining-regions' (J1, J2 and J3).

In Community Acquired (CA)-MRSA and Livestock Associated (LA)-MRSA ST398 strains homologous SCCmec type V (5C2&5) elements have been identified. Most cassettes share the conserved organization of orfX-(J3 containing a type 5 ccr gene complex)-mec gene complex-(J2)-type 5 ccr gene complex containing ccrC-(J1)-orfY.

Methods: Two ST398 MRSA strains isolated from patients in Sweden, 22 ST398 MRSA strains isolated from people having direct or indirect contact with pigs and 5 ST398 MRSA isolates from animals were analyzed for the presence of SCCmec by PCR. Strains were typed using Pulsed-Field Gel Electrophoresis (PFGE), Multi Locus Sequence Typing (MLST) and Multiple-Locus Variable number tandem repeats Analysis (MLVA). PCR combined with sequence analysis was used to determine the composition of the J1 region of SCCmec type V (5C2&5).

Results: Sequence analysis of the J1 region from the ST398 patient isolate UMCG-M4, showed a region of ~13 kb that is homologues to the J1 regions present in the type V (5C2&5) elements of *S. aureus* strains PM1 and JCSC 5952. This region is followed by a ~12 kb region completely homologous to a region flanking orfY in SCCmec of *S. aureus* (LA)-MRSA ST398 strain SO385. Both regions are separated by an integration site sequence for SCC which is also present in the J1 regions of other cassettes that carry a different gene content suggesting a hotspot for recombination. A PCR based approach to compare the composition of SCCmec elements showed that of 29 MRSA ST398 isolates from animals and human most contain a type V (5C2&5) cassette, while some contain a cassette of yet unidentified composition. Although some differences were observed within the J1 region, the ~12 kb fragment mentioned above is conserved among all analyzed ST398 isolates. Most LA-MRSA isolates contain a cassette with similar composition as the SO385 strain. All patient related, PVL positive ST398 isolates, have a composition identical as the SCCmec from UMCG-M4. PFGE as well as MLVF and MLVA analyses indicate that patient isolates belong to a separate cluster different from animal related isolates.

Conclusions: These results support the hypothesis that SCCmec elements are very mosaic especially within the J1 regions. More importantly, typing of the strains suggests a possible host related variation of the SCCmec type V (5C2&5) especially within the J1 region.

P042

Genomic Sequence Footprinting (GSF): a high-throughput target pipeline for the development of innovative vaccines and antimicrobials

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Introduction: The bacterial respiratory tract pathogen *Streptococcus pneumoniae* is an important cause of otitis

media, pneumonia, sepsis, and meningitis. The World Health Organization (WHO) estimates that pneumococcal infections are responsible for over 2 million deaths each year. The main risk factors for pneumococcal disease are age, i.e. young infants and the elderly, and an affected immune system, e.g. splenectomized patients and individuals with a concurrent viral respiratory tract infection. Vaccination and antibiotic treatment are important strategies to prevent and treat pneumococcal invasive disease, but the rapid spread of pneumococcal non-vaccine serotypes that are often antibiotic resistant throughout the community threatens their long-term success.

Method: To identify novel bacterial targets for therapy and prophylaxis, Genomic Sequence Footprinting (GSF) was set up as an innovative target pipeline for design of novel intervention strategies. GSF exploits next generation DNA sequencing innovations to generate sequence footprints of bacterial transposon mutant libraries. Changes in library footprints after stress exposure signify genes that are conditionally important for pathogen survival. The GSF technology was validated by identification of *S. pneumoniae* genes specifically required for growth and survival in ambient air outside the human host. These genes likely play a direct role in the transmission of this pathogen between individuals.

Results: GSF analysis of the challenged *S. pneumoniae* mutant libraries reproducibly identified at least 8 genes that were attenuated for pneumococcal growth in CO_2-poor, ambient air conditions. The outcome of the GSF screen was validated by growth inhibition of single *S. pneumoniae* directed mutants in the challenge condition. A mutant in pneumococcal carbonic anhydrase (PCA) had the most pronounced CO_2-dependent growth phenotype. PCA was previously shown to catalyze the reversible hydration of CO_2 to bicarbonate (HCO_3^-). In CO_2-poor conditions this enzymatic reaction is essential for retention of endogenous CO_2 inside the bacterial cell to support metabolic pathways, such as fatty acid biosynthesis. The growth defect of the other *S. pneumoniae* directed mutants was less pronounced and the role of these GSF target genes in the challenge condition remains elusive.

Conclusion: The GSF technology was successfully setup as a target pipeline for novel antimicrobial therapy and prevention. As a proof of concept experiment we identified several *S. pneumoniae* factors that are important for pathogen growth and survival outside the host. These putative transmission factors could aid the design of innovative intervention strategies to prevent acquisition of novel pathogens by the human host. It is foreseen that application of GSF to conditions that reflect other relevant aspects of human disease and pathogen acquisition will enhance the development of other innovative strategies to combat infectious diseases.

P043

Spo0A links DNA-replication with sporulation by direct binding to the origin of replication in *Bacillus subtilis*

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Introduction: When starved, *Bacillus subtilis* cells can enter the developmental program of endospore formation by activation of the master transcriptional regulator Spo0A. Importantly, the mothercell and endospore both inherit a single copy of the chromosome during sporulation and correct chromosome copy number is crucial for the production of mature and fully resistant spores. Accordingly, DNA-replication and sporulation must be tightly coordinated. Spo0A has been shown to be able to prevent unwinding of the origin of replication (*oriC*) and thus initiation of replication *in vitro*, but a study of another group indicates that Spo0A is not capable of inhibiting DNA-replication *in vivo*. We intend to resolve this conundrum and investigate whether Spo0A functions as a direct link between DNA-replication and sporulation.

Methods: ChIP-PCR, surface Plasmon resonance, EMSA, time-lapse and fluorescence microscopy, qRT-PCR.

Results: Spo0A directly binds to a number of specific Spo0A-binding sites that are present near the *oriC*. Cells lacking these Spo0A-binding sites are perturbed in chromosome copy numbers due to over-initiation of replication. The resulting spores exhibit reduced fitness.

Conclusions: Our results support the hypothesis that Spo0A regulates DNA-replication *in vivo*.

P044

A structural rearrangement during the assembly of *Bacillus cereus* pili

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Background: *Bacillus cereus* elaborates pili at its surface by sortase-mediated cross linking of the structural repeating subunit BcpA, while the ancillary subunit, BcpB, is positioned at the tip. Although the biochemical principles of gram-positive pilus assembly have been elucidated, very little is known about the native structures of these fibers. The objective of this study was to visualize pili of *B. cereus* by high resolution transmission electron microscopy (TEM) and to compare these structures with the partial x-ray structure of recombinant BcpA, its subdomains and BcpB.

Methods: Wild-type or mutant ($Asn_{163}Ala$) pili were isolated from *B. anthracis* Sterne Δ*srtA* bacteria containing the *bcpA-srtD-bcpB* operon on inducible plasmids. Hexahistidyl tagged recombinant BcpA, CNA_1 and BcpB were produced in *Escherichia coli*. Recombinant proteins and pili were dialyzed and viewed by negative staining TEM.

Results: Pili are flexible filaments formed from head-to-tail coupled BcpA monomers, capped by BcpB. TEM revealed that each BcpA subunit is comprised of four domains – CNA_1, CNA_2, XNA, and CNA_3. Positional assignments of the Ig-like fold of CNA_2 and CNA_3 as well as the XNA jellyroll domain within pilin subunits was derived by fitting x-ray structural and TEM data. The CNA_1 domain assumes a circular domain with a diameter of 8.9 1.6 nm (n=126) within the full length BcpA precursor, comparable to the diameter of the rCNA_1 protein (9.1 1.6 nm, n= 65). Upon sortase-catalyzed linkage of major subunits, CNA_1 acquires the intramolecular amide bond at Lys_{37}-Asn_{163} and adopts a contracted dimension of 4.7 0.8 nm (n= 102) in native pili, while an $Asn_{163}Ala$ mutant revealed a CNA_1 domain with a diameter of 6.8 2.4 nm (n=106) in mutant pili.

Conclusion: Studying pilus biogenesis with a combination of single-particle high resolution TEM together and X-ray crystallography we gained insights into the structural composition of major pilin subunits within native pili of *B. cereus*. The CNA_1 domain of BcpA contributes to pilus assembly by first enabling the formation of sortase-catalyzed intermolecular amide bonds and then promoting intramolecular amide bonds that allow pili to adopt a protease resistant state.

P045

Screening and confirmation methods for carbapenemases in *Enterobacteriaceae*

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Objectives: In 2010, our group published a guideline for detection of carbapenemases in *Enterobacteriaceae* (IJAA 2010). This guideline recommends a meropenem screening breakpoint of =0.5 mg/L or a zone diameter of =23mm (10ug disk loading), or, alternatively, the less specific ertapenem (=0.5 mg/L). Carbapenemase inhibition tests with boronic acid (BA) were recommended for KPC and with EDTA or dipicolinic acid (DPA) for metallo-carbapenemases (MBL). The aim of this study was to evaluate meropenem and ertapenem as screening carbapenems, and to compare our guideline breakpoints with EUCAST clinical breakpoints. In addition, the inhibition tests with BA and DPA for carbapenemase confirmation were evaluated, and the CICA-Beta-Test as MBL detection test.

Methods: 61 carbapenemase producers were included (44 *K. pneumoniae*, 7 *E. coli*, 5 *Enterobacter* spp., 3 *S. marcesens*, 2 *P. mirabilis* of which 30 KPC, 25 MBL (16 VIM, 6 GIM, 3 NDM), 4 KPC/VIM combined, 1 SME, 1 OXA-48) and 136 negative controls (32 AmpC, 84 ESBL, 3 ESBL/plasmid AmpC combined, 8 K1 hyperproducing K. oxytoca, 2 non ESBL TEM/SHV, 7 *E. coli* without beta-lactamase). MICs of meropenem and ertapenem were determined using broth microdilution (Merlin, Germany). Carbapenem zone diameters were determined using 10ug discs (Mast, UK) and tablets (Rosco, Danmark). The confirmation tests were obtained from Rosco. Cica-Beta-Tests were obtained form Mast.

Results: Carbapenemase screening with a meropenem and ertapenem MIC =0.5 mg/L had a sensitivity of 100% and 92%, respectively ($p>0.05$), and a specificity of 96% and 76%, respectively ($p<0.001$). Screening with the EUCAST clinical breakpoint of meropenem (I/R=4 mg/L) had a sensitivity of 92% and a specificity of 99% (comparison with meropenem =0.5 mg/L not significant, NS). Screening with a meropenem zone diameter =23mm around a disc (Mast 10ug) and tablet (Rosco, 10ug) had a sensitivity of 100% and 97% (NS), respectively and specificities of 99% and 98%, respectively (NS).

Confirmation of KPC production using BA + meropenem combination tablets had a sensitivity of 85% and a specificity of 100%. Confirmation of MBL production had a sensitivity of 79% and a specificity of 100%. When the KPC/VIM double positive isolates were excluded, the sensitivity of APBA/meropenem for KPC production was 97%, and the sensitivity of DPA/meropenem for MBL production was 92%. The sensitivity of the Cica-Beta tests for MBL detection was 41% and the specificity of 34%.

Conclusion: For carbapenemase detection, screening with meropenem is more sensitive and specific than screening with ertapenem. The meropenem =0.5 mg/L screening breakpoint for carbapenemases was 100% sensitive versus 92% using the meropenem EUCAST clinical breakpoint. The inhibition tests with BA for KPC and with DPA for MBLs are sensitive and specific, although those tests failed to detect isolates producing both VIM and KPC. Cica-Beta strips require further development for detection of MBLs.

P046

Assessment of therapeutic efficacy by quantitative PCR in experimental models of Invasive Aspergillosis

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Introduction: Invasive aspergillosis (IA) is an increasingly common infection of both immunocompromised and non-immunocompromised patients. Experimental models of infection have been used to explore pharmacodynamic and pharmacokinetic properties (PK/PD) of antifungal agents as well as the comparative utilities of diagnostic markers. Survival is still considered the gold standard effect measure but has the disadvantage that the large number of animals is needed to determine the dose-response relationships and PK/PD. Measurement of fungal load using culture method is unreliable.
Methods: We performed a systematic literature review to investigate the feasibility of using fungal load determination by real-time quantitative PCR (qPCR) as an effector measure in experimental models of invasive aspergillosis. The English language literature was searched using terms: Invasive Aspergillosis, experimental model, fungal burden, quantitative PCR and therapeutic efficacy. Targets and correlation with other markers were compared.
Results: A total of 11 papers were found that convincingly demonstrate non-culture based tools, in particular real-time quantitative PCR (qPCR) has appeared to be quite promising for assessment of fungal burden in experimental models of IA. In comparison, qPCR has a large dynamic range and better diagnostic capability and have shown that is superior to galactomannan enzyme immunoassay (GM-EIA) and quantitative culture (CFU). The majority of these assays target multicopy ribosomal DNA (rDNA) genes (18S, 28S, and 5.8S) and internal transcribed spacer (ITS) regions (ITS1 and ITS2) particularly for *Aspergillus fumigatus*, in order to maximize sensitivity and specificity.
Conclusion: A limited number of studies describe the use of real-time qPCR for determination of fungal burden. The use of this molecular marker for outcome measure in Pk/Pd relationships requires further studies.

P047

Sugar metabolism discriminates virulent serotype 2 *Streptococcus suis* strains from avirulent strains

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Introduction: *Streptococcus suis* is a zoonotic pathogen that causes infections in piglets with symptoms like meningitis, arthritis and pericarditis. Thirty-three serotypes have been described for *S. suis* of which serotype 2 is most frequently isolated worldwide. Within serotype 2 different phenotypes exist: pathogenic, weakly pathogenic, and non-pathogenic strains can be found. Comparative genome hybridization has shown that whereas weakly pathogenic and pathogenic serotype 2 strains are genetically similar, non-virulent serotype 2 isolates are genetically distinct. These isolates are genetically more similar to serotype 7 isolates. To understand the difference in virulence between the phenotypes more thoroughly, transcriptional analysis was performed on representatives of the phenotypes of serotype 2.
Methods: Representative isolates of each serotype 2 phenotype were grown exponentially in Todd-Hewitt broth. RNA from three independent cultures was pooled and hybridised to a *S. suis* array (Agilent). Microarray data were normalised, and subsequently analysed using KegArray. Metabolic potential of serotype 2 and 7 strains was determined by growing strains in Trypton-Vitamin (TV) minimal medium supplemented with 16 different sugars using a BioScreen (Thermo Labsystems).
Results: Transcriptional analysis of three *S. suis* serotype 2 strains representing the three different phenotypes revealed considerable differences in gene expression. When the list of differentially expressed genes was studied in more detail, several metabolic processes occurred to be differentially regulated between the phenotypes. Pathway analysis using KEGGarray revealed expression differences in vitamin synthesis, purine and pyrimidine synthesis and in several phosphotransferase systems (PTS). To study these metabolic differences, five isolates of each phenotype were grown in minimal TV-medium containing 2% of different sugars as carbon source. Monosaccharide, disaccharide, trisaccharide and polysaccharide sugars were included in the analysis. Based on the previous CGH results, 5 serotype 7 isolates were also included in the sugar growth assay. The results showed that avirulent serotype 2 strains as well as serotype 7 strains are more versatile in their potential to grow on the different sugars than the weakly pathogenic and pathogenic serotype 2 strains.
Conclusion: It was shown that *S. suis* serotype 2 strains of different phenotypes differ in their gene expression after growth in Todd-Hewitt. Pathway analysis revealed that several genes are involved in metabolic processes, as was confirmed by growth of the strains in minimal medium containing different sugars as carbon source. In conclusion there seems to be a relation between metabolic versatility with respect to sugars and virulence of strains. This has been described for other streptococci as well. In particular for *S. equi* strains there is clear evidence that gaining virulence resulted in a loss of metabolic versatility. This is the first indication that something similar might be true for *S. suis*. More research into the link between virulence and metabolism of *S. suis* isolates is required to prove this.

P048

Suspected horse-to-human and human-to-human transmission of a methicillin-resistant *Staphylococcus aureus* ST398 of spa-type t588

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Introduction: Methicillin-resistant *Staphylococcus aureus* (MRSA) is emerging worldwide among humans and animals. In this case study we describe the suspected transmission of an MRSA of ST 398 between a horse, a dog, a nurse and a patient.

The case: An 83 year-old male patient was hospitalized and treated with meropenem because of an urosepsis with an ESBL-producing *E. coli*. Two weeks later a follow-up urine sample was sent to the laboratory. An MRSA resistant to tetracyclines, ciprofloxacin, gentamicin, cotrimoxazole and susceptible to erythromycin, clindamycin, rifampicin and fusidic acid was cultured. Cultures from the nose, throat and perineum from the patient were also MRSA-positive. *Spa*-typing of the isolate by the RIVM showed the rare *spa*-type t588 belonging to the livestock-associated MLST ST398. The patient did not have any contact with animals like pigs or calves. Personnel at the hospital directly involved in care for the patient were screened and one nurse was found to be MRSA-positive. Her housemates and animals (seven horses and a dog) were screened for MRSA. MRSA with an identical susceptibility pattern was isolated from the nares of her husband, child, dog and horse C. A second horse (N), also tested MRSA positive but this isolate had additional resistances to clindamycin and erythromycin. Horse N had been treated with antimicrobials recently due to a wound infection and a lung infection, but no samples were taken at that time. Repeated cultures from the nurse and her housemates were MRSA-negative after eradication therapy. No attempt was made to eradicate the MRSA from the animals.

The seven isolates from the patient, the nurse, her housemates, horses and dog were further characterized by PCR targeting the *fem*A gene and the *nuc* gene, *mec*A PCR, ST398-specific PCR, *spa*-typing and PFGE using *Cfr*9I as restriction enzyme. All isolates were identified as *Staphylococcus aureus*, were *mec*A positive, belonged to MLST ST398, and had indistinguishable PFGE patterns using *Cfr*9I except for the isolate from horse C which had a slightly different pattern. The *spa*-types from all isolates were closely related: all human isolates had *spa*-type t588 (08-16-02-24-25), the isolates from horse C and the dog had *spa*-type t4628 (08-02-24-25), the isolates from horse N had *spa*-type t-034 (08-16-02-25-02-25-34-24-25).

Colonization of people in contact with infected or colonized horses has been widely reported and MRSA ST398 of *spa*-type t588 is cultured regularly from equine samples. Livestock-associated MRSA are rare in humans in the region where the patient lives and human-to-human transmission of MRSA ST398 is uncommon. MRSA of ST398 is also rare in dogs.

Conclusion: In this case is it likely that transmission from nurse to patient occurred at the hospital. As *spa*-type t588 is rare in humans and dogs but common in horses, the horses are the most likely source of the infection. We suspect that the nurse got colonized through contact with her horse and transmitted the MRSA to her patient.

P049

Dimerization of the *Clostridium difficile* anti-sigma factor tcdc

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Background: *Clostridium difficile* is the main cause of nosocomial diarrhoea.

Well known virulence factors for *C. difficile* are the presence of the two large clostridial toxins, TcdA and TcdB and the binary toxin. In addition, high virulence is associated with deleterious mutations in the gene coding for the negative regulator of toxin expression, TcdC.

Toxin expression is positively regulated by the sigma factor TcdR, which is essential for recognition of tcdA and tcdB promoters by the RNA polymerase. TcdC has been described to act as an anti-sigma factor, which means that it prevents toxin promoter recognition by the RNA polymerase/TcdR complex, and thus is responsible for inhibition of transcription of the toxin genes.

Methods: To investigate the biochemical properties of TcdC, TcdC and mutants thereof were expressed in *E. coli* and purified. Mutants had deletions of the hydrophobic N-terminus and the proposed dimerization domain. In addition, some point mutations were introduced in the putative dimerization domain.The dimerization of the mutants was compared to wild type TcdC using crosslinking followed by SDS-PAGE and gel filtration.

Results: TcdC formed dimers and multimers. By deletion of the putative dimerization domain of TcdC, the dimerization of TcdC was indeed strongly impaired.

Conclusions: TcdC dimerizes through a coiled-coil domain, as was previously suggested. We propose that the dimerization of TcdC is essential for its function.

P050

Marine nitrifiers: ecophysiology and interactions

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Under oxygen limited conditions, like those of the oceans oxygen minimum zones (OMZs), different functional groups of nitrogen cycle microorganisms may co-exist such as anammox bacteria, nitrifiers and denitrifiers. This project focuses on ammonium oxidation under limiting oxygen conditions. Although nitrification was previously assumed to be mediated by bacteria, archaeal ammonium oxidizers (AOA) have been postulated as key players in the marine nitrogen cycle. Whether AOA dominate marine nitrification and what physiological traits determine their dominance needs to be elucidated. Detailed physiological studies of the microbial mediators of marine nitrification are impeded by the lack of cultured representatives. Enrichments of marine nitrifiers from the North Sea were established to mitigate this deficiency. Highly enriched (>80%) cultures of marine ammonium-oxidizing bacteria (AOB; a new Nitrosomonas species) and archaea (AOA; a Nitrosopumilus maritimus-like species) as well as nitrite-oxidizing bacteria (NOB, a new species of Nitrospira sp) and anammox bacteria were obtained in batch bioreactor set-ups. Serial dilution series have been established to obtain pure cultures. With the thus generated pure cultures key physiological parameters (e.g. affinity constants for oxygen and ammonium) will be determined and competition experiments performed to yield new insights into the microbial roots of the global marine nitrogen cycle, and their adaptation to low oxygen concentrations.

P051

Staphylococcus epidermidis O-47 constitutively expressing Green Fluorescent Protein (GFP) to study Biomaterial-associated Infections (BAI) in mice

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Introduction: Contamination and subsequent infection are disastrous complications of any implanted biomaterial. Implantation of a biomaterial provokes an inflammatory response known as the 'foreign body response'. Additionally, the host immune system is triggered upon infection. *Staphylococcus epidermidis*, the major cause of biomaterial associated infection (BAI), has been shown to survive inside macrophages around biomaterials implanted in mice, and was retrieved from peri-catheter tissue in humans.

The BMM (BioMedical Materials) NANTICO (Non-adherent ANTImicrobial COatings) program aims to develop and evaluate biomaterial coatings to prevent infection. An *S. epidermidis* strain constitutively expressing Green Fluorescent Protein (GFP) is constructed for the *in vivo* analysis of bacterial survival in the BAI soft tissue mouse model.

Methods: Strain construction: Plasmid pWVW189GFP was transformed into the *S. epidermidis* O-47 strain. It carries the GFP gene under control of a constitutive *Staphylococcus aureus* promoter. The transformation was optimized by pre-treatment with achromopeptidase (ACP). The transformant was analyzed *in vitro* on GFP expression (by FACS analysis), plasmid stability, and for effect of the presence of GFP on the growth rate.

In vivo competition assay: One group of 9 mice was used in the mouse BAI model. A SEpvp catheter segment was inserted subcutaneously at each side of the back. A final inoculum of 2x107 CFU of a 1:1 mixture of *S. epidermidis* wild type and GFP mutant was injected along each implant. Mice were sacrificed after 4 days and a standardized biopsy of 12 mm in diameter was taken from each implantation site comprising the implant with surrounding tissue. The tissue samples were homogenized and quantitatively cultured. Plates were inspected for growth after incubation for 24 h at 37C. Distinction between the wild type and GFP-expressing strains was based on fluorescence.

Results: The transformant had a high level of GFP expression; this strain remained highly GFP-positive upon repeated culture without selection pressure for plasmid maintenance. The expression of GFP had no adverse effect *in vitro* on growth in bacterial and cell culture media.

The *in vivo* competition assay between the *S. epidermidis* O-47 wild type and GFP mutant strain was performed in the BAI mouse model. Both strains were retrieved at almost identical numbers from the biopsies after 4 days in most of the biopsies. At this time point, numbers of bacteria are known to vary, because their clearance is variably advanced. However, independent of the total numbers of colony forming units (CFUs) retrieved, wild type and GFP mutant were retrieved in almost equal numbers.

Conclusions:

1. An *S. epidermidis* O-47 strain, constitutively and stably expressing GFP, was constructed.
2. Plasmid pWVW189GFP was stably maintained in *S. epidermidis* O-47 without selective pressure.
3. GFP had no effect *in vitro* on growth of *S. epidermidis* O-47 in the tested media.

4. The expression of GFP had no influence on the fitness of *S. epidermidis* O-47 *in vivo*.

P052

Comparison of biofilm formation between major clonal lineages of methicillin-resistant *Staphylococcus aureus*

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Introduction: Infections with methicillin resistant *S. aureus* (MRSA) are a major health threat, both in hospital and community settings. One of the survival strategies employed by bacteria is to form biofilms, thereby facilitating a prolonged persistence of infection. The aim of the present study was to investigate whether there are differences in biofilm formation between the various epidemic clones of MRSA and the role, if any, of genetic characteristics like particular SCC*mec* (staphylococcal cassette chromosome *mec*) types or clonal complex (CC), and presence of virulence factors like the Panton Valentine leukocidin (PVL) or the arginine catabolic mobile element (ACME).

Methods: Log phase cultures of 76 isolates belonging to 13 different MRSA clones (EMRSA-15 CC22-MRSA-IV, n=7; EMRSA-16 CC30-MRSA-II, n=4; South-West Pacific CC30-MRSA-IV(-PVL), n=5; USA600 CC45-MRSA-II, n=3; Berlin CC45-MRSA-IV, n=11; Southern Germany CC5-MRSA-I, n=6; New York/Japan CC5-MRSA-II, n=4; pediatric CC5-MRSA-IV, n=5; European CC80-MRSA-IV-PVL, n=4; Iberian CC8-MRSA-I, n=4; Hungarian/Brazilian CC8-MRSA-III, n=12; USA500 CC8-MRSA-IV, n=8; USA300 CC8-MRSA-IV-PVL-ACME, n=3) were studied on a static and a continuous flow (BioFlux, Fluxion, USA) assay. Polystyrene peg plates (Nunc, Thermo Scientific, USA) were seeded in triplicate, incubated for 72 hrs, and the OD_{492} of the crystal violet-acetic acid eluate was measured. BioFlux microchannels were seeded in duplicate, and the medium was flown at 0.5 dyne/cm^2 for 17 hrs followed by live/dead BacLight staining (Invitrogen, Life Technologies, USA). A strain was considered a biofilm former if (i) the OD value was=75% of the positive control (ATCC25923) in the static assay, and (ii) microchannels showed biofilms. Biofilm structure was observed by confocal microscopy.

Results: Overall, 16 strains (21%) were biofilm formers both in the static assay and shear flow assay. Of these, majority harboured SCC*mec*IV (n=13, 81%), and 9 belonged to CC8. All USA300 strains and 5 out of 7 EMRSA-15 strains were positive in both assays. Strains harbouring SCC*mec* type IV, and specifically the CC8-MRSA-IV-PVL-ACME (USA300) clone, produced significantly higher biomass in the static assay (P <0.001, Mann-Whitney-Wilcoxon tests) than the other clones. The South-West Pacific clone did not form biofilms in either assay. Presence of PVL did not significantly correlate to better biofilm formation (P=0.197), while strains harbouring ACME (USA300) did produce higher amounts of biomass (P=0.012).

Conclusions: (i) We show here that, SCC*mec* type IV harbouring MRSA, and specifically the USA300 and the E-MRSA-15 clones, are prolific biofilm formers. (ii) The presence of ACME in the USA300 clone might have facilitated biofilm formation, and needs further investigation.

P053

Antimicrobial resistance of *Escherichia coli* isolated from nursing home residents in the southern part of the Netherlands

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Introduction: The choice for empiric antibiotic treatment should be based on current surveillance data. However, for nursing homes these data are not widely available. In many cases the choice for empiric treatment is based on surveillance data from hospitals. This will result in a relatively high use of inappropriately prescribed broad-spectrum antibiotics which will contribute to an increase of antimicrobial resistance and a more prevalent production of extended spectrum beta-lactamases (ESBLs). Therefore, a surveillance of antimicrobial resistance of *Escherichia coli* and ESBL producing isolates from nursing homes residents in the province of Limburg in the Netherlands was conducted. This study is part of the EurSafety Health-net EMR project.

Methods: *E. coli* strains were isolated from urine samples collected from asymptomatic nursing home residents in 2010. Quantitative antimicrobial susceptibility testing was performed using a micro broth dilution method. Susceptibility breakpoints were defined by the European Committee on Antimicrobial Susceptibility (EUCAST). The prevalence of ESBL producing isolates was determined with a combination disk diffusion test according to NVMM guidelines.

Results: A total of 188 *E. coli* isolates were collected from four nursing homes. The number varied from 33 to 64 isolates per home. Resistance to nitrofurantoin was lowest

(0.5%), while resistance to amoxicillin and amoxicillin-clavulanate was 47% and 22%, respectively. Two isolates were resistant to ceftazidime but only one was a confirmed ESBL producer. Resistance to the carbapenems was not detected. Resistance to ciprofloxacin and norfloxacin was 17% and 21%, respectively and resistance to trimethoprim and trimethoprim-sulfamethoxazole was 20% and 19%. Multidrug-resistance ranged from 8.5% of the isolates resistant to three classes of antibiotics to 1.5% resistant to five antibiotic classes.
Differences in resistance between the four nursing homes were observed. Resistance to amoxicillin and amoxicillin-clavulanate ranged from 39% to 63% (p=0.013) and from 14% to 31% (p=0.023), respectively. Ciprofloxacin and norfloxacin ranged from 11% to 24% (p=0.111) and from 11% to 28% (p=0.05). Resistance to trimethoprim and trimethoprim-sulfamethoxazole ranged from 6% to 31% (p=0.005) and from 6% to 28% (p=0.013).

Conclusions:

1. Resistance among *E. coli* isolates from nursing homes residents was higher for most antibiotics compared with those from primary care patients. In particular, the resistance to the quinolones was relatively high among nursing homes isolates, which was probably due to a higher use of antibiotics among nursing homes residents compared with general practice patients.
2. Only one isolate (0.5%) was ESBL positive.
3. No Carbapenemase producing isolates were found.
4. Resistance varied significantly between the different nursing homes, which is likely due to differences in use. This emphasizes that antibiotic resistance in nursing homes is home specific and that local antibiotic resistance surveillance is important to guide an optimal empiric choice.

P054

Telbivudine exerts no antiviral activity against HIV-1 *in vitro* and in humans

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Introduction: HIV/HBV co-infected individuals who need to be treated only for their HBV infection have limited therapeutic options, since most approved anti-HBV agents have a risk of selecting for drug-resistant HIV mutants. *In vivo* data are inconclusive as to whether telbivudine (LdT) may exert antiviral effects against HIV. Thus, we investigated in further detail the antiviral activity and the biochemical properties of LdT against HIV-1.
Methods: To investigate the activity of LdT against HIV-1 in humans we analyzed viral dynamics and genotypic and phenotypic resistance development in two HIV/HBV co-infected individuals with no prior antiviral exposure. To investigate the activity of LdT against HIV-1 *in vitro*, LdT susceptibility for HIV-1 wild type strains as well as drug-resistant strains was determined. Furthermore, we studied whether the 5'-triphosphate form of LdT (LdT-TP) can act as a substrate for wild type HIV-1 RT.
Results: In the two patients studied, LdT treatment did not result in a significant decline of HIV-1 RNA load nor in selection of genotypic or phenotypic resistance in HIV-1 RT. *In vitro* virological analyses demonstrated that LdT had no activity (EC50 >100 M) against wild type HIV and drug-resistant variants. Biochemical analyses demonstrated that LdT-TP is not incorporated by wild type HIV-1 RT.
Conclusions: Based on the *in vivo* and *in vitro* evidence obtained in this study, we conclude that LdT has no anti-HIV-1 activity and is currently the only selective anti-HBV agent among the five FDA approved nucleoside/nucleotide analogues for treatment of HBV infections in HIV-infected individuals.

P055

Significant increase, but no seasonality in ESBL producing *Escherichia coli* and *Klebsiella pneumoniae* in 47 hospitals in the Netherlands

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Introduction: Recently, a significant increase was reported in the incidence of infections caused by ESBL positive *E. coli* (ECO) and *K. pneumoniae* (KPN) and *K. oxytoca* in 2 German hospitals from 2005 to 2009. Furthermore, a positive association between incidence and mean monthly temperature was observed. The aim of this study was to determine whether we could confirm these findings in Dutch hospitals: an increasing incidence of infections caused by ESBL positive *Enterobacteriaceae* and a relative increase in the summer months.
Methods: Analyses were performed on the database of the national Infectious Disease Surveillance Information System on Antibiotic Resistance (ISIS-AR) that collects monthly all data present in the laboratory information systems on clinically relevant micro-organisms. At the moment of analysis, 18 laboratories serving 47 hospitals (33% of Dutch hospital beds) provided data. From January 2008 until July 2010 the first clinical isolate per hospital

patient was included. An isolate was defined ESBL positive if reported as such or as third-generation cephalosporin (intermediate) resistant. The incidence of ESBL positive ECO and KPN and the monthly variability for 2008 and 2009 were determined. Differences in percentage ESBL positive isolates per month were tested using Chi-square, and for time trend analyses the Cochran-Armitage test was used.

Results: In the 10 quarters study period, for ECO (n=30.935 isolates) as well as for KPN (n=5.078 isolates), the trend test showed a significant increase in the percentage ESBL positive isolates, from 3.4 to 4.8% ($p<0.0001$) and from 4.3 to 7.7% ($p<0.001$) respectively in Dutch hospitals.

During 2008 and 2009, in total 967 ESBL positive ECO and 218 ESBL positive KPN were reported to ISIS-AR. No monthly difference was observed for either ECO ($p=0.08$) or KPN ($p=0.67$). The variability of the monthly incidence for KPN (median 5.2%; range 3.0-8.9%) was higher than for ESBL pos ECO (median 3.8%; range 3.1-5.8%).

Conclusion: From January 2008 to July 2010, the quarterly incidence of ESBL positive ECO and KPN isolates in Dutch hospitals has increased significantly. No seasonality was observed in the incidence of both species during the period 2008/2009.

P056

Typing of group A *Streptococcus* in the Netherlands 2005-2010

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Introduction: Group A *Streptococci* (GAS) can cause a variety of infections, ranging from acute pharyngitis to toxic shock syndrome, puerperal fever and necrotizing fasciitis. Typing of GAS was performed at the National Institute for Public Health and the Environment (RIVM) as part of a national surveillance program until 2003. However, this program was discontinued in 2004. Since then, typing could only be performed on isolates sent in on voluntary basis. In the study we present an overview of the typing results in the past six years.

Methods: During these six years, 815 isolates were submitted to the RIVM. These GAS isolates were typed using the *emm*- and T-typing method. Since 2004, the *emm*-typing method has replaced the older M-typing (reverse line blot) method, overcoming its limitations with unambiguous, more discriminatory sequence data. Furthermore, presence of two toxin genes (*Spe*A and *Spe*C) was determined and limited epidemiological data was collected.

Results: After the surveillance program had been discontinued, a dramatic drop in submitted isolates was observed, compared to previous years was observed. Most prevalent emm-types over this period were types 1, 3, 6, 28 and 89. The trend seen over these years was first a rise of emm-types 3 and 89, replacing type 1 as most frequently isolated type and subsequently emm type 1 returned as the most frequently found type. Of all strains, 90% possessed at least one of the two toxin genes (*Spe*A and *Spe*C) tested for. An elevation in the number of submitted strains was seen in 2006, when more than twice the average amount over the other five years was received. However, typing revealed no rise of one single type, instead different types were elevated at different hospitals.

Conclusion: Partly due to the voluntary basis on which the strains are sent to the RIVM and the dramatic decline in the amount of submitted isolates, it remains unclear what caused the rise of submitted isolates in 2006, the emergence of emm-types 3 and 89 and reappearance of type 1 as most prevalent type. For this reason we advocate the re-initialisation of the national GAS-surveillance as it existed before 2004.

P057

Uptake, trafficking, and survival of *Campylobacter jejuni* in human epithelial cells

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Campylobacter jejuni is one of the leading causes of bacterial enterocolitis worldwide. Infection usually occurs by ingestion of contaminated chicken meat. Severe (bloody) diarrhea, inflammation, and abdominal pain for a period of 5-7 days are common symptoms of a *C. jejuni* infection. In a small number of cases severe sequelae can arise, such as the paralyzing auto-immune neuropathy Guillain-Barr syndrome. The pathogenesis of *C. jejuni* pathology remains elusive. *C. jejuni* colonizes the mucus layer and invades the epithelium. It is assumed that the invasion damages the integrity of the intestinal epithelial lining and is accompanied by immune activation. The mechanism behind invasion and the intracellular fate of the bacteria are unknown. Recently, it was discovered that when *C. jejuni* is starved the bacteria go underneath cultured epithelial cells and invade these cells in high numbers. This process of subvasion followed by invasion makes it for the first time possible to study the molecular mechanism of invasion and the fate of *C. jejuni* in highly infected cells. The goal of the present study was to investigate the uptake, trafficking and intracellular survival of *C. jejuni* using CaCo-2 intestinal epithelial cells as a model. These cells were grown in a transwell system to obtain polarized

monolayers that were infected with GFP expressing *C. jejuni*. Bacterial invasion and translocation across the polarized monolayer were followed by confocal laser microscopy and measurement of the transepithelial resistance (TEER). Intracellular trafficking of the bacteria was determined using specific staining of cellular compartments. Bacterial survival was measured using the gentamicin survival assay. Results demonstrate that *C. jejuni* translocates across the epithelial monolayer without disrupting the tight junctions. Following subvasion, *C. jejuni* invaded non-polarized CaCo-2 cells in a micro-tubule dependent manner. Once inside, they resided mainly in endolysosomal compartments. However, bacterial recovery from the intracellular compartment was only successful within the first 48 h of infection, as determined by the gentamycin survival assay. In our hands, invasion was not accompanied by NF-κB activation as measured via a NF-κB reporter luciferase assay. The absence of RNA upregulation of IL-8 or IL-1 in infected cells confirmed these results. Overall, our data suggest that, at least under the conditions employed, there is no long term-survival of intracellular *C. jejuni* in epithelial cells. Yet, *C. jejuni* may prevent the induction of a local innate immune response.

P058

FluGEM: an intranasal vaccine in the protection against influenza

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Mucosis B.V. is a Dutch biotechnology company developing innovative mucosal bacterial like particle (BLP) based vaccines that can be applied needle-free via the nose. BLP is a recently developed adjuvant consisting of bacterial particles produced by acid treatment of the bacterial cells of the food grade bacterium *Lactococcus lactis*. The result is a cell-shaped cell wall matrix. The primary goal is to develop BLP as a novel adjuvant matrix for use in vaccines administered via the mucosal route (intranasal) and validate the platform by obtaining proof of concept using BLP together with Split Virion Influenza antigen (FluGEM).

Here we show in a mouse model that FluGEM induces both a robust mucosal and systemic immune response sufficient to protect against an otherwise lethal influenza challenge. Moreover, we show that the GEM maturates and activates dendritic cells via TLR2.

Clearly, FluGEM is a promising and attractive influenza vaccine candidate: easy to administer and raising a potent mucosal and systemic immune response, thus already protecting against influenza at the port of entry.

P059

Exposure to interferon beta for 18 hours induces a long-lasting antiviral condition in human epithelial cells

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Introduction: In general, type I interferons (IFNs) exert a pronounced protective effect against viral infections when given prophylactically. Thus IFNs are attractive candidates for preventing seasonal infections and reducing the incidence of virus-mediated exacerbation in chronic obstructive pulmonary disease (COPD) patients. Yet, therapeutic prescription of type I IFNs has remained limited due to significant side effects appearing upon repetitive and/or chronic use. Along these lines, we recently observed that IFNbeta pre-treated epithelial cells were markedly protected against viral infections, even when IFNbeta was removed from the medium before infection. The length of the protection after drug removal of the drug was the subject of this study.

Methods: Human epithelial cells (A549) were treated for 18h with IFNbeta (125U/ml) to induce an antiviral state. Additionally A549 cells were similarly pre-treated with poly(I:C) (50μg/ml), which has been shown to induce type I IFN production by stimulation of either Toll-like receptor 3 (TLR3) or melanoma-differentiation-associated gene 5 (MDA5). Hereafter, the IFNbeta- or poly(I:C)-containing medium was removed and replaced by normal medium. Cells were subsequently infected with the Human Rhinovirus 1B (HRV-1B; MOI 0.1) at 24, 48, 72 or 168 hours after the pre-treatment period. At 48 and 72h p.i., the cytopathogenic effect (CPE) was determined by visual inspection. Also, cells were collected and the number of HRV-1B RNA copies was determined by RT-qPCR.

Results: CPE was pronouncedly reduced at 24, 48, and 72h post-IFNbeta removal. At 168h the protective effect was clearly diminished. Moreover, HRV-1B RNA copy numbers were reduced by 85% when IFNbeta was removed 24h before infection. Surprisingly, despite the increase in CPE, HRV-1B RNA copy numbers were still reduced by 50% at 168hr post- IFNbeta removal. In contrast, no antiviral effects of poly(I:C) were observed at any time point.

Conclusion: These data implicate that a single treatment with IFNbeta, but not with poly(I:C), induces a sustained antiviral effect which lasts at least for 72h. Such prolonged protection may open new opportunities for prophylactic treatment, e.g. of COPD patients at risk of viral exacerbations, without the risk of side effects common after daily administration of type I IFNs.

P060

In vitro evaluation of the impact of *Kluyveromyces marxianus fragilis* b0399 on the human gut microbial ecology

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Introduction: *Kluyveromyces marxianus* is lactic yeast of industrial interest, used in the production of different fermented or diary products. Since its utilization as a candidate probiotic strain has been suggested, evaluation of its potential health-promoting activities and the impact on the human gut microbial ecosystem is required.
Methods: A three-stage *in vitro* continuous culture system simulating the proximal, transverse and distal region of the human colon was employed, and *K. marxianus fragilis* B0399 was dosed at a daily concentration of 10^6 CFU/ mL for a period of 14 days. FISH analysis on thirteen main intestinal bacterial groups was employed to evaluate the impact of the intervention on the gut microbiota of patients affected by irritable bowel syndrome (IBS), while SCFA production was monitored by HPLC. The cytotoxic potential of the fermentation supernatants along the dietary intervention was assessed using a HT-29 cell growth curve assay.
Immunomodulatory activity of *K. marxianus fragilis* B0399 towards PBMC was tested using Bio-PlexTM Cytokine Assay, whereas its ability to adhere NCM460 colonic cells was assessed using a qPCR-based assay.
Results: The administration of *K. marxianus fragilis* B0399 to the *in vitro* colonic model system does not alter drastically the overall composition of the gut microbiota. Level of total bacteria did not change over the course of the intervention, whereas an increase of bifidobacteria ($P<0.05$) occurred in the first and second stage of the fermentative system. An increase in the concentration of propionic acid has been shown as main outcome of the impact of the yeast on the overall SCFA production. Furthermore, no significant changes in the cytotoxic potential of the fermentation supernatants were found in the second and third stages of the colonic model system, as assessed by EC50 measurement. Conversely, the dietary intervention led to a decrease of the cytotoxicity of the metabolites in the first stage representing the proximal colon.
Cytokine assay demonstrated that K. marxianus fragilis B0399 induced a pro-inflammatory response of PBMC (increase in IL-6, TNFα, MIP-1a, IFNγ and IL-1β). Conversely, it ameliorated the overall LPS-induced inflammation (decrease in IL-6, TNFα and MIP-1a). In addition, results provided in this study demonstrated that the lactic yeast strain is strongly adhesive to NCM460 colonic cells.
Conclusions: This study provides findings supporting the utilization of the candidate probiotic strain *K. marxianus fragilis* B0399 for human purposes. *K. marxianus* while is not altering the overall biostructure of the gut microbiota, is able to increase the bifidobacterial concentration and the production of SCFA propionate. A decrease of the cytotoxic potential in the first stage of the colonic model was also found. In addition, results provided in this study suggest that *K. marxianus fragilis <B0399* is strongly adhesive to NCM460 cells and it is able to ameliorate a low grade inflammatory status by downregulating the production of pro-inflammatory cytokines and growth factors in NCM460 cells and PBMC.

P061

Small mutations allow *Bordetella pertussis* to resurge in the face of intensive vaccination

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Introduction: The gram-negative bacterium *Bordetella pertussis* is the causative agent of whooping cough or pertussis, an infection of the respiratory tract. Despite high vaccination coverage since the 1950s, whooping cough has resurged and has become one of the most prevalent vaccine-preventable diseases worldwide. In the Netherlands, the infection frequency has been estimated to be 6% per year. Since 1996, a dramatic increase in pertussis notifications has been observed in the Netherlands and we have proposed that both waning vaccine-induced immunity and pathogen adaptation have contributed to this phenomenon. Comparative genomics and phylogenetic analyses were used to study adaptation of B. pertussis populations to vaccination.
Methods: 284 Dutch *B. pertussis* isolates were used in this study, isolated from the period 1950-2008. Genotyping of a number of virulence-associated genes was performed, including the promoters for pertussis toxin (ptxP) and for the type III secretion system gene bteA (involved in toxin secretion) and the genes for the pertussis toxin subunit A (ptxA), pertactin (prn), serotype 2 (fim2) and serotype 3 (fim3) fimbriae. Phylogenetic analyses of all strains was carried out based on 87 single nucleotide polymorphisms (SNPs).
Results: In particular four alleles (ptxP, ptxA, prn and fim3) were associated with shifts in the *B. pertussis* population. Phylogenetic analyses revealed a stepwise adaptation in which *B. pertussis* accumulated small mutations. The earliest mutations involved ptxA, followed

by prn, ptxP and fim3, respectively. Whole genome sequencing suggested that changes in these genes were associated with clonal expansions and increased strain fitness. No evidence was found for the acquisition of novel genes.

Conclusion: In the last 60 years, changes were found in the *B. pertussis* population with respect to virulence-associated genes resulting in antigenic divergence between circulating strains and vaccine strains and increased pertussis toxin production.

Adaptation involved accumulation of small mutations or SNPs.

The shifts in *B. pertussis* populations suggest that these small mutations significantly affected strain fitness.

No evidence for acquisition of novel genes, plasmids or pathogenicity islands in the B. pertussis genome were found suggesting that the B. pertussis gene repertoire is already well adapted to its current niche and required only fine tuning to persist in the face of intensive vaccination.

P062

Molecular analysis of MRSA isolated from patients sampled multiple times suggests long term persistent MRSA carriage

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Introduction: Since 2002, virtually all Dutch methicillin-resistant *Staphylococcus aureus* (MRSA) isolates are sent to the National Institute for Public Health and the Environment (RIVM) for molecular typing. All typing data is stored in a database and analysis of the database revealed that a considerable number of the isolates originated from patients that have undergone multiple hospital admissions and as a result have been sampled at multiple time points. It is unknown whether the repeated isolation of MRSA from patients is caused by re-colonization with the same or a new strain or that it represents prolonged or persistent MRSA carriage. Therefore, we characterized these multiple isolates by multiple-locus various number of tandem repeat analysis (MLVA) and staphylococcal protein A (*spa*)-typing and supplemented this data with multi-locus sequence typing (MLST) and pulsed field gel electrophoresis (PFGE) analysis of a subset of these samples.

Methods: During the study period 2007-2010, nearly 13,000 isolates have been typed with *spa*-typing and 10,500 by MLVA. Approximately 40% of these isolates were livestock-associated MRSA (LA-MRSA). Interrogation for the presence of isolates of patients sampled more than once revealed 1935 pairs of isolates. To ensure that each pair of isolates was obtained by sampling of the patient during distinct hospital admissions, only isolates with submission dates of at least 6 months apart were included. As a result 477 pairs comprising 319 pairs of MRSA and 158 pairs of LA-MRSA were selected.

Results: In approximately 76% of the pairs of MRSA and LA-MRSA isolates the MLVA-profile and the *spa*-type remained unchanged. In 10% of the MRSA isolates one of the various number of tandem repeat (VNTR) loci had changed (single-locus variants, SLVs). Double- and multiple-locus variants (DLVs and MLVs) were found in 2% and 12% of the pairs, respectively. Among the LA-MRSA pairs 16% were SLVs, 5% DLVs and only 1% MLVs. Among SLV pairs the *spa*-locus was the most variable locus in MRSA (41%) and even more so in LA-MRSA (92%). Unchanged, SLV and DLV pairs of MRSA and LA-MRSA showed little or no variation in MLST (STs). However, pairs in which more than 2 loci differed (MLVs) all yielded different STs. PFGE of unchanged and SLV MRSA pairs and of unchanged LA-MRSA pairs revealed that these pairs had nearly identical profiles. In contrast, the SLV LA-MRSA pairs all showed major variations in PFGE analysis.

Conclusion: The majority of MRSA isolates from patients sampled at multiple time points showed either unchanged or highly related profiles when using MLVA, MLST and *spa*-typing. This suggests either long term persistent MRSA carriage or re-colonization with the same strain. PFGE of the LA-MRSA isolates revealed that many isolates that were typed as identical by MLVA and *spa*-typing clearly were different strains. This emphasizes the need for a new and more accurate molecular typing technique for LA-MRSA.

P063

Optimization and validation of sequential multiplex PCR deployed to determine *Streptococcus pneumoniae* polyserotype culture composition

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Introduction: *Streptococcus pneumoniae* is a human commensal and a pathogen causing respiratory and invasive infections. The ultimate virulence factor of pneumococcus is the polysaccharide capsule with 90+ serotypes identified. The serotype distribution in the human population is not random since only few serotypes are predominating in carriage and invasive pneumococcal disease (IPD). In 2006 a 7-valent conjugate vaccine targeting the serotypes most common in IPD was implemented in the Netherlands, effectively eradicating the vaccine types, yet inducing an emergence of previously

uncommon non-vaccine serotypes. This phenomenon suggests the presence of dynamic interactions between serotypes competing for a niche within the individual host. To gain insight into this interaction simultaneous detection of all serotypes present is required.

Aim: the aim of this study was to adapt the Sequential Multiplex (SM) PCR that was originally designed as alternative to the conventional Quellung assay, into a protocol optimized to determine qualitatively multi-serotype composition of mixed pneumococcal cultures.

Methods: The set of 40 serotype-, serogroup- or cross-serotype/serogroup-specific primer pairs targeting 66 different serotypes as published by CDC (www.cdc.gov/ncidod/biotech/strep/pcr.htm) was tested for specificity in polyserotype sample settings by exclusion of each targeted serotype individually. Next, primers sensitivity was tested in multiplex reactions composed of groups of 3-5 primer pairs per PCR reaction using the same set of DNA templates. Finally, the SM PCR protocol was applied to determine the serotype composition of 62 primary nasopharyngeal cultures which were initially processed by conventional methods. Cultures were 5 to 14 days old by the time of harvest, DNA purification and SM PCR analysis. Primers pairs universal for all capsule loci (*cpsA*) and for pneumolysin (*ply*) were used to identify the presence of pneumococcal DNA in general.

Results: No false positive results were observed in single-primer pair or in SM PCR assays. The SM PCR was prone to generate false negative results when an artificial mix of genomic DNAs was tested. With SM-PCR we confirmed the serotyping results of the conventional culturing and serotyping of the clinical samples in 16 out of 18 samples (89%). The false negative results were observed in samples containing pneumococci of serotype 19A. DNA templates of both these cultures were also negative for *ply* specific PCR product. In addition, twenty-seven samples that were negative for *S. pneumoniae* by conventional methods were positive for *cpsA* and/or *ply* product in SM-PCR, 11 of which also generated a serotype specific signal. The mean number of serotypes detected by SM PCR for culture positive samples was 1.26; and 1.32 for all samples positive for any serotype specific signal, with up to 3 different serotype-specific products per sample.

Conclusion: We commonly detected presence of multiple serotypes in nasopharyngeal cultures of healthy individuals. Although Multiplex Sequential PCR seems to be at least as good as conventional culture to detect serotype present, it allows only partial insight into the multiserotype composition as it has proven to underestimate the presence of certain serotypes.

P064

Evaluation of a second generation point-of-care real-time PCR for influenza virus type A and B detection and A(H1N1) 2009 identification

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Introduction: Diagnostic tests for influenza virus infections vary from time consuming virus isolation, required for antigenic characterization, to antigenic point-of-care (POC) tests used for rapid diagnosis. However, antigenic POC tests are relative insensitive and hence unsuitable for individual patient diagnosis. First generation Cepheid (Sunnyval, CA) Xpert Flu POC qRT-PCR offered a good alternative to antigenic POC tests, but had some limitations (Jenny et al. J Clin Virol. 2010;49:85-9). Second generation Xpert Flu assay is now available and here we report the results of its evaluation.

Methods: The Xpert Flu assay detects influenza virus types A and B and identifies specifically the A(H1N1) 2009 virus. The assay kit consists of a single-use cartridge, nucleic acid binding reagent in a squeeze ampoule and a disposable pipette to transfer specimen into the cartridge. A 4-site Cepheid GeneXpert Dx System was used. For the in-house qRT-PCR nucleic acid was purified from specimens using a MagnaPure LC system with the MagnaPure LC total nucleic acid isolation kit. qRT-PCRs for influenza virus types A and B and H1 2009 were performed on a Lightcycler 480 with the Taqman EZ RT-PCR core reagent kit for one-step qRT-PCR. In total, 71 specimens, of which a subset with different loads, were tested including a respiratory panel of 14 different viruses and 2 bacteria, the 2010 QCMD influenza subtyping panel (n=10), an in-house influenza panel made for quality control for Dutch laboratories during the 2009 pandemic (n=10), influenza negative (n=11) and positive (n=17) clinical specimens and cultured Influenza virus specimens (n=7) covering almost all human influenza virus type A subtypes and influenza B viruses of the Yamagata and Victoria lineages.

Results: The hands-on time of the Xpert Flu assay was 1 minute and the assay took 1 hour and 17 minutes to complete. Compared to the first generation of the Xpert Flu assay the second generation performed better with the H5 and H9 subtypes and in addition included influenza B virus detection. Except for the 10-6 dilution of cultured A(H9N2) with matrix gene Ct value of 34.52 in the in-house qRT-PCR and a 10-2 dilution of a clinical specimen containing influenza B virus Yamagata lineage with NS gene Ct value of 35.54 in the in house qRT-PCR, all other specimens were correctly diagnosed in the Xpert Flu assay. One invalid result appeared, but the repeat run was correct and a cause for the invalid result could not be identified.

Conclusions: The second generation Xpert Flu assay: 1) is easy to perform and rapid and generates easy to interpret results; 2) has similar analytical sensitivity and specificity as our in-house qRT-PCR for detection of influenza virus types A and B and identification of H1 2009 virus and therefore is a good alternative to antigenic POC tests; 3) is an improvement compared to the first generation Xpert Flu but still misses identification of seasonal A(H1N1) and A(H3N2); and finally, 4) is most useful for POC or off office hour diagnostics and not for high throughput use.

P065

Evolution of network stochasticity as bet-hedging adaptations to fluctuating environments

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Isogenic populations of unicellular organisms often show heterogeneity in response to environmental stress. This is caused by stochasticity – or noise – in underlying multistable regulatory networks. A noisy' phenotypical output in a genetically identical population is an adaptation to randomly varying environments and is a risk-spreading strategy; so-called bet hedging.

Mathematical evolutionary theory predicts relations between the population phenotype distribution and the frequency, magnitude and predictability of changes in the environment. A bet-hedging strategy is increasing fitness when the environment changes unpredictably, relatively often and the penalty for maladaptation to the new environment is high.

As a model system we study sporulation in *Bacillus subtilis*. Only a fraction of the cells will produce a highly resistant endospore while other cells remain capable of proliferating. Sacrificing vegetative cells for spores reduces colonization potential but increases survival rates in case of a sudden and rapid environmental change.

Our goal is to elucidate the dynamics of gene regulation networks that control distribution and timing of sporulation in *B. subtilis* by using environmental fluctuations to shape the bet-hedging strategy. For that we use multi-generation experiments with fluctuating selection pressures. Mutant strains with successful strategies will be analysed using, e.g., single cell analysis, mutant-genome resequencing, subpopulation sorting and transcriptomics. Furthermore, evolutionary modeling and network modeling (environmental input plus intrinsic and extrinsic noise components) have been initiated.

P066

SA hygiene chip: novel DNA microarray for outbreak detection of *S. aureus*

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Fast detection of *Staphylococcus aureus* upon patient admission to a hospital is crucial for management of infection, in particular when there is an outbreak. Nowadays, a variety of typing methods are available and the choice of largely depends on whether *S. aureus* is studied at population or at (local) outbreak level and on the availability of the method to the investigators. It is generally known that the currently used methods suffer from different drawbacks. DNA microarrays with their ability to simultaneously detect thousands of distinct DNA sequences have been used on a large scale. However, they include far more probes than required for typing which makes these arrays too expensive and complex for routine use.

We evaluated a 64 selected probes-DNA microarray on 312 clinical human *S. aureus* strains including ICUs and outbreaks isolates, as well as isolates taken from patients over 7 years period. These isolates have been genotyped previously by MLVA and spa-typing. Without minimizing the fidelity of the original design of 375 probes the number of probes was reduced to 64. General performance criteria for molecular typing were employed and spa-typing was used as reference method. The 64 probes microarray demonstrated good typeability and reproducibility with a discriminatory power slightly higher than spa-typing. It correctly identified the outbreak isolates and provided stable in-vivo markers. This study demonstrates that the use of limited number of probes microarray is sufficient and robust for *S. aureus* outbreaks detection and has its potential for population structure analysis by using the same array with different cut-off level. Inclusion of specific genes such as virulence factors or resistance genes may further enhance the utility of the microarray.

P067

Comparison of pattern recognition methods in nasopharyngeal microbial flora

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Introduction: It is widely accepted that microflora of healthy humans in general represent a balanced ecosystem. It is also believed that an imbalance in microbial communities might lead to overgrowth and invasiveness

of potential pathogens, for example in respiratory infections. To identify interactions between members of the microflora, it is crucial to understand the patterns of colonization. However, studies on the application of pattern recognition methods on complex microflora are still limited.

Methods: We tested several approaches for pattern recognition including clustering analysis methods and ordination methods and applied them to a microflora data set obtained through 454-sequencing of approx. 100 nasopharyngeal samples. First, we normalized the data using a compositional normalized method (central log ratio method). Then hierarchical clustering with 17 measures of distance was applied at different taxonomical levels. Simultaneously we applied factor analysis, correspondence analysis and graph pattern method on this data set, respectively.

Results: The clustering method with canberra distance and Pearson correlation distance, factor analysis and graph pattern method performed especially well for detecting operational taxonomic unit (OTU) patterns. Moreover, most of these methods produced comparable results.

Conclusion: Bacterial interaction within nasopharyngeal microflora could easily be tested with existing clustering methods or factor analysis. These approaches may also be useful in studying colonization patterns in other (human) microbiome data sets.

P068

Resistance mutations in the viral protease alter the *in vitro* resistance profiles of bevirimat

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Background: Bevirimat is a maturation inhibitor that inhibits HIV-1 replication by blocking the viral protease-mediated cleavage of the capsid/p2 (CA/p2) junction. Bevirimat resistance mutations are selected in the CA/p2 cleavage site (CS, gag aa 358, 363, 364, 366), but mutations in the QVT-motif (aa 369-371) just downstream of the CA/p2 CS also impair bevirimat activity. We show that resistance mutations in the viral protease alter BVM resistance patterns and can also have an effect on the impact of the bevirimat resistance mutations.

Methods: We selected a panel of seven recombinant viruses that contained PI resistance mutations in the viral protease and/or gag CS and displayed a broad range of replicative capacities (RC). Multiple *in vitro* selections were performed in which the viruses were cultured in SupT1 cells in the presence of increasing bevirimat concentrations. From the four most frequently occurring resistance mutations (Gag V362I, A364V, S368N and V370A) we generated site-directed mutants in different genetic backgrounds. The effect of these individual mutations on BVM resistance and viral replication was investigated.

Results: The rate of selection of bevirimat resistance mutations appeared not to be affected by the presence of PI resistance mutations or differences in viral RC. A difference was observed in the mutations selected when comparing either wild-type (Hxb2) protease variants with viruses with PI resistant proteases. In >90% of cultures with wild-type proteases Gag mutation A364V was selected, whereas in the background of protease inhibitor (PI) resistant proteases, a much more diverse pattern predominantly consisting of Gag mutations V362I, A364V, S368N and V370A was selected. None of these mutations had an effect on replication in wild-type viruses, but substitutions A364V and V370A conferred much higher resistance levels (>150 fold) than substitutions S368N (7 fold) and V362I (3 fold). However, the precise level of resistance was influenced by mutations in protease. The A364V mutant was the most resistant virus in all backgrounds but seemed to impair replication in viruses with mutations in protease. In particular, it had a detrimental effect on replication in already replication deficient virus.

Conclusions: This study shows that PI mutations in the viral protease alter the bevirimat resistance pathway selected. It also shows that individual BVM resistance mutations in Gag have a different impact depending on protease background. It partly explains the differences in BVM resistance profiles observed for wild-type proteases and PI resistant proteases. The almost exclusive selection of Gag A364V by wild-type proteases can be explained by its high level of resistance and negligible effect on replication. The more diverse BVM resistance profile selected by PI resistant proteases is a combined result of enhanced BVM resistance and the differential effects on viral replication. These data are a clear example of the complex interactions between HIV protease and its substrate Gag and their continuous co-evolution.

P069

Increased detection of *Campylobacter* by using the Luminex xTAG®-GPP multiplex PCR for diagnosing infectious gastro-enteritis

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Introduction: Infectious gastro-enteritis (GE) is a major diagnostic challenge as it can be caused by parasites, bacteria and viruses. The Luminex Gastro-enteritis Pathogen Panel (xTAG-GPP) detects 18 most common GE causing pathogens including viruses (norovirus GI and

GII, adenovirus, and rotavirus), bacteria (*Campylobacter, Salmonella, Shigella, E. coli* O157, *Vibrio*, and *Yersinia*), toxins (*Clostridium* toxins A/B, Shiga toxins 1 and 2, and ETEC toxins) and parasites (*Giardia, Cryptosporidium* and *Entamoeba histolytica*). This study shows the *Campylobacter* results of a prospective application of this assay.

Methods: A total of 300 samples submitted for routine culture of bacterial pathogens, multiplex real-time PCR for viruses, and/or parasites were analyzed by the xTAG-GPP assay. The Luminex xTAG-GPP assay positive *Campylobacter* samples were confirmed by real-time PCR targeting *Campylobacter* spp. (16s rRNA gene), *C. jejuni* (MapA gene) and *C. coli* (CeuE gene).

Results: The xTAG-GPP assay was positive for *Campylobacter* spp. in 94 of 300 samples (31.3%). Only 10 (10.6%) of these positives were detected by bacterial culture. Of the remaining 84 positive samples, bacterial culture was either not requested (43 samples, 51.2%), negative (26 samples, 31.0%) or information about bacterial culture was lacking (15 samples, 17.9%). All ten xTAG-GPP and culture positive samples were confirmed by real-time PCR and revealed *C. jejuni* specific amplification (median Ct-value 26.1, range 21.1 - 32.7). Real-time PCR on the remaining 84 xTAG-GPP *Campylobacter* spp. positive samples showed a positive result in 2 samples for *C. jejuni* (Ct-values 17.5 and 28.2), 2 positive for *C. coli* (Ct-values 28.3 and 33.0), and 2 positive for both *C. jejuni* (Ct-values 23.3 and 30.2) and *C. coli* (Ct-values 24.1 and 27.1). Another 52 samples were positive in the *Campylobacter* spp. real-time PCR (median Ct-value 40.2, range 16.2 - 44.1), but negative in both the *C. jejuni* and *C. coli* real-time PCR. Only 4 of these samples showed a Ct-value below 35 and the median Ct-value of culture negative samples was slightly lower than that of samples on which culture was not requested (39.1 and 40.9, respectively). The remaining 26 xTAG-GPP *Campylobacter* spp. positives (27.7%) could not be confirmed by the real-time PCRs performed in this study.

Conclusion: The xTAG-GPP assay results in a large increase in detection of *Campylobacter* spp. In some of these samples *C. jejuni* and/or *C. coli* specific DNA amplification was detected by real-time PCR, but in most samples real-time PCR detected low amounts of another *Campylobacter* species or was negative. Further research is required to investigate the clinical relevance of the detected *Campylobacter* species.

P070

Chlorate and nitrate reduction by *Alicycliphilus denitrificans* strain BC

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Alicycliphilus denitrificans strain BC is a bacterium capable of benzene degradation coupled to chlorate reduction. This characteristic makes the strain interesting for application in *in situ* bioremediation of environments polluted with benzene. Aeration of polluted anaerobic soil sites will stimulate degradation of benzene, however, this is costly and difficult to apply due to low oxygen solubility. Sodium chlorate is highly soluble, which allows addition via the groundwater. Furthermore, chlorate is an ideal electron acceptor and since molecular oxygen is formed during chlorate reduction, controlled release of oxygen is possible. Chlorate can be reduced by chlorate reductases, but also by nitrate reductases. It is not yet known how the synthesis and activity of these enzymes are regulated. The aim of this study is to obtain more insight in the mechanisms of chlorate and nitrate reduction used by *A. denitrificans* strain BC. Growth experiments and enzyme assays were performed. Cultures were anaerobic and, depending on the experiment, acetate and nitrate or chlorate was added. For growth experiments, time courses of OD660 and acetate, oxygen, nitrate, nitrite, chlorite and chloride concentrations were determined. Furthermore, enzyme activity of the chlorate reductase was assayed in crude extracts of *A. denitrificans* strain BC cells grown on acetate and chlorate and on acetate and nitrate. The specific growth rates of cultures grown routineously on acetate are about 0.56 and 0.95 day^{-1} with nitrate and chlorate as the electron acceptors, respectively. Interestingly, cultures grown on acetate plus chlorate had an increased growth rate when inoculated with cells pre-grown on acetate plus nitrate, compared to cultures inoculated with cells pre-grown with acetate plus chlorate. Moreover, optical cell density of *A. denitrificans* strain BC grown on acetate and chlorate was three times higher when inoculated with cells pre-grown on acetate plus nitrate compared to cells pre-grown on acetate plus chlorate. This indicates that an active or activated nitrate reductase has a positive influence on growth with chlorate. In agreement with this, the activity of the chlorate reductase in crude extracts of *A. denitrificans* strain BC from cells grown on acetate and nitrate was 1.7 U/mg protein compared to 0.28 U/mg protein in extracts from cells grown on acetate and chlorate.

P071

Raltegravir resistance pathways selected *in vivo* are not predisposed in the genetic background of the integrase gene

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Background: Therapy failure in HIV-1 patients treated with the integrase inhibitor raltegravir is generally associated with one of three resistance pathways marked by selection

of one of the primary resistance mutations Y143C/R, Q148K/H/R or N155H. The determinants that favor a particular pathway are still poorly understood. No clear correlation between subtype or drug-levels and the pathway selected has been found. We investigated if a specific integrase background determines the raltegravir resistance pathway selected during virological failure.

Methods: We collected longitudinal samples from two patients experiencing virological failure during a raltegravir containing regimen. The viral integrase genes from pre-raltegravir baseline samples were amplified and cloned into an Hxb2 reference virus lacking the integrase gene. Both patient isolates were subtype B. Multiple resistance induction studies were performed with the recombinant viruses and Hxb2 wild-type controls, in presence of increasing raltegravir concentrations to a final concentration of 1024 nM in passage 10. Evolution of integrase genes was monitored at several passages.

Results: *In vivo*, in patient 1 (pt1) raltegravir resistance was selected through the Q148 pathway and in patient 2 (pt2) via the N155 pathway. During *in vitro* selections, pt1 baseline isolate recombinant viruses selected for all three resistance pathways. Pt2 recombinant viruses (N155H pathway *in vivo*) predominantly selected Q148 mutations. The Hxb2 wild-type viruses showed diverse resistance patterns, including 148 and 155 mutations. Regardless of the selected pathway, multiple resistance mutations were observed in all viral cultures. In contrast to the random selection of the resistance pathways the selection of particular amino acids at the resistance related codon was influenced by variations in the baseline sequence. As such, pt2 viruses selected only the G140S variant, which is considered a fitter variant and required only a single nucleotide change. In contrast, in Hxb2 only the less fit G140A variant was selected because the G140S required two nucleotide changes from baseline.

Conclusions: The combined approach of both *in vitro* and *in vivo* raltegravir resistance selection supports a random selection of the raltegravir resistant pathway independent of the integrase background. Whether or not the genetic differences between subtypes will affect the mutational patterns selected remains to be evaluated.

P072

A simplified universal assay for HIV-1 drug resistance genotyping in Africa

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Background: In an effort to reduce the cost of drug resistance testing, while maintaining a full genotypic profile for all antiviral drugs in African first-line reverse transcriptase (RT) inhibitor based HAART regimens, a new genotyping assay, compatible with all major HIV-1 subtypes, was developed. The assay achieves population based genotyping in a single round RT-PCR targeting a region harboring all vital drug resistance mutations for RTIs, and will thus provide maximal data with minimal cost and effort while still enabling the use of existing algorithms for resistance interpretation. This assay was developed and evaluated for its use in resource-limited settings.

Methods: An amplicon obtained from a single RT-PCR, was sequenced using a single forward and reverse primer to produce full double stranded coverage from codon 41 to 238 of RT. The assay was optimized for specificity and analytical sensitivity using a panel of reference viruses for HIV-1 subtypes A, B, C, D, A/E, F, G, and H, after which it was applied to 137 plasma samples from primarily African HIV infected individuals harboring various viral loads and HIV-1 subtypes. The assay was subsequently transferred to an African reference laboratory in Uganda for local evaluation. A further 133 plasma and 21 dried blood spot samples were amplified, of which a subset of 53 were sequenced.

Results: All major HIV-1 subtypes could be detected in the RT-PCR with an analytical sensitivity of 3.70 log copies/ml in plasma. Application of the assay on 137 clinical samples comprising of subtypes A (13%), B (15%) C (40%), D (11%), AE (7%), AG (7%), and rare subtypes H, G and F (7%), viral load ranging from log 2.83-6.92 (median 5.17), was 100% successful. The assay was successfully transferred to the Ugandan laboratory, with preliminary data indicating 100% success with plasma samples =3.70 log. The DBS samples had a viral load range of log 3.46-6.63copies/ml (median 5.56), and demonstrated 100% amplification success, sequencing results are pending.

Conclusions: We have developed a simplified, subtype-independent HIV-1 genotyping assay for the detection of all major mutations associated with RTIs, using only a single round RT-PCR amplification and 2 sequencing primers. This assay greatly reduces cost, contamination risk and hands-on time, making it an option for both high throughput and resource limited settings.

P073

Quantitative comparison of *Streptococcus pneumoniae* in patients with community acquired lower respiratory tract infections and in matched healthy controls

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Introduction: *Streptococcus pneumoniae* (*S. pneumoniae*) is a common cause of community-acquired lower respiratory tract infections (CA-LRTI) but also colonizes healthy individuals. Quantitative changes might determine the aetiology of *S. pneumoniae* in CA-LRTI. However, there are as yet no definitive cut-offs available to distinguish between colonization and infection. Defining the 'infectious' load range of potential pathogens like *S. pneumoniae* is necessary for the development of rapid molecular diagnostic tools that can confirm the bacterial aetiology of CA-LRTI and allow targeted antibiotic use. This study aimed to establish an infection threshold by quantifying *S. pneumoniae* in nasopharyngeal swabs (NPS) obtained from patients with symptomatic CA-LRTI and compare these to age- and gender-matched healthy controls.

Methods: NPS obtained from patients with CA-LRTI were screened on blood agar with 5g/mL gentamicin and those found to be positive for *S. pneumoniae* (n=33) were included in this study. All samples obtained from healthy controls matched to these patients based on age, gender and inclusion date (n=29) were also included. NPS were collected from CA-LRTI patients and matched healthy controls in primary care centres in 14 European countries as part of GRACE (Genomics to combat resistance against antibiotics in CA-LRTI in Europe, Network of Excellence, FP6-LSHM-CT-2005-518226). Pneumococcal growth was semi-quantitatively classified as 1^{+}-5^{+} following inoculation in 5 quadrants on blood agar with 5g/mL gentamicin. *S. pneumoniae* were identified with biochemical tests. DNA from all 62 NPS was extracted using EasyMAG (bioMrieux) and bacterial loads in terms of DNA copies were determined using quantitative real-time SYBR-Green PCR (qRT-PCR) targeting the *S. pneumoniae* intergenic region, *spn9802*. Clinical samples were tested in triplicate on real-time PCR. Standard curves were set up using serially diluted *spn9802* PCR product of *S. pneumoniae* ATCC 49619. The spectral absorbance of the purified PCR product was converted into DNA copies/L using the Avogadro's constant and molecular weight of the standard.

Results: *S. pneumoniae* was detected on qRT-PCR in all 33 (100%) CA-LRTI patients and in 5 out of 29 (17.2%) healthy controls. Global average loads of *S. pneumoniae* in CA-LRTI patients (7.3E+07 DNA copies/mL, standard error of mean (SEM) 4.9E+07) were significantly higher than in healthy controls (2.6E+06 DNA copies/mL, SEM 2.5E+06) ($P<0.001$, Mann Whitney test). However, there was a large overlap between the pneumococcal load in patients with CA-LRTI and the 5 matched healthy controls that harboured *S. pneumoniae*. Loads of *S. pneumoniae* isolated from patients varied from 5.8E+03 to 1.6E+09 DNA copies/mL, while the maximum for colonized individuals was 7.3E+07 DNA copies/mL. On average, 1^{+}-3^{+} growth on semi-quantitative culture correlated to 2.9E+06 DNA copies/mL (standard deviation (SD) 3.1E+06) and 4^{+}-5^{+} growth to 1.6E+08 DNA copies/mL (SD 4.0E+08).

Conclusion:

1. Patients with CA-LRTI showed 28-fold higher average loads of *S. pneumoniae* than colonized individuals.
2. However, 17.2% of healthy controls were colonized with *S. pneumoniae* which makes it difficult to clearly define an infection threshold that differentiates between colonization and infection in patients and colonized individuals.
3. Results obtained with semi-quantitative culture and quantitative real-time PCR showed a good correlation.

P074

Clinical significance of rhinovirus, the most frequently detected respiratory virus in hospitalized children

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Background: Human rhinoviruses (HRVs) constitute the largest genus in the *Picornaviridae* family and are the most common cause of acute respiratory infection ('common cold'). More than 100 serotypes have been described belonging to three genotypes. Recent developments in molecular diagnostic tools have led to easy detection and identification of a large number of divergent HRV strains. However, rhinovirus diagnostics are generally not incorporated into routine clinical use. Also, the lack of clinical and epidemiological data hampers the interpretation of molecular diagnostic findings.

Methods: As part of a prospective study into viral respiratory tract infections in children hospitalized in the Beatrix Children's Hospital, we collected clinical data of all children in whom respiratory samples were taken for detection of 15 respiratory viruses. We characterized HRVs by sequencing the VP4/VP2 region of the HRV genome. Positive RT-PCR and sequence analysis was related to clinical symptoms, including the presence of co-infection with other respiratory viruses and underlying illness.

Results: From September 2009 till January 2011, 1455 respiratory samples were collected, from 644 patients with 904 disease episodes. In 916 samples (63.0%), one or more viruses were detected of which 610 samples (66.6%) tested positive for HRV. HRV was the single respiratory virus detected in 446 samples (48.7%), relating to 254 disease episodes. The second and third most frequently detected mono-infections were from respiratory syncytial virus A/B (57 disease episodes) and influenza A H1N1nv (22 episodes). Sequence analysis of HRVs revealed equal amounts of HRV genotype A and C, with predominance of these genotypes over HRV genotype B. The presence of

co-infecting respiratory viruses appears to influence the association of HRV with clinical symptoms. In patients with underlying pulmonary illness, HRV is associated with more severe respiratory illness.

Conclusions: HRVs are the most frequently detected viruses in respiratory samples taken from children hospitalized in a Dutch tertiary hospital. Co-infection with other respiratory viruses and underlying pulmonary disease appears to influence the contributing influence of HRV on clinical symptoms.

P075

Chitinase activity is increased in immunocompromised rats with invasive pulmonary aspergillosis (IPA)

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Introduction: Chitin is ubiquitous in nature and is mainly found in the exoskeleton of parasites and in the cell wall of fungi. In reaction to chitin-containing pathogens, chitinases are produced in the human host. In the past it has already been demonstrated that chitinases can be produced in the human lungs in reaction to fungal pathogens. At that time only one chitinase was described. Nowadays at least two types of chitinases have been described in humans: chitotriosidase (an endochitinase) and AMCase (an exochitinase). Chitotriosidase levels are increased in Gaucher patients, whereas increased AMCase levels are associated with allergic diseases and asthma.

In the present study it was investigated whether these two chitinases are induced in the lungs of immunocompromised rats with invasive pulmonary aspergillosis (IPA). Furthermore, we also determined whether treatment with the antifungal agent caspofungin (CAS) alters the chitinase production, since CAS inhibits growth of the fungus. In a previous study we have shown *in vitro* that CAS alters the -glucan/chitin ratio in the fungal cell wall.

Methods: IPA was induced in immunocompromised (cyclophosphamide-induced) or immunocompetent female RP rats by respiratory inoculation of *A. fumigatus*. Chitinase levels were determined in 6 groups of rats. Group 1 was immunocompromised, infected and not treated. Group 2 was immunocompromised, infected and treated with CAS. Group 3 was immunocompromised, not infected and not treated. Group 4 was immunocompetent, infected and not treated. Group 5 was immunocompetent, infected and treated. Group 6 was immunocompetent, not infected and not treated (nave rats). At day 1, 3 and 6 serum samples were taken and rats were euthanized for lung homogenization. Chitotriosidase activity, AMCase activity and galactomannan (an *A. fumigatus* antigen) index were determined in serum samples and in lung homogenates.

Results: Chitinase activity in the lung was increased in immunocompromised infected rats compared to immunocompromised non-infected rats. Both chitotriosidase and AMCase activity were increased at day 6 (5.5-fold, $p<0.001$ and up to 10.5-fold, $p<0.001$, respectively). Treatment with CAS reduced the increase in chitotriosidase activity (1.9-fold lower than untreated rats, $p=0.021$). Strikingly, the chitinase activity seemed in accordance with the galactomannan index. In immunocompetent rats, chitinase activity was not significantly increased after inoculation of *A. fumigatus*. Immunocompromised untreated infected rats had up to 14x higher chitinase activity in the lung than their immunocompetent colleagues. Chitinase activity was much higher in lung homogenates than in serum. Serum chitinase activity did not correlate with chitinase activity in the lung.

Conclusion: Fungal infection with *A. fumigatus* significantly increased the chitinase activity in immunocompromised rats. In lung homogenate, both chitotriosidase and AMCase are increased, most likely due to a high fungal burden. Treatment with CAS tempers the increase of chitinase. As we learned from former experiments, immunocompetent rats did not develop IPA after respiratory inoculation of *A. fumigatus*. This could explain why a significant increase in chitinase activity was not observed in these rats. Based on the observations from the present study, inhibition of chitinases as novel therapeutical approach could be investigated.

P076

Impact of changes in antibiotic use on the molecular epidemiology of macrolide resistance in group A *Streptococcus*

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Introduction: Macrolide resistance in group A *Streptococcus* (GAS) can be caused by different mechanisms of which drug efflux by a *mef*-encoded pump and target site modification are the most important. Methylation of the target site is encoded by *erm*(A) or *erm*(B) genes and generally results in a low-level resistant, inducible and a high-level resistant, constitutive phenotype, respectively. We followed the temporal changes in the molecular epidemiology of macrolide-resistant GAS during 1999-2009 in Belgium and related these data to fitness of predominant strain-genotypes and use of macrolide, lincosamide, streptogramin B (MLS) and tetracycline antibiotics.

Methods: GAS (n=11819) isolated from patients with pharyngitis or invasive disease during 1999-2009 in Belgium were screened for macrolide-resistance, genotyped for *erm*(A), *erm*(B) and *mef*(A), and for *emm*

types by PCR-sequencing and reverse line blotting. Outpatient MLS+tetracycline use data expressed in million packages were collected by the Belgian Institute of Health and Disability Insurance for the period 1997-2008. Generalized linear models were used to assess the association between MLS+tetracycline use and macrolide resistance, and between MLS+tetracycline use and proportions of the predominant strain-genotype between 1999 and 2009. The relative fitness of the emerging strain-genotype among macrolide-resistant GAS was investigated based on competition experiments with the strain-genotypes of macrolide-resistant GAS that dominated during 1999-2007 in Belgium.

Results: A total of 849 (7.2%) confirmed GAS isolates were macrolide-resistant. Yearly prevalence of macrolide resistance among GAS decreased from 1999 to 2006 (13.5%, 12.2%, 11.5%, 17.5%, 8.9%, 7.3%, 4.5%, 3.3%, respectively) after which resistance was stable at low levels of 3.0%-3.7% till 2009. Proportions of *mef*(A)-associated *emm*12, *emm*4 and *emm*1, and *erm*(B)-associated *emm*28, *emm*22 and *emm*11 among macrolide-resistant GAS decreased from 86.4% in 1999 to 10.6% in 2009. Meanwhile *erm*(A)-associated *emm*77 emerged in 2006 and yearly prevalence (2006-2009) of this strain-genotype among macrolide-resistant GAS was 5.7%, 14.3%, 32.3% and 59.6%, respectively. MLS+tetracycline use decreased from 4.32 million packages in 1997 to 2.03 million packages in 2004 and remained stable at this level till 2008. Using a two year time lag, we found a significant association between MLS+tetracycline use (1997-2007) and the proportion of macrolide-resistant GAS (1999-2009) ($P<0.001$). MLS+tetracycline use (1997-2007) was negatively correlated to proportions of strain-genotype *erm*(A)-*emm*77 macrolide-resistant GAS (1999-2009) ($P<0.001$). Maintaining the volume of MLS+tetracycline use below the critical threshold of 2.356 million packages in Belgium (0.62 packages/1000 inhabitants/day) seems to facilitate a positive selection of the strain-genotype *erm*(A)-*emm*77 among macrolide-resistant GAS. This inducible *erm*(A)-*emm*77 strain-genotype seems to carry a lower fitness cost than the constitutively expressed *erm*(B) and *mef*(A) genotypes that dominated macrolide-resistance in GAS in the past.

Conclusions:

1. A decrease in MLS+tetracycline use in Belgium was not only associated with a decrease in macrolide resistance among GAS but also with the selection of the low-level resistant, inducible *erm*(A) harbouring resistance mechanism among macrolide-resistant GAS.
2. Maintaining the volume of MLS+tetracycline use below a critical threshold can select for milder macrolide resistance mechanisms among GAS and could be a novel strategy to control antibiotic resistance.

P077

Microbial composition of various components of closed aquaculture systems: analysis of biofilters and microflora of fish intestines

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Aquaculture is becoming increasingly important for the production of fish but maintenance of an optimal water quality poses challenges for aquaculture worldwide. Especially the accumulation of nitrogen compounds, which are produced by fish, is a major problem in closed aquaculture systems. Strict environmental rules for concentrations of these compounds in the effluent water and the high toxicity of these compounds forces the aquaculture to reduce the nitrogen in the effluent water which can be reached in different manners: 1) A more efficient removal of the nitrogenous compounds from the water or 2) Reduction of the nitrogen input.

Biofiltration is often used to remove nitrogen compounds from the water. However, at the moment we know very little about the contribution of various groups of nitrogen cycle bacteria to the removal of nitrogenous compounds in aquaculture systems. The microbial biodiversity of an aquaculture biofilter was investigated using 16S rRNA gene analysis, fluorescence in situ hybridization (FISH) and activity assays. This revealed the presence of various members of nitrogen-cycling bacteria, including a new type of anammox bacteria and ammonium- and nitrite-oxidizers.

Reduction of nitrogen accumulation in the aquaculture can also be achieved by decreasing the protein concentration in food. Most fish are fed high protein content food to maximize growth. Part of the protein can be exchanged for plant material; however it is important to know if fish can convert the plant components in usable metabolites. Therefore the intestinal microflora of common carp (Cyprinus carpio) was investigated using culture-independent methods (16S rRNA gene sequencing by Roche 454 pyrosequencing). This revealed the presence of a large population of microorganisms able to ferment plant components although these fish were fed a high protein diet. Furthermore, members of the nitrogen cycle bacteria were found to reside in the gut of fish pointing to new in situ possibilities to reduce nitrogen emissions in aquaculture.

By investigating the bacterial composition in various components of closed aquaculture systems, we hope to get more insights in the nitrogen cycling in these systems, thereby increasing the efficiency of fish aquaculture.

P078

Selective bacterial pre-enrichment increases the clinical sensitivity of PCR directly on whole blood samples in *S. aureus* bacteraemia

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Introduction: Blood stream infection (BSI) is a serious life-threatening disease, requiring early appropriate antibiotic treatment. Golden standard in diagnosis is blood culture, which usually requires at least 48 hours before definite results are available. Molecular diagnostics directly on whole blood samples have the potential to significantly speed up diagnosis and subsequent optimal therapy, but have so far not been able to achieve a clinically acceptable sensitivity. This is largely due to the use of small volumes, since larger volumes show an inhibitory effect of human DNA. The MolYsisTM-tool (Molzym) is a pre-test enrichment method which selectively eliminates human cells. This could create the possibility to increase the initial blood volume and subsequent diagnostic sensitivity. We investigated the clinical use of this tool in patients with *S. aureus* bacteraemia (SAB).

Methods: A cohort of 22 consecutive patients with culture-proven SAB was studied. Temporarily stored whole blood samples (EDTA), simultaneously drawn during collection of the blood culture, were divided into two aliquots: a standard input volume (SI) of 0.2 ml, and a high input volume (HI) of 1 ml. For the SI; no pre-test enrichment, and isolation of bacterial DNA with the easyMAGTM-platform (Biomrieux) was performed. For the HI; bacterial enrichment and subsequent isolation of bacterial DNA using the MolYsis CompleteTM-tool was performed. After DNA isolation, *S. aureus*-specific DNA was measured in the same quantitative PCR-set-up (Applied BiosystemsTM ABI-PRISM 7500). PCR was performed in duplicate, results were considered positive if =1 yielded a positive result.

Results: Using SI, the *S. aureus*-specific PCR was positive in 15/22 (68%) of the samples, compared to 20/22 (91%) in the HI. Of the seven blood-culture positive samples that were false-negative using SI, six (86%) were PCR-positive when using HI. One sample was positive with SI, but negative with HI; this sample had a very low bacterial load (Ct-value >41). Direct comparison of Ct values for SI and HI showed that for each blood sample, the Ct value in HI was lower than in SI ($p<0.01$). The mean decrease in Ct value was 2.29 (range 0.16 to 3.97). These findings indicate that the HI samples structurally contain a higher amount of bacteria, enabling detection of *S. aureus*-positive samples that have loads below the detection limit in SI.

Conclusions:

- The pre-test enrichment method decreases PCR-inhibition, enabling the use of 5-fold larger test volumes.
- The use of larger volumes increases the input of bacterial DNA into the PCR, which raises the signal of false-negative samples to above detection limit.
- Incorporation of a pre-test enrichment method into the workflow increases the diagnostic sensitivity of PCR directly on whole blood samples to a clinically useable range (>90%).

P080

HSV-1 excretion in ulcerative stomatitis; a prospective study on prevalence, quantity and the role of viral resistance

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Introduction: Stomatitis, including oral mucositis, is a frequent complication in patients receiving a hematopoietic stem cell transplant (HSCT). Its causes can be diverse, including toxicity from chemotherapy and irradiation, infection by bacterial and fungal pathogens and reactivation of herpes simplex virus type 1 (HSV-1). It is unknown whether HSV-1 excretion necessarily causes oral ulceration. In addition, it is unclear whether prolonged HSV-1 excretion despite antiviral treatment is caused by the profound immunosuppressed status of HSCT recipients, or due to resistance of the virus to the antiviral medication.

The aim of this prospective study in adult HSCT recipients was to investigate the relationship between the presence of oral ulcerations and the duration and amount of HSV-1 excretion and the presence of resistant HSV-1 strains.

Methods: Data were collected during hospitalization of 49 consecutive adult patients that underwent HSCT. Patients did not receive prophylactic therapy with (val)aciclovir. Follow up was performed twice weekly: at each follow-up mucositis (lesions on non-keratinized oral mucosa) and lesions on keratinized oral mucosa were scored and a standardized oral rinsing sample was taken. HSV-1 loads were determined by internally-controlled real-time PCR and a beta-globin PCR was performed as a control for cell concentration in the samples. For the study of antiviral resistance, the first sample of each patient that was HSV-1 DNA positive and subsequent samples from patients remaining positive despite antiviral treatment for at least 5 days were studied. Resistance was determined by cycle

sequencing analysis of the thymidine kinase and DNA polymerase genes of the viral isolates. Possible predictors of ulcerative stomatitis were investigated in repeated measures logistic regression.

Results: HSV-1 was detectable in 47% of samples from patients with ulcerative stomatitis and in 16% of samples from patients without oral ulcerations. Detection of HSV-1 was a significant predictor of ulcerations on non-keratinized mucosa (OR 2.44, p-value 0.04) and of ulcerations on keratinized mucosa (OR 5.02, p-value <0.01). No threshold of the HSV-1 load could be established above which all samples were derived from patients with ulcerative stomatitis.

Twenty-three patients had at least one HSV-1-positive sample during the study period of whom 18 received antiviral treatment. In nine patients (50%), samples remained HSV-1 positive after 5 days of antiviral treatment. In isolates from 2 of these patients resistance-associated mutations were found.

Conclusions: HSV-1 is a relevant factor in both mucositis and ulcerations on keratinized oral mucosa after HSCT. A quantitative relationship was not found. Persistent HSV-1 replication despite antiviral treatment is common, but is due to resistance in a minority of patients.

P081

The power of Multiple-Locus Variable-number tandem repeat Fingerprinting of polymorphisms (MLVF): Discrimination of *S. aureus* isolates with the same USA300 PFGE profile

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Introduction: The rapid spread of community-acquired methicillin resistant *Staphylococcus aureus* (CA-MRSA) represents a serious threat for public health worldwide. Of particular concern is the CA-MRSA clone USA300, which is predominant in the United States of America. Pulsed-field gel electrophoresis (PFGE) is the gold standard for the typing of MRSA and other *S. aureus* strains in epidemiological studies, but it recently showed clear limitations in distinguishing between the 'classical' USA300 clone with the *spa* type t008 (ST8-IVa, Panton-Valentine leukocidin positive) and a related clone with the *spa* type t024 (ST8-IVa, and PVL negative). The aim of this study was to determine the applicability of Multiple-Locus Variable number tandem repeat Fingerprinting (MLVF) in the discrimination of these two related clones.

Methods: 78 USA300-like isolates with different molecular characteristics and epidemiology were collected at the Statens Serum Institute (SSI) in Denmark. Seven staphylococcal variable number tandem repeat genes (*sdrC*, *sdrD*, *sdrE*, *clfA*, *clfB*, *sspA* and *spa*) were subject to an established multiplex PCR with subsequent electrophoretic separation using an Agilent 2100 Bioanalyzer. Data analysis was carried out using the Gel Compare II software from Applied Maths with a tolerance of 0.7% and an optimization of 0.5%.

Results: MLVF grouped the 78 isolates into 2 distinct clusters: A (n=40) and B (n=38), when a cut-off value was set at 60%. Importantly, all isolates of cluster A had the *spa* type t024, whereas all isolates of cluster B had the *spa* type t008. Furthermore, both clusters were composed of several patterns (4 patterns in cluster A and 9 in cluster B). Specifically, 30 isolates yielded the same MLVF pattern in cluster A, and the same was true for 25 isolates in cluster B. Thus, the majority of the strains within each cluster are genetically very closely related. Notably, a major difference in the MLVF banding patterns related to the *spa* gene, reflecting the spa types t024 and t008 in cluster A and B, respectively.

Conclusions: MLVF has the discriminatory power needed to distinguish between the classical USA300 clone and closely related lineages with the same PFGE profile. Additionally, MLVF allows a further differentiation of isolates with the same *spa* type into subclusters. The subclustering of isolates is indicative of molecular differences that can be relevant for further epidemiological studies. An additional advantage of MLFV over PFGE is that it is a fast and cheap high-throughput typing tool.

P082

Comparison of norovirus detection using RIDA® Quick norovirus kit, ImmunoCardSTAT®!norovirus kit versus norovirus real-time RT-PCR

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Introduction: Norovirus is a major cause of gastroenteritis outbreaks in hospitals, nursing homes, schools and kindergartens, but also on cruise liners and hotels. The virus is usually transmitted through the faecal-oral route. Environmental transmission in hospitals involves areas such as floors, work surfaces, light switches and door handles, with the virus surviving for prolonged periods. Because norovirus is highly infectious and outbreaks can lead to ward closure and increased patient stay, an accurate and rapid detection of norovirus is essential. Presently norovirus diagnosis is performed using real-time ReverseTranscription (RT)-PCR, which is sensitive and specific, but also time consuming and expensive.

The objective of this study is to evaluate the RIDA Quick norovirus assay and the ImmunoCardSTAT!norovirus assay compared to norovirus RT-PCR for rapid diagnosis during a norovirus outbreak.

Methods: In this study a selection of 55 stool-samples, selected based on RT-PCR results, were tested retrospectively with the two rapid assays. Twenty nine were positive and 26 were negative for norovirus based on the RT-PCR results. Among the norovirus negative samples, there were samples selected positive for adenovirus type 1, echovirus type 3, coxsackievirus type B3 and rotavirus (all n=1). Both assays were performed according to manufacturer's instructions. For the detection with the real-time RT-PCR stool suspension were prepared in phosphate-buffered saline and nucleic acid was extracted using the MagNA Pure LC Total Nucleic Acid Kit (Roche Diagnostics). RT was performed using TaqMan Reverse Transcription Reagent kit (Applied Biosystems) with random hexamers. Of the obtained cDNA an aliquot was amplified using TaqMan Universal Master Mix (Applied Biosystems) and the PCR was run in an Applied Biosystems 7500 Real Time PCR system. Primers and probe set as described by Hoehne et al. were used.

Results: Of the 29 positive norovirus RT-PCR samples, the RIDA Quick norovirus assay reported 21 (72,4%) positive and the ImmunoCardSTAT!norovirus assay reported 19 samples (65,5%) positive. The 26 norovirus RT-PCR negative samples were all (100%) found negative by RIDA Quick norovirus assay, while the ImmunoCardSTAT!norovirus assay reported 2 (7,7%) samples positive.

Conclusions: Results were obtained in 20 minutes with both the ImmunoCardSTAT!norovirus assay and the RIDA Quick norovirus assay. In these selected stool-samples the RIDA Quick norovirus assay was slightly more sensitive and specific than the ImmunoCardSTAT!norovirus assay. Both tests are less sensitive than the real-time RT-PCR but both rapid tests can be used for diagnosis in outbreak settings where multiple patients are tested to assertain wheter norovirus is the causative agent of the outbreak. However the low sensitivity of both tests makes them unsuitable for diagnosis in individual cases.

In a previously published prospective study of 537 stool-samples, with the same RT-PCR as comparison, the RIDA Quick norovirus assay scored a sensitivity of 57,1% . This suggests an over-estimation of the sensitivity in the sample selection that was studied.

P083

Maintenance of gut bacterial community structure during domestication of the zebrafish

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Experimental analysis of gut microbial communities and their interactions with vertebrate hosts is conducted predominantly in domesticated animals that have been maintained in laboratory facilities for many generations. These animal models are useful for studying coevolved relationships between host and microbiota only if the microbial communities that occur in animals in lab facilities are representative of those that occur in nature. We performed 16S rRNA sequence-based comparisons of gut bacterial communities in zebrafish sampled from their natural habitat and from lab facilities in different geographic locations. Patterns of gut microbiota structure in domesticated zebrafish varied across different lab facilities in correlation with historical connections between those facilities. However, gut microbiota membership in domesticated and wild-caught zebrafish was strikingly similar, with a shared core gut microbiota. The zebrafish intestinal habitat therefore selects for specific bacterial taxa despite radical differences in host provenance and domestication status.

P084

Identification of genes involved in mycobacterial capsular alpha-glucan production by random transposon mutagenesis

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Tuberculosis, caused by the bacterium *Mycobacterium tuberculosis*, is the most deadly bacterial disease worldwide and claims over 1.7 million lives each year. *M. tuberculosis* possesses a variety of immunomodulatory factors that influence the host immune response. When the bacillus encounters its target cell, the outermost components of its cell envelope are the first to interact. *Mycobacteria*, including *M. tuberculosis*, are surrounded by a loosely attached capsule that is mainly composed of proteins and polysaccharides. Although the chemical composition of the capsule is relatively well known, its biological function and the route by which the capsule is produced are only poorly understood. The aim of this study was to obtain further knowledge on mycobacterial capsule biogenesis by identifying genes involved in its production. We focussed on alpha-glucan, which is, in *M. tuberculosis*, the major capsular polysaccharide.

The approach we used was to created random transposon mutant banks in three representative mycobacterial species: the non-pathogenic species *Mycobacterium smegmatis* and the two pathogenic species *Mycobacterium marinum* and *M. tuberculosis*. Obtained tranposants (n=30,000) were then screened for an altered capsule production with an antibody against alpha-glucan in a double filter screening assay. In addition, the mutants were exposed to iodine vapours which stains alpha-glucan and thus provides an alternative screening approach. In total, we identified 140 transposon mutants that showed either an increased or reduced alpha-glucan production. After reconfirming the mutant phenotypes using double-filter and whole-cell spot blot assays, the affected genes were identified using ligation-mediated PCR. Amongst the genes identified were glgC, which encodes for glucose-1-phosphate adenylyltransferase and glgB encoding for glycogen branching enzyme. Both these gene products are known to be involved in capsular alpha-glucan production. Furthermore, the glgC mutation was identified in both *M. smegmatis* and *M. marinum* thus validating our screening approach. Our current research is focussed on further characterising the mutant phenotypes allowing the mutants to be classified and select for interesting mutants that will be investigated in further depth.
In conclusion, we have identified a panel of mutants in relevant mycobacterial species with various defects in capsular alpha-glucan production. Further investigating these mutants will not only provide insight into how complex structures, such as large hydrophilic (water-soluble) polysaccharides, are synthesised and transported across the extremely hydrophobic (water-repelling) mycobacterial cell-wall, but will also provide important tools for investigating the role of the capsule during infection.

P085

Microbial community transcriptome dynamics in anaerobic gut microcosms exposed to prebiotic oligosaccharides

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The human gastrointestinal tract hosts microbial communities of astonishing density and complexity. This commensal microbiome, including members of Bacteria, Archaea, Eukarya, and their viruses, is fundamental to human health. It protects against gastrointestinal infections and exerts beneficial effects on the host immune system and energy metabolism. Prebiotics are non-digestible carbohydrates, which allow selective modulations of the composition and/or activity in the gastrointestinal microbiota that confers benefits upon host health. Here, we characterize the transcriptional response of key members of the human gut microbiota incubated in the presence of prebiotic oligosaccharides. Using a high-throughput colon microcosm system and a multi species microarray covering the complete genomes of *Akkermansia muciniphila, Bacteroides vulgatus, Bifidobacterium longum*, and *Bifidobacterium catenulatum*, we show these bacteria native to the human distal colon exhibit differential transcription profiles when exposed to fructooligosaccharides (FOS).
Notably, there were no genes implicated in carbohydrate metabolism that were upregulated in *A. muciniphila* and *B. vulgatus* during growth in the presence of FOS. In line with known prebiotic effects of FOS, *B. longum* showed increased transcription of xylulose-5-phosphate fructose-6-phosphate phosphoketolase (*Xfp*) under FOS availability. Surprisingly, *B. catenulatum*, which is more prevalent in the microbiota of formula fed infants and adults showed increased Xfp transcription when FOS was added.
In order to develop and apply non-digestible oligosaccharides in food products, it is imperative that we understand the mechanisms by which these compounds selectively stimulate the growth specific members of the intestinal microbiota. Analysis of the transcriptional response of model colon microbiota subjected to FOS availability illuminates the fermentation pathways and uptake mechanisms that operate in these microorganisms.

P086

Optimization of 16S amplicon preparation from low bacterial density communities; working at the boundary of microbiota detection

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Introduction: Detailed analysis of microbiota community structure can be obtained through barcoded small subunit sequencing. Our work is focused on the nasopharyngeal colonisation in infants in relation to *Streptococcus pneumoniae* vaccination. However, analysis is often challenged by limited amounts of bacterial material that can be obtained in sampling (103-104 bacteria/ml). Optimal methods for sample processing and preparation of 16S-amplicons are essential for adequate analysis of these samples. The aim of this study was to 1) find the most optimal DNA-extraction method for low density communities, and 2) determine if 16S-rDNA recovery from those communities influences the accurate detection and composition of the complete microbiome.
Methods: We sampled the nares, saliva, naso- and oropharynx of four healthy individuals. We also included

a 20-species mock reference community. Dilution series were prepared ranging from 10^6-10^2 cfu/ml to identify the lower DNA quantity limit for accurate microbiota profiling. To establish the optimal DNA-extraction method, we tested three commercial kits (Epicentre Masterpure, Qiagen DNeasy, Mobio Powersoil) and a phenol bead-beating protocol combined with Agowa Mag mini. Bacterial DNA recovery efficiency was determined using RT-PCR. Efficiency to extract DNA from the various taxa at all bacterial concentration levels was determined through GS-FLX-Titanium-Sequencing of the 16S V5-V7 region.
Results: Large differences in DNA extraction efficiency were observed between different methods. The two best performing protocols extracted 10 to 100 fold more DNA. Interestingly, these extraction efficiencies were dependent on the origin of the samples, probably reflecting differences in bacterial taxa present. These differences in extraction efficiency were also reflected in the phylogenetic coverage that was obtained. Background bacterial DNA might interfere with adequate microbiota profiling when samples contain <700-1000 fg DNA. Furthermore, below this critical value, 454 PCR amplicon levels were not always reflective of template DNA.
Conclusion: This study aimed to optimize and interpret microflora profiles of low-density communities in humans. We show the limits for accurate microbial profiling: limits that might vary, and are strongly influenced by the methods for handling samples. Our findings should be considered for all microbiota studies.

P087

A functional proteomics approach to immune evasion

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In bacteria secretion systems are crucial virulence determinants, however, our knowledge on the functions of secreted proteins in innate-immune evasion is limited. The existing routines for the identification of the proteins involved are founded on homology (gene sequence, gene location, protein structure) with known inhibitory proteins and inherently provide only a limited set of proteins. The research presented here aims at the development of a functional-proteomics based approach that facilitates identification of truly novel proteins with immune-modulating capacity within the secretome of the human pathogen *Staphylococcus aureus*.
Secreted proteins that specifically interact with phagocyte receptors and with serum components deposited on the cell surface (i.e. opsonization; labeling of particles for clearance) likely contribute to the pathogen's ability to evade the host-immune system. Secreted proteins are recovered from *S. aureus* culture supernatants using ion-exchange chromatography and are subsequently biotinylated. These concentrated and labelled proteins are shortly incubated with the neutrophils and opsonized particles to facilitate binding. Following washing of the target particles the proteins are eluted (high salt or low pH) and captured on streptavidin-coated beads by means of their biotin label. The proteins of interest are identified by peptide-mass fingerprinting (on-bead digestion).
The secretome of *S. aureus* strain N315 was successfully extracted from the culture supernatant by Ion-Exchange Chromatography and biotin labelled. Small-scale experiments were setup to test the capacity of these proteins to specifically interact with the target particles. In a control experiment binding of the protein CHIPS, a secreted inhibitor of the neutrophil associated C5a-receptor, to and elution from neutrophils was analyzed by western blotting. Based on the estimated number of C5a receptor approximately 50% of CHIPS is recovered. Similar experiments using the purified secretome indicate about 15-20 proteins specifically bind neutrophils or opsonized particles. A preliminary on-bead digestion and mass-spectrometric analysis indicates two of the recovered proteins are the well known immune-modulatory proteins Staphylococcal Complement Inhibitor (SCIN) and Immunoglobulin G binding protein A (SpA). In future experiments both the amount of targets particles and secretome will be increased in order to identify the novel proteins of interest.
The data presented above show the basic strategy behind this novel approach is working and provide a starting point for the functional-based identification of novel putative immune-modulating proteins.

P088

Postprandial microbial succession in the chicken digestive tract

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The microbiota in the gastrointestinal tract (GIT) of chicken plays an important role in health and bird performance. Understanding the interplay between the gut microbiota and bird performance and health is essential to establish sustainable chicken production. Improvements in chicken gut health will result in enhanced bird performance and reduce potential poultry related food borne pathogens such as *Campylobacter* and *Salmonella*. It further provides a fundamental insight into host microbe interactions in relation to nutrition and health.
We used barcode pyrosequencing of 16S rRNA gene amplicons to probe the composition of the highly diverse

and dynamic microbiota of the chicken GIT, and identify the influences of host, diet and environment. The goal of this study was to characterize the microbiota of the different compartments of the chicken GIT and to reveal its dynamics upon refeeding after an overnight feed withholding.
During the experiment, commercial broiler (Ross 308) male chicks were kept under controlled housing and fed diets that meet or exceed the nutrient requirements. At 36 d of age (market age), feed was withheld overnight and five birds were humanely euthanized at 0, 2, 4 and 6 hours post re-feeding. Samples were obtained from all GIT compartments, including crop, proventriculus/gizzard, duodenum, jejunum, ileum, caecum and colon and frozen immediately. Following DNA isolation, the V6 hypervariable region of the small subunit ribosomal RNA gene was PCR amplified and subsequently subjected to pyrosequencing.
Sequence analysis showed the presence of 7 different bacterial phyla comprising 115 different genera. Depending on the GIT compartment, differences were observed in the structure of the microbiota as well as its dynamics. The microbiota of the caecum differed strongly from all other GIT compartments. In particular *Lachnospiraceae*, *Bacteroidaceae* and *Riminococcaceae* were dominant, while hardly present in other compartments. In the proximal part of the GIT, crop, stomach, duodenum, jejunum and ileum, *Lactobacillaceae* increased postprandially, while the presence of chloroplast sequences from the feed is strongly decreasing in time and downstream the proximal GIT. Moving down stream the small intestine, the presence of *Streptococcaceae* and *Enterococcaceae* become more dominant.
The observed alterations of the gut microbiota during feeding and fasting emphasize the need to consider both microbial and host cellular responses to nutrient flux. These findings are currently integrated to define system biology biomarkers that can be applied in the rapid determination of feed intervention effects on bird performance and food safety targets.

P089

Step-wise development of carbapenem resistance in *Pseudomonas aeruginosa*

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Background: In the absence of carbapenem hydrolyzing enzymes, carbapenem resistance in clinical *P. aeruginosa* strains is often multifactorial. However, most *in vitro* mutagenesis studies assess the development of single-step mutants upon exposure to fixed concentrations of these drugs, and it is unclear how such mutants relate to clinical strains. Here, we describe the step-wise development and stabilization of resistance to ertapenem and imipenem/meropenem in *P. aeruginosa* exposed to ertapenem in varying concentrations mimicking therapeutic serum levels.
Methods: Small inocula (106 cells) of 10 clinical *P. aeruginosa* strains (with meropenem MICs <0.25 mg/L, imipenem MICs <1.5 mg/L and ertapenem MICs= 4 mg/L) were incubated at 37C in RPMI-medium with ertapenem in concentrations that reflect the *in vivo* concentrations achieved with the standard daily dose (1 h in each 16, 8, 4 and 2 mg/L, 4 h in 1 mg/L and 16 h in 0.5 mg/L of ertapenem) for three consecutive days (days 1 - 3). If visible growth (clouding of the medium) was observed on day 1 and/or 2, an aliquot of the cultures was plated on LB-plates without antibiotics (called day 1/day 2 isolate). All cultures (with or without visible growth) were plated on day 3 (day 3 isolate). Thereafter, the isolates where continuously cultured for 30 days in the absence of antibiotics. MICs for ertapemen, imipenem and meropenem were determined by E-tests at various time points.
Results: 5/10 of the ertapenem-treated strains yielded day 1 and/or day 2 isolates, and 9/10 day 3 isolates. In all of the isolates, the MICs for ertapenem and at least one of the other carbapenems were elevated, but the changes were more prominent in the day 3 than in the day 1 or 2 isolates for all but one strain. The resistant phenotype was stable after 30 days of culturing in 8/9 of the day 3 isolates but lost in 4/5 of the day 1 or 2 isolates.
Conclusions: These data show that even though *P. aeruginosa* can become resistant to one or more carbapenems very quickly, the phenotype often develops and stabilizes gradually. A detailed molecular analysis of the day 1, 2 and 3 isolates of a given strain will help to determine the nature and sequence of the underlying genetics changes. The method of drug exposure used in this study resembles clinical exposure, but further research is needed to establish weather the mutants also resemble clinical mutants.

P090

Cowpox virus protein CPXV012 inhibits antigen presentation by interfering with the function of the MHC class I peptide loading complex

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Viral antigen presentation by MHC class I molecules on the cell surface is one of the principal mechanisms to trigger antiviral immune responses by patrolling T cells.

Not surprisingly, many viruses have evolved unique ways to tamper with this mechanism. For example, proteins encoded by various herpesviruses interfere with the peptide loading of MHC-class I molecules by targeting components of the peptide loading complex (PLC) including the ATP-driven transporter associated with antigen processing (TAP). The PLC mediates transport of proteasome derived peptides over the ER membrane and subsequent loading onto MHC class I molecules in the ER. Inhibition of the PLC retains MHC class I in the ER and thus blocks presentation of viral antigens on the cell surface.

Recently, it has been shown that cowpox viruses also inhibit MHC- class I antigen presentation, and thereby CD8 T cell recognition of infected cells *in vivo*. Using gene knock-out studies, the viral proteins CPXV012 and CPXV203 were identified to be responsible for this effect. The 9kDa protein CPXV012 inhibits antigen transport into the ER lumen by blocking TAP by an unrecognized mechanism.

In this study, we aim to identify the mechanism by which CPXV012 inhibits the peptide loading complex. Preliminary results show that CPXV012 binds to the PLC by an ATP-dependent mechanism. Furthermore, using a fluorescence recovery after photo bleaching (FRAP) assay, we show that CPXV012 increases the diffusion rate of the PLC. This suggests that ATP-dependent binding of CPXV012 to the PLC either changes the conformation of TAP or alters the composition of the PLC. These studies give a glimpse into the way a recently discovered viral protein of only 70 amino acids is able to efficiently disrupt a large multimeric key component in antiviral immunity.

P091

Pneumococcal immune evasion by Zinc Metalloprotease C

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Introduction: *Streptococcus pneumoniae* is a commensal of the human upper respiratory tract but also a pathogen responsible for over a million deaths annually. The bacterium resides in the nasopharynx, and although colonization is asymptomatic, it can spread from this site and cause a wide range of diseases from otitis media, through pneumonia, septicemia and meningitis. The ultimate virulence factor of *S. pneumoniae* is the polysaccharide capsule and there are a number of surface proteins known to contribute to bacterium pathogenicity, relatively little is known about the role of secreted proteins in pneumococci virulence. In this study we tested whether pneumococci produce secreted proteins inhibiting leukocyte recruitment to the site of infection.

Methods: Human leukocytes were isolated from peripheral venous blood of healthy donors using a Ficoll/Histopaque gradient We isolated the entire secretome of whole genome sequenced invasive pneumococal strain TIGR4 and conducted an antibody inhibition assay with 62 monoclonal antibodies directed against the active sites of surface receptors, including all known receptors involved in the process of transmigration of leukocytes. Fishing with recombinant PSGL-1-fc was performed with protein-G beads in the isolated TIGR4 secretome. Western blotting of PSGL-1was performed with two different monoclonal antibodies. TIGR4 knock-out of ZmpC was generated with a Janus-type cassette. ZmpC presence in different *S. pneumoniae* serotypes was detected by PCR. ZmpC was isolated by FPLC chromatography; anion exchange and size exclusion chromatography. Static adhesion studies were performed with calcein-labeled neutrophils on immobilized p-selectin.

Results: The screening showed the presence of a component in the secretome of TIGR4 that could inhibit the binding of a monoclonal antibody directed against the active site of P-selectin Glycoprotein Ligand-1 (PSGL-1). PSGL-1 has been identified as the principal ligand for P-selectin. It is expressed on most leukocyte including neutrophils and it has been shown to be critical for leukocytes rolling in the recruitment of leukocytes to the site of infection. Purified TIGR-4 secretome could also inhibit static adhesion of neutrophils to P-selectin, indicating that there is a functional active inhibitor of PSGL-1 present. Additionally, fishing with recombinant human PSGL-1 resulted in degradation of the receptor, indicating the role of a specific protease. Furthermore, addition of EDTA (a divalent cation chelator) resulted in loss of receptor degradation. Purification of this inhibitor was done by FPLC chromatography resulted in the isolation of a 200kD inhibitor. The purified protein was analysed by mass-spectrometry and identified as Zinc metalloprotease C (ZmpC). ZmpC presence in *S. pneumoniae* serotypes correlates a 100% with the ability of cleaving PSGL-1, furthermore a TIGR4 ZmpC knock-out could not cleave PSGL-1 anymore.

Conclusion: *Streptococcus pneumoniae* is able to produce an inhibitor of PSGL-1, thereby block the transmigration process; leukocytes are unable to leave the blood stream, and therefore won't reach the side of infection, weakening the innate immune response.

P092

The zebrafish as a model to study bacterial-host interactions in IBD: bacterial composition and immune cell recruitment to the intestinal mucosa

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Background & Aims: The pathogenesis of inflammatory bowel disease involves dysfunctional mucosal immune responses to commensal bacteria in genetically predisposed hosts. Interactions between host cells and bacteria are complicated, and it poses a challenge to assess their relative contribution to intestinal pathology. Therefore, we developed a novel model for enterocolitis in zebrafish to study bacterial host interaction in greater detail, in a simplified system.
Methods: Enterocolitis was induced by intra-rectal administration of the hapten oxazolone in adult zebrafish pre-treated with antibiotics (either vancomycin or colistin sulphate). Intestinal inflammation was evaluated by histological and flow cytometry analyses, and cytokine profiling with quantitative PCR. Changes in the composition of the intestinal microbiota following antibiotic administration were assessed by 16SrRNA sequencing and bacterial load was quantified by culture on non-selective media.
Results: Zebrafish pre-treated with vancomycin had bacterial populations dominated by *Fusobacteria* and reduced enterocolitis scores. This associated with a reduction in the recruited neutrophils and eosinophils. In contrast, zebrafish given colistin sulphate had a predominance of *Proteobacteria*. These zebrafish had reduced eosinophil and lymphocyte infiltration. However, the percentage of neutrophils wasnot reduced and thesecolistin pre-treated fish still displayed severe enterocolitis.
Conclusion: In zebrafish with oxazolone-induced enterocolitis, components of the intestinal microbiota affect the severity of disease and composition of the intestinal infiltrate. Future studiesusing thiszebrafish system will aim to investigate how specific bacterial species are able to recruit differentimmune cells.

P093

TcdR regulates toxin expression

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Introduction: *Clostridium difficile* is a gram-positive spore forming rod, which can cause a wide variety symptoms. The rate and severity of *Clostridium difficile* infection (CDI) has increased in recent years and is associated with the rise of *Clostridium difficile* PCR-Ribotype (Type) 027. The highly virulent *Clostridium difficile* Type 027 strains are hyperproducers of toxin A (enterotoxin) and toxin B (cytotoxin). Supposing that hyper production of toxin A and toxin B is responsible for the increased severity of CDI. The pathogenicity locus (PaLoc) contains the encoding genes for toxin A and B enclosed by the positive regulator (tcdR) and the negative regulator (tcdC) genes. Hndsberger et al., showed that transcription levels of the PaLoc genes in *Clostridium difficile* VPI10643 strain are growth dependent. The early logarithmic phase is associated with high transcription levels of tcdC gene and weak expression of transcription levels of the tcdR, toxin A and B genes. The late logarithmic and stationary phase is associated with low transcription levels of the tcdC gene and high transcription levels of tcdR, toxin A and B genes.
Materials and Methods: *Clostridium difficile* strain 630 was grown under anaerobic conditions on blood agar plates. Single colonies were used to inoculate pre-reduced Brain Heart Infusion broth supplemented with Yeast extract and L-cysteine. Samples for RNA preparation and toxin detection were taken after 2, 4, 6, 9 and 24 hours post inoculation. Total RNA was stabilized by adding methanol. RNA was extracted using Trizol (Invitrogen) according manufactories protocol, followed by a DNase step. Synthesized cDNA was used in a real-time PCR for detecting transcription levels of the PaLoc genes. The levels of toxin expression were determined with a commercial ELISA (Techlab) according manufactures protocol.
Results: Northern blot analysis revealed that the isolated RNA was intact. Transcription levels of the toxin genes and tcdR are increasing in time. While the transcription levels of tcdC increases until the logarithmic phase and decrease in the late logarithmic phase and stationary phase. However, tcdC/tcdR ratios are decreasing in time. We detected increasing levels of total toxin expression in time.
Conclusion: 1) We could not demonstrate a correlation between tcdC and the other PaLoc genes as suggested by Hundsberger et al., 2) However, considering the decreasing tcdC/tcdR ratios in time and increasing levels of toxin expression, suggest that increased transcription levels of tcdR are important for toxin production

P094

Mumps virus outbreak; update from Laboratory for Infectious Diseases, Groningen (NL)

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Introduction: Mumps is an acute viral infection characterised by fever and swelling of the salivary glands. Complications may include inflammation of the testicles and of the central nervous system.
Mumps vaccination programme, vaccination on age of 14 month and 9 years together with measles virus and rubella virus, was introduced in the Netherlands in 1987 with a coverage of >93%. Since the introduction of this vaccination the incidence of mumps virus infections has strongly declined. Despite this, outbreaks have occurred

in the Netherlands as well as in surrounding countries. The current outbreak in students was first notified in December 2009 and had a peak incidence in March-April 2010. Most cases were found in the provinces Utrecht and Zuid-Holland. Between December first 2009 and November third 2010, 391 cases were reported. The latest cases were mainly reported from Groningen.
In this study we present the results of mumps virus testing in 2010 in our laboratory.
Methods: Data were collected between January 2010 and December 2010. In total 198 samples from 181 patients were examined by real-time RT-PCR and 119 samples were also tested by shell vial culture followed by immunofluoresence with monoclonal mumps virus antibodies. Positive samples were sent to the Dutch National Institute for Public Health and the Environment (RIVM) for further typing.
Results: Eight samples were tested in the first quarter of the year, in the second, third and fourth quarter 35, 51 and 104 samples were tested respectively.
A total of 102 samples, from 99 patients, were found positive for mumps RNA by real-time RT-PCR and 2 sample, from 2 patients, were found positive for mumps by culture only. Of the comparison between RT-PCR and culture 48 are positive and 56 are negative in both tests, 13 were RT-PCR positive and culture negative and as mentioned before 2 were culture positive and RT-PCR negative.
Most of the samples were sequenced by the RIVM. In some of these samples a mutation was found, resulting in a 2 bp difference compared to the wild-type mumps virus.
Conclusions: There is still a mumps epidemic in Groningen. Most patients are students living in the city of Groningen and most of the mumps positive patients have at least been vaccinated once. More research is needed to determine whether the cause of this epidemic waning immunity or due to changes in the viral genome, resulting in immune escape. 60 more samples were sent to the RIVM for genotyping and these results will be presented at this meeting.

P095

Immunogenicity and protectivity of *Staphylococcus aureus* and *Streptococcus pneumoniae* proteins in relation to nasopharyngeal colonization and vaccination

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Introduction: Nasopharyngeal colonization with potential pathogens is a prerequisite for respiratory and invasive diseases with amongst others *Streptococcus pneumoniae* and *Staphylococcus aureus*. To develop novel immunization strategies against these pathogens understanding the immunological correlates of natural carriage is essential. Because colonization rates of *S. pneumoniae* and *S. aureus* are inversely correlated and seem to shift after pneumococcal conjugate vaccination (PCV-7) we decided to study the natural humoral response against surface proteins in relation to carriage with both pathogens in PCV-7 immunized and non-immunized.
Methods: We performed a prospective population-based cohort study. Sera collected from 105 and 156 colonized and non-colonized children of respectively 12 and 24 months old were analyzed for IgG against 40 staphylococcal and 18 pneumococcal proteins using flow cytometry-based technology. Nasopharyngeal swabs were obtained from these children at the ages of 6 weeks, 6 months, 12 months, 18 months and 24 months and cultured for respiratory pathogens with conventional methods. Half of these children were immunized with PCV-7 at 2, 4 and 11 months of age.
Results: In *S. aureus* colonized children the levels of IgG against the proteins ClfA, Efb, SCIN, SEH and SSL_5 were significantly higher at both 12 months and 24 months ($p<0.01$), whereas in pneumococcal colonized children IgG levels against the proteins BvH3, CbpA, ENO, NanA, PdBD, PLY, PpmA, PsaA, PspA, SlrA, sp0189 and sp1003 significantly increased compared to non-colonized individuals ($p<0.001$). Increasing age was associated with a higher response against almost all pneumococcal proteins and a lower response against more than half the staphylococcal proteins ($p<0.01$). Anti-protein antibodies at the age of 12 months did not protect against colonization in the following year. In addition, no cross-protection could be observed between anti-protein antibodies to pneumococcal and staphylococcal carriage and vice versa. In contrast, a positive association ($p<0.01$) was found between antibodies against 9/18 pneumococcal proteins and (persistent) colonization with pneumococci and against 6/40 staphylococcal proteins and staphylococcal colonization, respectively, which were primarily antibodies against toxins and surface proteins. Finally, there were no differences in humoral anti-protein responses between vaccinated and non-vaccinated children.
Conclusions: Many pneumococcal and staphylococcal proteins appear immunogenic in children under 24 months of age. Unfortunately, at 12 months of age neither of the anti-protein antibodies showed to be protective nor cross-protective against pneumococcal and staphylococcal colonization in the second year of life. Antibody levels against *S. aureus* proteins appear to decrease with age, whereas antibodies to *S. pneumoniae* proteins increase with age, which both correlate with colonization dynamics. PCV-7 vaccination had no influence on antibody responses in this age group.

P096

Phenol-soluble modulins promote colony spreading in *Staphylococcus aureus*

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Introduction: The gram-positive bacterium *Staphylococcus aureus* is primarily a commensal species that is frequently encountered among the human microbiota. However, *S. aureus* is also one of the leading causes of infections both within health care settings and the community. The ability of *S. aureus* to cause infections depends on a diverse array of cell wall-associated and extracellular virulence factors. The expression of many of these virulence factors is coordinated by the accessory gene regulator (agr) quorum sensing system, which responds to cell density-dependent stimuli. We have recently shown that an intact agr system is required for the movement of *S. aureus* over surface-air interfaces by colony spreading. Since the involvement of surfactants in colony spreading seemed likely, we investigated in the present studies whether agr-regulated phenol-soluble modulins (PSMs) with known surfactant properties promote colony spreading in *S. aureus*.

Methods: One *S. aureus* laboratory strain, one methicillin-sensitive (MSSA) clinical isolate, one community acquired methicillin-resistant (CA-MRSA) isolate, and a set of isogenic PSMa mutants were tested for colony spreading on soft agar plates consisting of 0.24% TSA. Plates were dried in a safety cabinet for 20 min and 2 l of an overnight culture was spotted onto the centre of each plate. After inoculation of bacteria, the plates were dried for 10 min and incubated overnight at 37C. Chemically synthesized PSMa peptides were used to complement the mutant strains.

Results: All the parental strains tested were able to spread on soft agar plates and to secrete PSMs, which is consistent with the fact that they have a functional agr system. The colony spreading of all tested PSMa mutants was dramatically decreased. However, the spreading of these mutant strains was restored when synthetic PSMa peptides were added to the samples prior to spotting on spreading plates.

Conclusions: Our present studies clearly show that agr-regulated PSMs promote colony spreading in *S. aureus*. Since PSMs have been implicated in virulence, our findings suggest that colony spreading could also be a virulence mechanism that might contribute to the successful spreading of *S. aureus* strains in the human host.

P098

Characterization of two novel CTX-M-25 group Extended-Spectrum β-Lactamases, CTX-M-94 and -100, recovered from patients at a tertiary care hospital in Israel

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Introduction: CTX-Ms are the most prevalent extended-spectrum -lactamases (ESBLs) in *Enterobacteriaceae* causing hospital acquired infections. Antibiotic selection pressure can cause mutations in $bla_{CTX\text{-}M}$ and emergence of novel phenotypes. We characterized two novel CTX-M variants in *E. coli* isolated from stool samples of two elderly patients admitted at the Tel Aviv Sourasky Medical Center, Israel. Both patients were undergoing treatment with cephalosporins prior to isolation of the novel CTX-M variants.

Methods: The *E. coli* strains, isolated from stool samples inoculated on -lactamase screening agar (MacConkey/Drigalski agar supplemented with ceftazidime and cefotaxime, respectively), were identified using mass spectrometry. ESBL presence was confirmed by the double disk synergy test (cefotaxime, ceftazidime, cefepime, and amoxicillin/clavulanate) and PCR-sequencing of -lactamase genes ($bla_{CTX\text{-}M}$, bla_{TEM}, bla_{SHV}). Primers specific for the IS*Ecp1* element identified the genetic context of the CTX-M variants and their extrachromosomal location was confirmed by amplification from plasmid DNA. The variants were cloned into a pCR-BluntII-TOPO vector and electrotransformed into *E. coli* TOP10 cells to ensure a clean background without additional -lactamase genes. Single point mutations (A77V, G240D) were introduced in cloned CTX-M-94 by site-directed mutagenesis. Susceptibility to -lactams was determined for transformed (wild types and mutants) *E. coli* by Etest. Isoelectric focusing was done to estimate the pI.

Results: The novel CTX-M variants, designated CTX-M-94 and -100 (Lahey), showed >99% homology with known CTX-M-25 group subtypes, CTX-M-25 and -39. Both variants differed from CTX-M-25 by amino acid substitution V77A, and from CTX-M-39 by amino acid substitution D240G. CTX-M-94 differed from all CTX-M-25 group subtypes by an additional amino acid substitution (F119L; Ambler numbering). CTX-M-94 (A77, L119, G240) conferred high-level resistance to cefotaxime (MIC 64 g/ml) and piperacillin (MIC >256 g/ml), intermediate resistance to amoxicillin/clavulanate (MIC 8 g/ml), and was susceptible to ceftazidime (MIC 4 g/ml). CTX-M-100 (F119) conferred only intermediate resistance to cefotaxime (MIC 12 g/ml) and piperacillin (MIC 32-48 g/ml), and was susceptible to amoxicillin/clavulanate (MIC 3 g/ml).

Mutagenesis experiments of CTX-M-94 showed that susceptibility to cefotaxime was lowered from high-level resistant (MIC 64 g/ml) to intermediate resistant (MIC 16 g/ml) and susceptible (MIC 4 g/ml) after introduction of A77V and G240D substitutions, respectively. Similarly, susceptibility to amoxicillin/clavulanate was lowered from intermediate resistant (MIC 8 g/ml) to susceptible (MIC 3 g/ml) for both mutants. Furthermore, after introduction of the G240D mutation, the MIC value for ceftazidime dropped to 0.5 g/ml. The pI of CTX-M-94 and -100 was found to be 7.3-7.5.

Conclusion: This is the first report of CTX-M-94 and -100, novel members of the CTX-M-25 group. The F119L substitution that is present in CTX-M-94, but not in other CTX-M-25 group subtypes, is associated with increased resistance to piperacillin. The D240G that is present in both CTX-M-94 and -100, is associated with reduced susceptibility to ceftazidime. Interestingly, the three substitutions (V77A, F119L, and D240G) identified in CTX-M-94 have a synergistic effect on resistance to cefotaxime and amoxicillin/clavulanate.

P099

Epstein-Barr virus protein BGLF5 contributes to innate immune evasion by downregulating Toll-like receptor 9

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Toll-like receptors (TLR) are innate pattern recognition receptors that sense various microbial components. These receptors play an important role in initial detection of viral infections. Upon ligand binding, TLRs initiate immediate antiviral defense mechanisms and play a role in shaping the adaptive immune response.

The human herpesvirus Epstein-Barr virus (EBV) is a large enveloped DNA virus that persistently infects more than 90% of the adult world population. Clinically, EBV is the causative agent of infectious mononucleosis and is associated with several malignant tumors. After primary infection, EBV establishes lifelong latency in memory B cells, characterized by expression of up to 10 viral proteins. Occasionally, new viral particles are produced following entry into the productive phase of infection that involves expression of all ~90 proteins encoded in the EBV genome. Among these are dedicated immune evasion molecules that ensure viral escape from adaptive immunity. Since EBV particles are recognized by members of the TLR family, we hypothesize that EBV has also acquired strategies to counteract innate detection, thereby facilitating establishment of primary infection and lifelong persistence in the host. The aim of this study is to determine if and how EBV interferes with TLR-mediated recognition.

Using our unique *in vitro* system to identify and isolate productively infected B cells, we found that EBV infection interferes with expression of several TLRs: during latency mRNA expression of TLR1 and 10 was reduced, whereas productive infection affected TLR1, 6, 7, 9, and 10. Downregulation of TLR9 was most pronounced, with >85% reduction at both the RNA and protein level in productively infected B cells. Importantly, EBV particles were able to trigger TLR9 signaling. We identified the EBV protein BGLF5 as contributing to TLR9 downregulation. BGLF5 blocks cellular protein synthesis through mRNA degradation (host shut-off'). Indeed, efficient degradation of TLR9 mRNA was observed in the presence of recombinant BGLF5 protein. Transfection of BGLF5 into 293-TLR9 cells caused a dose-dependent reduction in TLR9 protein level up to 50%, providing evidence for a functional consequence of TLR9 mRNA degradation by BGLF5 in cells. However, this result also suggests that other EBV gene products complement BGLF5, together resulting in the robust downregulation observed in infected B cells (>85%).

In conclusion, we found that during productive EBV infection levels of in particular TLR9 are strongly downregulated. BGLF5 contributes to reduced TLR9 levels through mRNA degradation. Additional EBV molecules are likely to be operative to achieve the drastic phenotype in infected B cells. This reflects the situation observed for adaptive immune evasion, which is effectuated by the concerted action of multiple gene products. Importantly, we observed that EBV particles trigger TLR9 signaling, revealing reduction of TLR9 levels during infection to be a potentially useful immune evasion strategy used by the virus. As hypothesized, EBV thus interferes with innate immunity at the level of TLR detection.

P100

Zebrafish as high throughput model for biomaterial-associated immune responses and infection

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Introduction: Biomaterial-associated infections (BAI) are a major problem in modern medicine. Recently it has been shown that the main causative agent of BAI, *Staphylococcus epidermidis*, not only produces a biofilm as a defense mechanism against the host immune system but also has

the ability to invade the tissue surrounding the biomaterial causing persistent infections. This project aimed at establishing an *in vivo* model to investigate the pathogenesis of *S. epidermidis* and *S. aureus* in zebrafish embryos in combination with implanted biomaterials. To allow for high-throughput and minimize variability, the yolk of the early-stage embryo was chosen as the injection site for all experiments, and polyvinylpyrrolidone$_{40}$ (PVP$_{40}$) was the only inoculum carrier used.

Methods: The yolk of zebrafish embryos was injected with standardized amounts of *S. epidermidis* O-47 pWVW189 (a plasmid carrying the Green Fluorescent Protein gene) and *S. aureus* RN4220 pWVW189, and infected embryos were examined using a fluorescence stereo microscope and quantitatively scored based on phenotype. Bacterial burden was determined by crushing and culturing the infected embryos. In addition we investigated the possibility of analyzing the bacterial burden of infected zebrafish embryos using the Complex Object Parametric Analyzer Sorter (COPAS XL, Union Biometrica Inc. USA). The intensity of fluorescent signal, gathered from a pool of 5 embryos, was compared with the numbers of bacteria measured by colony forming units (cfu) counts of the same group of embryos.

Results: Injection of *S. aureus* resulted in a much stronger infection than that obtained with *S. epidermidis*, with no embryos surviving past day 4. A challenge-dose-dependent bacterial growth was observed over the experimental time course of 120 hours for both *S. aureus* and *S. epidermidis*. The COPAS flow cytometer provided a reliable readout of bacterial load, which correlated accurately with the number of cfu's present in the infected embryos.

Conclusion: In the zebrafish embryo model *S. aureus* clearly is more virulent than *S. epidermidis*, resembling the virulence in other animal systems and in human patients. When combined with biomaterial implantation, yolk injection and COPAS individual embryo bacterial load determination offer excellent prospects for high throughput analysis of biomaterial-associated immune responses and susceptibility to infection.

P101

Molecular diagnosis of *Salmonella sp.*, *Shigella sp.* and *Campylobacter jejuni* compared to culture

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Introduction: Diagnosis for detection of *Salmonella*, *Shigella* and *Campylobacter jejuni* via culturing is regularly more slow than disappearance of the complaints. However, molecular diagnosis using real time PCR can be much faster, is easy to automate and, therefore, could spare hospitalisation time and costs, however carries the risk of over-sensitivity (detection of irrelevant loads). This study compares Ct values with culturing results, in order to determine comparable sensitivity.

Methods: A generally accepted real time PCR method for detection of *Salmonella* (target TtrC), *Shigella* (target IpaH) and *Campylobacter jejuni* (target MapA) was compared to standard culturing techniques (Selenite and Rappaport followed by Salmonella Shigella agar plus Desoxycholate agar, followed by TSI and Urea/indol, versus Charcoal Cefoperazone Deoxycholate agar). The PCR was performed on over 1400 clinical feces samples besides on feces plus o/n selenite culture. PCR positive samples for the respective organisms were ordered based on the Ct (including selenite), and the culturing results (positive or negative) were indicated.

Results: Ct values at which culturing does not likely give rise to a positive result differ between organisms and are estimated at 37 for *Salmonella*, 20 for *Shigella* and 37 for *Campylobacter jejuni*. Overnight culture in selenite results in a Ct value of around 20 in case of alive positive *Salmonella*, irrespective of the Ct value in the original feces (albeit below 37). Knowledge of PCR result improves culture outcome by 20% for *Campylobacter jejuni*.

Conclusions: PCR Positives with Ct values<30 can nearly always be cultured, except in case of dead material and maybe some *Shigella*, and can be reported the next day. With Ct values above 30 positive culture results become less likely and the error rate of the PCR increases, so PCR positives should be confirmed, which means that in about 30% of the positives the results will be delayed. The numbers for *Salmonella* and *Shigella* are still too low, but the Campylobacter *jejuni* results show that even with a Ct below 35 it is still very likely to get a positive culture (knowledge of the PCR result increases this likelihood, leading to an increase in strains for sensitivity determination).

P102

Identification of mycobacterial outer membrane proteins

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The causative agent of tuberculosis, *Mycobacterium tuberculosis*, is an atypical gram-positive bacterium with an unique cell envelope that consists of two hydrophobic layers. The

outer layer or mycomembrane consists mainly of long fatty acids, mycolic acids, intercalated with many unusual free lipids, resulting in a thick permeability barrier. This complex cell envelope, which is an essential part of the intrinsic resistance of mycobacteria to many antibiotics, poses a problem towards the secretion of virulence factors and the uptake of nutrients. Despite the evident importance of outer membrane proteins for secretion processes and nutrient uptake in Gram negative bacteria, very few of these proteins are identified in mycobacteria. So far, the only proteins known to reside in the mycomembrane are the general porin MspA specific to *Mycobacterium smegmatis* and the two channel-forming proteins OmpA and Rv1698 of *M. tuberculosis*. These mycobacterial outer membrane proteins (MOMPs) do not have structures similar to the gram-negative outer membrane proteins, as they reside in an unique lipid bilayer. Specific structural characteristics of MOMPs have not been elucidated yet. Due to their location on the outer layer of the bacteria, these proteins represent attractive drug targets, but their discovery is complicated by the difficulties in obtaining clean fractions of mycobacterial inner- and outer membranes.

For the identification of novel MOMPs, we have developed a reproducible method for differential fractionation of mycobacterial proteins of the inner- and outer membrane. During these studies we discovered that we can specifically obtain proteins from the outer layer of *Mycobacterium marinum* cell envelopes using differential solubilisation with specific detergents. We have analyzed these extracted fractions by mass spectrometry (LC-MS) to determine which proteins are located in the outer mycomembrane. The extracted fractions reproducibly contained the two known outer membrane proteins and were enriched for lipoproteins and many other mostly unknown proteins. Analysis of a number of these candidate MOMPs for their expression and localization in the cell envelope has revealed specific features of these proteins.

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Can clinical data and laboratory findings predict 30-day mortality of *Clostridium difficile* infected patients?

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Introduction: *Clostridium difficile* infections (CDI) are the leading cause of antibiotic associated diarrhoea. Mortality associated with CDI varies from 6% to 19% but can increase up to 30% when a pseudomembranous colitis is present. General patient characteristics such as age, comorbidity and type of *Clostridium difficile* have been used to predict the outcome of CDI. Recently, also laboratory findings and physical examination were proposed as predictors by the European Society of Clinical Microbiology and Infectious Diseases. We aimed to identify if these variables were associated with the 30-day mortality.

Method: In this ZonMw funded project, we included patients with a positive test for the toxin of *C. difficile* in nine hospitals in the Netherlands between March 2006 and May 2009. Demographic characteristic, clinical and laboratory parameters were collected using patient records, the electronic medical information system and consulting the physician in charge. We performed a univariable logistic regression with all cause mortality after 30 days and a severe course of CDI (death, ICU admission, collectomy) as an outcome.

Results: In total, 395 CDI patients were included of whom 64 died within 30 days (16.2%). 45 (12%) had a severe course of their CDI. Age and Charlson index were associated with both outcomes. Additionally, leucocytosis (OR 3.7 for mortality), low albumin (OR 7.0 for mortality) and a rise in creatinin of >50% (OR 4.9 for mortality) were strong predictors of mortality and a severe course. Presence of an ileus (OR 3.7) and hypotension (OR 2.7) were significantly associated with a severe course. Hypotension (OR 2.6) was also associated with mortality after 30 days.

Conclusion: Physical examination and clinical data are important in predicting the course of a *Clostridium difficile* infection. However, laboratory values have the strongest association with mortality after 30 days and a severe course of CDI. Since a bed-side prediction rule is not yet available, these parameters should be evaluated in this development.

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Gene loss and gain in the evolution of MRSA ST398

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Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) sequence type (ST) 398 is a colonizer and pathogen of humans and mammals and is sometimes associated with community-acquired infections. Whole genome sequences of four ST398 strains including the reference MRSA ST398 were compared with other staphylococcal genomes in the public domain to assess variation in the sequence of proteins known or putatively interacting with the host.

Materials and Methods: To establish the genetic variations within MRSA ST398, whole genome sequence analysis was performed for three selected ST398 strains isolated from different geographical regions, hosts, and with distinct antibiotic profiles. Tagged GS-FLX Titanium sequence libraries were constructed for each sample, followed by one sequence run on the GS-FLX Titanium.

All samples were assembled *de novo* using 'Newbler' (454 run assembly software). Annotations were performed with Kodon software (Applied Maths) and by the RAST and JCVI servers. The newly sequenced ST398 strains were mapped against the reference MRSA ST398 (S0385) genome. Next, we have compared the MRSA ST398 with the other human- and animal-associated *S. aureus* genomes (n=66) available via integrated microbial genomes (IMG) and NCBI websites.
Results: The genomes of all four ST398 isolates were examined for 22 microbial surface components recognizing adhesive matrix molecules (MMSCRAMs) implicated in adhesion (ClfA, ClfB, Cna, FnbpA, FnbpB, IsdC, SasA-K, SdrC-E, Spa, and Pls) and 90 staphylococcal exoproteins including toxins, immune evasion molecules, and coagulases. Some genes encoding for MMSCRAMs were missing or truncated and several distinct variants were identified. Similar to the MMSCRAMs, several enterotoxins and phage encoded toxins including Panton-Valentine Leukocidin were absent in all four ST398 strains. Human-specific immune evasion molecules such as staphylococcal complement inhibitory protein (SCIN) and chemotaxis inhibitory protein, encoded on bacteriophages of the phi3 family, were absent. However, genes for formyl peptide receptor inhibitory protein, extracellular fibrinogen-binding protein, extracellular complement-binding protein, and a distinct homologue of the human SCIN, were identified in all four ST398 genomes. Notably, the reference MRSA ST398 strain isolated from a patient with endocarditis harbors a pathogenicity island carrying a homologue of a secreted coagulase, called von Willebrand factor-binding protein, which is present in three out of four ST398 isolates, but absent from the other 66 *S. aureus* genomes.
Conclusions: *S. aureus* ST398-encoded surface and immune evasion genes are substantially different from the human-specific *S. aureus* strains. Further vaccination-based strategies need to take these variations into account should they protect against different MRSA ST398.

P105

Genetic diversity of human rhinoviruses obtained from patients hospitalized with severe respiratory illness during the winter season 2009-2010

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Introduction: Human rhinovirus (HRV) is the leading cause of acute respiratory tract illness, associated mainly with mild common cold symptoms. However, current advances in diagnostic techniques have revealed their frequent involvement in serious lower respiratory tract diseases. More than 100 serotypes have been described and classified into two genetic groups, HRV-A and HRV-B based on nucleotide sequence homology. Recently, a large number of previously unrecognized and highly divergent rhinoviruses were discovered and the majority of them clustered into a separate genetic clade proposed as a novel HRV-C species. Several studies have reported a higher incidence and more severe disease associated with HRV-A and HRV-C strains. The objective of this study was to investigate the HRV species prevalence and genetic diversity among patients hospitalized with severe respiratory illness at the Leiden University Medical Center (LUMC) during the winter season 2009 - 2010.
Methods: A total of 95 respiratory specimens positive for HRV as determined by real-time RT-PCR were analyzed in this study. The samples were obtained from 94 patients including mainly young children and elderly people consulting or hospitalized with severe respiratory illness at the LUMC during the period December 2009-April 2010. A semi-nested RT-PCR aiming to amplify the VP1 capsid gene of all three HRV species was developed with degenerate primers targeting conserved motifs in VP3, VP1 and 2A HRV genes. HRV genetic diversity was assessed by sequence and phylogenetic analyses of an approximately 800 nt genome region including almost the complete VP1 gene.
Results: Successful VP1 amplification and sequencing was achieved for 55 samples (58%) with viral loads corresponding to cycle threshold values ranging from 15 to 35.5, mean value 27. The characterized strains included 25 (46%) HRV-A, 7 (13%) HRV-B, 20 HRV-C (36%), and 3 (5%) HEV isolates. Comparative sequence analysis revealed two pairs of HRV-A and HRV-C and one pair of HRV-B identical sequences. VP1 genetic diversity among rhinovirus isolates was estimated to be up to 32% for HRV-B, 36% for HRV-A and 52% for HRV-C. Phylogenetic analyses revealed multiple VP1 genotypes co-circulating.
Conclusion: Our data indicates a high prevalence of HRV-A and HRV-C viruses circulating among patients with severe respiratory illness admitted to LUMC during the winter season 2009-2010. A significantly higher degree of genetic variation was observed among HRV-C viruses.

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Detection of *Mycoplasma pneumoniae* in healthy children by real-time PCR – preliminary data from the mymic study

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Introduction: *Mycoplasma pneumoniae (Mpn)* is a common cause of respiratory tract infections (RTIs) in children.

In the past decade, PCR has become a widely used tool to detect *Mpn*, because of its speed and high specificity and sensitivity. It is generally assumed that a positive PCR result for *Mpn* (which merely equals the presence of *Mpn* DNA) in the respiratory tract is always indicative of infection. However, it is unknown whether asymptomatic carriage with *Mpn* exists. If carriage exists, the treatment of all Mpn PCR-positive patients will lead to unnecessary overuse of antibiotic treatment. This holds the risk of development of antibiotic resistance, which is a documented emerging problem in several countries.
In this study we aim to improve diagnosis of RTIs caused by *Mpn* in children by (1) investigating the existence of asymptomatic carriage and (2) finding distinctive characteristics of carriage and infection.
Materials and Methods: From June 2008 until December 2010 267 children suffering from an upper or lower RTI, visiting the emergency Dept. of the Erasmus MC-Sophia Children's Hospital or a general practitioner's office and 300 healthy children, during a planned admission in the hospital for elective surgery, were enrolled in the study. For each child with RTI, clinical symptoms and signs of the acute illness were registered and from all children pharyngeal and nasopharyngeal samples and serum were collected. All samples were tested for the presence of bacteria and viruses, and in particular the presence of *Mpn* (by real-time PCR and culture) and the presence of anti-*Mpn* antibodies.
Results: Thus far, real-time PCR and culture for *Mpn* and standard bacterial cultures have been performed for 523 participants. Mpn DNA was detected in 33 of the 236 children (14.0%) with RTI versus 49 of the 287 of the healthy children (17.1%). A relation between the presence of common bacteria and the presence of *Mpn* was not observed In addition, there was no significant difference in *Mpn* load between the children with or without an RTI, as determined by real-time PCR.
Discussion and Conclusion: We investigated the presence of *Mpn* (determined by real-time PCR) in healthy children and children with upper or lower RTI and found no significant difference in prevalence in both groups. Moreover, we could not find differences in the Mpn genomic DNA load in both groups. Our data indicate that real- time PCR, as a diagnostic test for Mpn, should be interpreted carefully, since positive results can also represent cases of (healthy) carriage. Nevertheless, whether true carriage with *Mpn* indeed exists has not been established. In principle, a positive PCR for *Mpn* could also indicate that an individual is recovering from an infection or that an infection has just started to develop. To address this issue, we are currently testing all *Mpn* PCR-positive individuals in a longitudinal fashion.

P107

Surveillance of pathogens involved in gastro-enteritis in nursing homes: results from a pilot study within a sentinel surveillance network for infectious disease in nursing homes

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Introduction: Infectious gastro-enteritis in elderly is associated with high mordity and increased mortality, especially among residents in nursing homes. Using the sentinel surveillance network for infectious disease in nursing homes (www.SNIV.nl), elderly care physicians are requested to register weekly all episodes of gastro-enteritis that occur in nursing homes. The aim of this study was to find other causes of infectious gastro-enteritis than the well known norovirus.
Methods: From 1 June 2009-to 1 May 2010 all 24 nursing homes that participated in SNIV were asked to collect in total 5 stool samples of residents with gastro-enteritis. Gastro-enteritis was defined as diarrhea with 3 or more episodes of loose stools within 24 hours, or diarrhea with 2 of the following symptoms: fever, vomiting, nausea, stomach ache, abdominal cramps, blood or mucus in stool. Vomiting was included separately as a marker for gastro-enteritis when accompanied with 2 of the following symptoms; fever, nausea, stomach ache, abdominal cramps, blood or mucus in stool. Stool samples were send to RIVM-LIS (PCR analysis to norovirus, rotavirus, sapovirus, astrovirus, and adenovirus), to LUMC-Leiden (culture and characterization of *Clostridum difficile*), and to Laboratory for Infectious Diseases-Groningen (molecular diagnostics for Campylobacter jejuni, *Salmonella* spp., *Shigella* spp., EIEC, STEC, *Yersinia enterocolitica*, and *C. difficile*).
Results: In total, 31 stool samples were collected form 11 participating nursing homes. Of all samples, 10 (33%) contained norovirus, 2 (6%) had rotavirus, 1 (3%) sample had astrovirus, and 1 (3%) sample was positive for *C. difficile*. In total 4 samples (13%) were positive for atypical enteropathogenic *E. coli* and 2 (6%) samples were positive for *E. coli*, carrying the virulence gene astA of which 3 (10%) were also positive for norovirus. In 14 (45%) of the stool samples no pathogen was detected.
Conclusion:
1. Norovirus (33%) and enteropathogenic *E. coli* (19%) were the most frequently found pathogens of diarrhea in residents of nursing homes.
2. This pilot study needs a year-round follow-up with an appropriate control group to determine the exact role of enteropathogenic *E. coli*.

P108

Variation in a surface-exposed region of the *Mycoplasma pneumoniae* p40 protein as a consequence of homologous dna recombination between repmp5 elements

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Introduction: *Mycoplasma pneumoniae (Mpn)* is a human pathogen that causes a range of respiratory tract infections. The first step in infection is adherence of the bacteria to the respiratory epithelium. This step is mediated by a specialized organelle, which contains several proteins (cytadhesins) that have an important function in adherence. Two of these cytadhesins, P40 and P90, represent the proteolytic products from a single 130-kDa protein precursor, which is encoded by the MPN142 gene. Interestingly, MPN142 contains a repetitive DNA element, termed RepMP5, of which homologs are found at seven other loci within the *Mpn* genome. It has been hypothesized that these RepMP5 elements, which are similar but not identical in sequence, may recombine with their counterpart within MPN142 and thereby provide a source of sequence variation for this gene. As this variation may give rise to amino acids changes within P40 and P90, the recombination between RepMP5 elements may constitute the basis of antigenic variation and, possibly, immune evasion by *Mpn*.
Materials and Methods: To investigate the sequence variation of MPN142 in relation to inter-RepMP5 recombination, we determined the sequences of all RepMP5 elements in a collection of 25 strains isolated between 1962 and 1995. Eight RepMP5 specific primer pairs were designed and used to amplify all RepMP5 elements by PCR. Each PCR product was sequenced using a primer walking strategy. Sequences were analyzed using the application SeqManTM II (DNASTAR) and alignments were made with the multiple sequence alignment program ClustalW.
Results: All strains contained an identical number of RepMP5 elements as reference strain M129. Each element contained bacterial subtype-specific nucleotide polymorphisms and strain-specific polymorphisms. In two strains, the RepMP5 element within the MPN142 gene contained an aberrant sequence indicative of an inter-RepMP5 recombination event. In both strains, the aberrant sequence was derived from a distant RepMP5 element, and in both strains this event induced amino acid changes in a surface-exposed part of the P40 protein. In five other strains, inter-RepMP5 recombination events were found without the involvement of MPN142.
Discussion and Conclusion: We conclude that recombination between RepMP5 elements is a common phenomenon that may lead to sequence variation of MPN142-encoded proteins. This is the first time that variation in the MPN142 gene has been established. Since the proteins encoded by this gene are surface-exposed and highly immunogenic, homologous DNA recombination of MPN142 could play an important role in immune evasive strategies of *Mpn*.

P109

Course of *Staphylococcus aureus* bacteraemia in relation to colonization with *Staphylococcus aureus*, studies in a mouse model

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Introduction: *Staphylococcus aureus* (*S. aureus*) can cause a wide range of infections, and bacteraemia is often a serious complication. Previous studies in patients showed that *S. aureus* bacteraemia was more frequent in carriers than in non-carriers, while bacteraemia-related death was lower in carriers. In 80% of cases, *S. aureus* strains causing bacteraemia were endogenous. In the present study, we investigated in a mouse model whether colonization with *S. aureus* is protective against death due to *S. aureus* bacteraemia, and the role of the *S. aureus* strain (homologous strain or heterologous strain) in this respect. In addition, we studied which antigen-specific antibodies were relevant in this protection.
Methods: SOPF BALB/c mice that were initially *S. aureus*-free were colonized with various inocula of *S. aureus* using different routes (intranasal, intestinal, intracutaneous) to induce persistent *S. aureus* carriage and an anti-staphylococcal antibody response (10 mice per group). A *S. aureus* clinical isolate (strain P) or the sequenced *S. aureus* strain 8325-4 were used. Five weeks later, bacteraemia was induced intravenously with *S. aureus* strain P using two inocula that resulted in either 40% or 100% mortality (mild or severe bacteraemia, respectively). Animal survival rate was evaluated during a period of 14 days. In the *S. aureus* colonized mice, on the day before induction of bacteraemia, the presence of anti-staphylococcal IgG levels against 14 different *S. aureus* antigens in serum were determined using Luminex technology.
Results: Only intracutaneous inoculation of *S. aureus* was successful in inducing *S. aureus* colonization of mice and resulted in *S. aureus* persistence in the intestinal flora for 5 weeks as well as in anti-staphylococcal IgG levels in blood. A mild skin infection developed at the inoculation site, which was healed 5 weeks later.
Mice that were colonized with *S. aureus* showed an increased survival rate during severe bacteraemia

caused by the homologous *S. aureus* strain compared to non-colonized mice. This protective effect was not observed in mice colonized with a heterologous *S. aureus* strain. Colonization of mice with *S. aureus* strain P resulted in anti-staphylococcal IgG levels against IsaA, Nuc, PrsA, and TbpA at 5 weeks after induction of colonization, while colonization with *S. aureus* strain 8325-4 resulted in IgG levels against IsaA, lipase, PrsA, and TbpA.
Median IgG levels against the *S. aureus* antigens Nuc and PrsA were significantly higher in mice colonized with *S. aureus* strain P compared to mice colonized with *S. aureus* strain 8325-4.

Conclusion:

1. Mild skin infection with *S. aureus* is an appropriate mouse model for *S. aureus* colonization, exhibiting persistence of *S. aureus* in the intestines and circulating anti-staphylococcal IgG levels in blood.
2. Colonization with *S. aureus* results in delay in mortality due to *S. aureus* bacteraemia provided bacteraemia is caused by the homologous *S. aureus* strain. Colonization caused by a heterologous *S. aureus* strain has no protective effect. Circulating IgG antibodies against Nuc and against PrsA at the onset of bacteraemia might contribute to this protection.

P110

Identification of TSV infection as the likely cause of *Trichodysplasia spinulosa*

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Trichodysplasia spinulosa (TS) is a rare cutaneous skin disease that typically affects immuno-compromised individuals, a feature suggestive of an infectious origin. Up till now, 15 cases of TS have been described in the literature. A viral cause was suspected but not identified until recently, when we discovered and unraveled the sequence of a new polyomavirus in a TS patient. This virus was tentatively called the *Trichodysplasia spinulosa*-associated polyomavirus (TSV), but confirmation of the presence of TSV in TS lesions of other patients is still lacking. We collected formalin-fixed paraffin-embedded sections from a series of 7 TS cases previously described in the literature and 4 new TS cases originating from Western Europe (n=1), Australia (n=1), Canada (n=1) and USA (n=8). These materials were analyzed for the presence and load of TSV infection with quantitative PCR specific for three different TSV genome regions (VP1, LT and NCCR). Furthermore with the use of overlapping PCR that generated small TSV DNA fragments, TSV DNA contigs were obtained from each of these samples. On the complete TSV contig sequences phylogenetic analysis was performed.
The presence of TSV was confirmed in all 11 TS cases analyzed (100%). Average TSV loads ranged from 10^4 up to 10^7 viral copies per cell. Assessing non-lesional skin from 2 TS patients revealed almost undetectable TSV loads. Upon comparison of the TSV genome contig sequences from 3 different TS patients, 99.8-100% similarity was observed between TSV-isolates indicative of close phylogenetic clustering. Contig sequences from the other 8 TS samples will be analyzed within short notice.
In conclusion, almost identical TSV sequences were found in high copy numbers in all TS lesions tested, whereas non-lesional skin contained virtually no TSV DNA. These data provide compelling evidence that *Trichodysplasia spinulosa* is indeed caused by active TSV infection.

P111

Carbapenem-resistant *Pseudomonas aeruginosa* after short exposure to therapeutic concentrations of ertapenem

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Introduction: Compared to other carbapenems, ertapenem has only weak activity against *Pseudomonas* and *Acinetobacter* spp. This is suggested to be an advantage because when coverage for *P. aeruginosa* is not required, as in most community acquired infections, the use of *Pseudomonas* sparing agents, such as ertapenem, reduces antibiotic pressure. Since the introduction of ertapenem, however, concerns have been raised about the potential of this drug to select for resistance to other carbapenems in nosocomial pathogens, most importantly in *P. aeruginosa*. *In vitro* studies have shown that under certain conditions, such cross-resistance can develop, but conditions resembling clinical settings have not been tested. In this study, we determined the effect of incubation of *P. aeruginosa* with ertapenem in a range of concentrations that mimics the *in vivo* serum concentrations during ertapenem therapy.

Methods: 12 clinical isolates of *P. aeruginosa* (all with meropenem MICs <0.25 mg/L and imipenem MICs <1.5 mg/L; 10 with ertapenem MICs 0.064-4 mg/L, categorized as ertapenem sensitive, and 2 with ertapenem MICs >32 mg/L; categorized as ertapenem resistant) were incubated at 37°C in RPMI-medium with ertapenem concentrations reflecting the *in vivo* concentrations achieved with the standard daily dose (1 h in each 16, 8, 4 and 2 mg/L, 4 h in 1 mg/L and 16 h in 0.5 mg/L of ertapenem), this was repeated on three consecutive days. As a control, the isolates were incubated following the same scheme but in the absence of ertapenem. MICs for ertapemen, imipenem and meropenem were determined by E-tests, and sensitivity to other antibiotics was determined by Vitek-2.
Results: After the incubation with ertapenem, nine of the ertapenem sensitive *P. aeruginosa* isolates exhibited markedly elevated ertapenem MICs (>32 mg/L). Moreover, all these strains displayed diminished sensitivity or frank resistance to imipenem (MICs 4->32 mg/L) and/or meropenem (MICs 1.5->32 mg/L) and in some cases, the MICs for ceftazidime and piperacillin were also elevated. The phenotype was stable after one month of continuous culturing in the absence of antibiotics in all but one strain. In the case of one ertapenem sensitive isolate (ertapenem MIC 0.064 mg/L), no viable cells were recovered after the ertapenem treatment. The incubation with ertapenem had no effect on the carbapenem MICs of the two ertapenem resistant isolates, and none of the tested isolates showed changes in the sensitivity to non-betalactam antibiotics.
Conclusions: These data show that exposing *P. aeruginosa* to clinically relevant concentrations of ertapenem can rapidly select for diminished sensitivity to all carbapenems. Thus far, there is no evidence that such selection would occur in clinical practice, but these results disclose the need for continued surveillance.

P112

A multi-strain tiled microarray approach for prokaryotic genotyping; the *Staphylococcus aureus* example

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Introduction: Over the last decade, microarray technology has matured into a reliable tool for eukaryotic comparative genome hybridization (CGH). Recently, due to the availability of prokaryotic next-generation sequencing data, (custom-made) microarray-based CGH also prominently entered the prokaryotic domain.
Methicillin-resistant *Staphylococcus aureus* (MRSA) is currently emerging both as a community-associated (CA-MRSA) and livestock-associated (LA-MRSA) pathogen. Successful adaptation of MRSA to a new environment presumes acquisition of novel genomic elements, which is extensively studied in the EU project 'CONCORD'.
Here, we present a multi-strain tiled microarray approach to investigate the genomics content of *S. aureus* by a novel high-resolution CGH analysis.
Methods: A flexible microarray design is the essence of our multi-strain CGH approach: microarray probes directed against the 27 currently sequenced *S. aureus* strains were designed in a tiled fashion to completely cover these genomes on both strands. The resulting probe library of 121.901 *S. aureus* specific probes plus the appropriate control probes were produced as a custom SA-CGH microarray , which we tested on the Roche-NimbleGen (12x135K format) as well as Agilent (4x180k format) platforms. The SA-CGH microarray (NimbleGen) was validated by a CGH experiment using 10 well-known SA strains (MRSA252, MSSA476, Mu50, MW2, N315, NCTC8325, Newman, RF122, SO385 and USA300) as test samples and one strain (MRSA252) as a reference. Next, the SA-CGH microarray (Agilent) was used in a real-life study to genotype a cohort of 84 sepsis-associated isolates of *S. aureus*.
Results: The custom SA-CGH microarrays performed excellently on both technology platforms, although we developed a slight preference for the Agilent platform as it displayed a lower background level. This is important for absent/present calling, which is essential in CGH analysis. We successfully determined an array-specific absent/present threshold based on the relation between signal intensities and bit scores by analyzing triplicate CGH data from the 10 well-known strains. As such, the SA-CGH microarray allowed correct identification of all strains.
In the sepsis *S. aureus* study we were able to: determine phylogenetic relations, which show a good correspondence to MLST typing; perform a full SCC-mec typing analysis; classify the isolates according to IEC phage typing; assign a best-matched well-known strain to each isolate; identify lacking chromosomal regions compared to all well-known strains; identified the core genome within the sepsis isolates; and identify antibiotics resistance genes for clindamycin and co-trimoxazole by a correlation between probe intensities and antibiotics resistance.
Conclusion: We have designed a high-resolution multi-strain *S.aureus* tiled microarray as proof-of-concept for an innovative prokaryotic CGH approach that provides a deep insight in the genomic content of non-sequenced samples. This flexible and affordable approach can be applied in a multi-strain manner to any micro-organisms with a known DNA sequence.

P113

Staphylococcus aureus colonization in patients with Epidermolysis bullosa

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Introduction: Patients with the inherited disease Epidermolysis bullosa (EB) develop skin blisters upon minor injury. Depending on the type of EB, the symptoms vary in severity from minor blistering of the skin to a lethal form involving other organs.

Because the natural barrier function of the skin is partially absent in EB patients, it was anticipated that their wounds would be colonized by different microorganisms. In this study we focused on colonization by *Staphylococcus aureus*. This bacterium is mostly a harmless commensal in about 11-32% of the human population. However, if *S. aureus* reaches the blood stream, this bacterium can cause a range of serious invasive diseases.

Methods: 52 EB patients with chronic wounds (n=20) or without chronic wounds (n=32) were recruited. For each patient, swabs were taken from the left nostril, right nostril, throat and three different wounds, and the presence of *S. aureus* was tested with standard diagnostic tools. The swabs were taken 3 times with about 6-monthly intervals. In total, we collected 443 *S. aureus* isolates, which were analyzed by Multiple-locus Variable Number of Tandem Repeats Analysis (MLVA) and spa-typing.

Results: In total, 90% of the EB patients with chronic wounds and 79% of the EB patients without chronic wound were colonized by *S. aureus* on at least one sampled body site. Wounds were colonized in 88% of the patients with chronic wounds and in 60% of the patients without chronic wounds. Comparisons of the strains isolated from each individual revealed a high variation in the MLVA types found within patients. In 67% of the patients with chronic wounds and 55% of patients without chronic wounds we found more than one MLVA type. Remarkably, these MLVA types are changing during time both in nose/throat and wounds, independently of the severity of the disease.

Conclusion: The *S. aureus* colonization rate in EB patients is significantly higher than in healthy individuals. EB patients with chronic wounds show the highest level of wound colonization, which is consistent with an impaired barrier function of the skin. Typing of the isolated strains suggests that the colonization of EB patients is not correlated to a particular *S. aureus* lineage. Instead, our results indicate that EB patients provide an attractive niche for colonization by *S. aureus* in general.

P114

Epstein-Barr virus BGLF5 acts as an RNase to cause host shutoff

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Introduction: Several (herpes)viruses encode so-called 'host shutoff proteins' that extremely effectively block cellular proteins synthesis following viral entry. Epstein-Barr virus (EBV) – the causative agent of infectious mononucleosis and several malignant tumors – likewise drastically impairs cellular protein synthesis during viral replication. This host shutoff phenotype results from mRNA degradation by the early lytic-phase protein BGLF5. Interestingly, BGLF5 is the EBV DNase, or alkaline exonuclease, which is present throughout the herpesvirus family. During viral replication, this DNase is essential for processing and packaging of the viral genome. In contrast to this widely conserved DNase activity, host shutoff is only mediated by the alkaline exonucleases of the subfamily of gamma-herpesviruses (including EBV and Kaposi's sarcoma-associated herpesvirus) and not of alpha- and beta-herpesviruses (such as herpes simplex virus and human cytomegalovirus, respectively). The reasons for this restricted activity are still poorly understood. In this study, we investigated the mechanism of BGLF5-mediated host shutoff.

Methods: Based on the recently resolved crystal structure of EBV BGLF5 and on other information, mutants were generated and analysed for their nuclease activities. To measure DNase activity, plasmid DNA was incubated with either *in vitro* translated or recombinant BGLF5. RNase activity towards *in vitro* synthesized transcripts was studied using recombinant BGLF5. Additionally, RNase activity was examined in cells transiently transfected to express BGLF5. GFP reporter protein levels (as measured by flow cytometry) and relocalization of the cytoplasmic pool of the polyA-binding protein PABPC (as visualized by immunofluorescence) were used as readouts for shutoff activity of the BGLF5 variants expressed in the transfected cells.

Results and conclusion: We observed that in the presence of Mn^{2+}, recombinant BGLF5 exerts RNase activity *in vitro*, degrading both cellular and viral transcripts, with or without a polyA tail. When analysing various mutants of BGLF5, we found, on the one hand, that a point mutation destroying DNase function also blocked RNase activity, implying both nuclease activities to share a catalytic site. On the other hand, other mutations were more selective affecting either DNA or RNA degradation, pointing towards genetic separation of these two functions.

In conclusion, EBV BGLF5 is a dual-function protein that combines nuclease activities towards DNA and RNA. Unraveling the mechanism of the additional RNase function in gamma-herpesviruses will contribute to our understanding of immune evasion, as we have shown that host shutoff results in a block in the synthesis of immunologically relevant molecules, reflected by for instance reduced human leukocyte antigen (HLA)-restricted antigen presentation to T cells or signaling through Toll-like receptors on EBV-producing B cells. This could lead to immune escape, thus prolonging the time for the generation of viral progeny.

P115

Epidemiology and genetic diversity of Dutch *Cryptococcus neoformans* isolates investigated by AFLP, microsatellite typing and *in vitro* antifungal susceptibility testing

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Background: *Cryptococcus neoformans* is frequently implicated in meningoencephalitis in immunocompromised (mostly HIV positive) individuals. In the Netherlands, the highest number of infections was observed in 1987 due to the increasing numbers of HIV infected patients. With the introduction of HAART the number of cryptococcal infections has decreased significantly. Here we present genotyping data on a collection of 300 *C. neoformans* isolates (serotypes A and D) using various genotyping methods. In addition, we determined *in vitro* antifungal susceptibility patterns.

Materials and methods: A standardized AFLP protocol was used. Mating- and serotypes were determined using the *GPA1*, *STE12* and *STE20* genes. Serotype A isolates were further studied using 9 microsatellite markers, and serotype D isolates using 7 microsatellite markers. The antifungal susceptibility patterns of amphotericin B, 5-flucytosine, fluconazole, itraconazole, voriconazole, posaconazole and isavuconazole were determined using the CLSI microdilution method.

Results: 300 *C. neoformans* isolates were isolated from 237 patients. Almost half the studied cases of cryptococcosis occurred in HIV-infected patients (*n*=115; 48.5%), followed by the patient group with other underlying diseases (*n*=50; 21.1%) and immunocompetent individuals (*n*=18; 7.6%), 54 (22.8%) of the episodes occurred in patients with no known immune status. PCR based mating- and serotyping for *C. neoformans* revealed that the majority of isolates were aA (*n*=219; 73.0%), the remaining isolates were aAaA (*n*=21; 7.0%), aD (*n*=38; 10.7%), aD (*n*=6; 2.0%), aAaD (*n*=6; 2.0%), aDaA (*n*=4; 1.3%) and aAaD (*n*=1; 0.3%). Two isolates were identified as *C. gattii* (0.7%).

By AFLP, the majority of isolates was AFLP1 (*n*=245; 81.7%), 55 were AFLP2 (12.0%), 14 were AFLP3 (4.7%) and one for each AFLP4 and AFLP6 (0.3%). Three isolates were found to be interspecies hybrids genotype AFLP8 (1.0%). A significant correlation between AFLP genotype and the patient's immune status was observed (*P*=0.0288).

Microsatellite typing for isolates with a serotype A (*n*=259) and serotype D (*n*=53) background revealed 196 and 32 genotypes, respectively. The highest genetic diversity was observed during the peak of HIV-associated cryptococcosis. No statistical correlation between clinical data and microsatellite clusters could be observed.

Overall MIC-ranges were 0.063-1g/ml for amphotericin B, 0.125->64g/ml for 5-flucytosine, 0.25-64g/ml for fluconazole, 0.016-0.5g/ml for itraconazole and posaconazole, 0.016-1g/ml for voriconazole and <0.016-0.5g/ml for isavuconazole. One 5-flucytosine resistant *Cryptococcus* isolate was cultured from a HIV-positive patient, a second strain isolated on the same day from CSF was found to be susceptible (MIC=8g/ml). Less susceptible (=16g/ml) for 5-flucytosine and fluconazole were nine and ten *C. neoformans* isolates, respectively.

Conclusions: cryptococcosis in the Netherlands is mainly caused by *C. neoformans* variety *grubii* (AFLP1) and to a lesser extent by *C. neoformans* variety *neoformans* (AFLP2) and the AD hybrids (AFLP3). The microsatellite panels for serotype A and D isolates was found to be a highly discriminatory tool to distinguish between isolates of the same AFLP genotype as well as to discriminate between successive isolates from the same patient. *In vitro* susceptibility testing shows that resistance or decreased susceptibility against the conventional antifungal compounds is not commonly found among Dutch *C. neoformans* isolates.

P116

A novel microsatellite typing method to investigate the population structure of the expanding *Cryptococcus gattii* AFLP6 outbreak

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Background: An unprecented outbreak of the basidiomycetous primary pathogenic yeast *C. gattii* emerged in the temperate climate of Vancouver Island (Canada) and

expanded subsequently further into the Pacific Northwest. Detailed genotyping using RFLP and AFLP fingerprinting revealed that the outbreak was caused by genotype AFLP6 strains, which was prior the outbreak a rare genotype of *C. gattii*. Since then, several studies have been carried out to investigate the origin of this outbreak using Multi-Locus Sequence Typing (MLST). However, MLST is a laborious and expensive technique, which prompted us to develop a novel panel of microsatellite loci to investigate the global population structure of *C. gattii* genotype AFLP6.

Material, Methods and Results: 225 globally collected *C. gattii* AFLP6 strains were further subgenotyped using AFLP fingerprinting into the major outbreak genotype AFLP6A (*n*=77) and the minor genotypes AFLP6B (*n*=138) and AFLP6C (*n*=10). The majority of isolates were mating-type alpha (*n*=183). Mating-type a strains were of South American origin. 87 STR-types were identified using a novel 10-loci microsatellite typing method. *C. gattii* outbreak AFLP6A strains clustered together in one MC, which was also the case for North American and Australasian AFLP6B strains. A third MC contains solely South American AFLP6B mating-type **a** isolates. South American isolates show the highest genetic diversity compared to strains from other geographical regions.

Discussion: The outbreak related *C. gattii* AFLP6 strains fell all into one MC, containing eleven different STR-types, which indicates that more genetic diversity exists within the outbreak cluster than was previously concluded from MLST analyses. The highest diversity in STR-types was observed among South American *C. gattii* strains and this population also harbors strains with the opposite mating-type **a** and a. This indicates that South America is likely to be the origin of the global *C. gattii* AFLP6 population.

P117

Flexibility of real-time PCR: expansion of a pathogen identification assay with *E. coli* probe

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Introduction: Broad-range real-time PCR and sequencing of the 16S rDNA gene region is a widely known method for the detection and identification of bacteria in clinical samples. However, due to the need for sequencing, identification of bacteria is time-consuming. In an earlier designed multi-probe assay for blood cultures, we could successfully determine seven clinically important bacterial pathogens. However, the assay could not identify one the most frequently found gram-negative bacteria, i.e. *E. coli*. The aim of our study was to extend the multi-probe assay, by designing a probe specific for *E. coli*.

Methods: The design of the *E. coli* probe was based on regions of identity within the 16S rDNA gene. The performed assay also included a universal probe within this region for the detection of all bacterial DNA. We used a collection of clinical blood cultures, of which gram-staining was performed. In total, 109 blood cultures, positive for gram-negative bacteria, were included and used for the validation of the *E. coli* probe.

Results: The assay was tested on a collection of 109 positive and 16 negative blood cultures. The universal probe and the probe targeting *E. coli* had a sensitivity and specificity of 100%. In 57 blood cultures, *E. coli* was correctly identified with the newly designed probe.

Conclusion: These additional results showed a good agreement between conventional testing and the expanded real-time PCR assay. Furthermore, this assay significantly reduced the time needed for identification. In conclusion, using this more complete panel of pathogen-specific probes offers a more rapid alternative for pathogen detection and could improve the diagnosis of bloodstream infections. The *E. coli* probe, in combination with the earlier designed probes, is now being used in the DOBBI trial for the identification of bacterial pathogens in blood cultures.

P118

Innovation: mobile phones to support quality (microscopy based) diagnostics, prelimanary results

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Introduction: A current phenomenon in sub-Saharan Africa is the introduction of cellular telephony which has revolutionized the provision of many services. Within the field of health care, mobile phones have already been applied in remote areas to bridge the distance gap and provide quicker access to care. Rural health care clinics, such as those found in Sub-Saharan Africa, are typically under-staffed and lack training and support. Microscopes are the main instruments used for diagnosis of infectious diseases, and while they are available at most levels of health clinics, the lack of education, confidence (other) resources and experience amongst the staff are the main grounds for miss-diagnosis of illnesses which is a cause of inappropriate treatment and thus poor patient care. These challenges will be addressed by combining microscopy with mobile networks. This system will create a knowledge sharing forum specifically designed for rural health care workers to improve microscopy based diagnostics. Here we will highlight new ways to support diagnostic testing through mobile phones.

Objectives/expected benefits: Reduction of costs for quality assurance (QA) and increased compliance. Reduction of cost in patient care as a result of reduced travelling and

improved patient treatment. Strengthened education of rural health care workers and improved diagnosis of illnesses and proper treatment. To strengthen abilities to monitor for laboratory performance and general awareness for central agencies (government, NGOs etc.).

Methods: Selection of regional/reference hospital and five remote satellite health centre laboratories, Uganda. Improvement of current (quality control) procedures can be determined by evaluating the existing and novel systems concurrently over a period of time.

Conclusions: Under-educated and under-experienced health care workers will be empowered to meet quality assurance standards and accurately and confidently diagnose parasitical and bacterial infections and receive health education where necessary. By increasing the connectivity of isolated health workers in low resource settings and supporting quality control activities, misdiagnosis will decrease and reduce patients suffering from improper treatment.

P119

Identification of bacteria from positive Bact/ALERT blood culture bottles with MALDI-TOF MS: a comparison of 3 bacterial isolation methods

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Introduction: Matrix-assisted laser desorption ionisation-time of flight mass spectrometry (MALDI-TOF MS) is a fast and reliable method for identification of bacteria from agar media. Direct identification from positive blood culture bottles should decrease time to result. In this study, 3 different methods for the direct identification of bacteria from positive blood culture bottles were compared.

Methods: A total of 70 positive aerobe Bact/ALERT bottles were included in this study until now; 29 bottles containing gram-negative rods and 41 bottles containing gram-positive cocci. Aliquots from all bottles were used for 3 bacterial isolation methods; 1 ml was used for Bruker's MALDI Sepsityper kit, 1ml was used for Molzym's MolYsis Basic 5 kit until the washing step, and 4 ml was used for the method described by Ferreira L. et al., [CMI 2010] which entails a few centrifugation steps and washing with water. All methods were followed by Bruker's long extraction protocol. All results were compared with routine diagnostic testing and MALDI-TOF MS directly from blood plates.

Results: No identification (spectral score <1.7) was obtained in 31 (44%), 35 (50%), and 15 (21%) of the bottles when Ferreira, Molzym's MolYsis Basic 5, and Bruker MALDI Sepsityper were used respectively. Of the bacteria with a spectral score =1.7, 39 (56%), 35 (50%), and 55 (79%) were identified correctly when Ferreira, Molzym's MolYsis Basic 5, and Bruker MALDI Sepsityper were used respectively. gram-positive cocci were correctly identified in 29/41 (71%) of the cases, however, gram-negative rods showed a correct identification in 26/29 (90%) of all bottles when Bruker's Sepsityper kit was used.

Conclusion: These preliminary data show, that of the methods tested, Bruker's MALDI Sepsityper works superior for direct identification of microorganisms from aerobe Bact/ALERT bottles; 55/70 bottles were correctly identified. Gram-negative pathogens show better results compared to gram-positive bacteria. The Sepsityper kit is easy to use and leads to results quickly, as compared to the current diagnostics.

P120

Detection of commonly encountered gram-positive cocci from clinical material of suspected sepsis patients by an in-house real-time PCR, a pilot study

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Introduction: Blood stream infections are serious medical conditions. Gram-positive microorganisms account for the majority of episodes of bacteremia in critically ill patients in the intensive care unit (ICU). Early detection and subsequent adequate treatment of blood stream infections is critical for successful patient outcomes. In this study, the feasibility was determined of the detection of commonly encountered gram-positive cocci (*Staphylococci* and *Enterococci*) in clinical material of sepsis suspected patients using an in-house real-time PCR.

Methods: Duplex real-time PCRs were developed and validated using 100 cultured isolates and more than 300 blood culture bottles (both positives and negatives) for the differentiation of *S. aureus* from coagulase-negative *Staphylococci* (CNS), and detection of the most commonly encountered *Enterococcus* spp. To be able to analyse whole blood samples of patients, five bacterial DNA isolation methods for whole blood were compared, and the most sensitive one was subsequently evaluated in the clinic.

Results: The PCRs showed 100% sensitivity and specificity on positive blood culture bottles containing gram-positive cocci. Double infections discovered with culture techniques were correctly identified with the described duplex PCR assays. The optimal DNA detection limit from whole blood was 10 CFU/ml for 1ml S.aureus spiked EDTA blood. One CFU/ml could be detected for 5 ml *S. aureus* spiked EDTA blood isolated with the Molzym Complete kit. The Molzym Complete kit was therefore used to

isolate bacterial DNA from 1ml EDTA blood of 30 patients suspected of having a blood stream infection, to show proof-of-principle. Of all 30 patient samples, 22 could be compared to blood culture results. Sixteen of 22 samples resulted in similar data compared to blood culture results (72,7%). Blood culture results of 6 (27.3%) samples were not comparable to PCR data. However, in 2 of these 6 cases (9%) PCR might have added value to sepsis diagnostics.

Conclusion: Developed real-time PCR assays were 100% sensitive and specific for the detection of *S. aureus*, CNS and *Enterococcus* spp. using positive blood cultures showing gram-positive cocci. Double infections were identified correctly as well. Preliminary results of this sensitive assay on remaining EDTA blood of suspected sepsis patients showed increased and faster detection of *S. aureus* and supports further evaluation on a larger patient group.

P121

Prolonged culture of orthopaedic samples in prosthetic joint infections results in increased sensitivity

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Introduction: In VMC 750 prosthetic joint implantations were performed in 2010.

In 2010 a new culture procedure for prosthetic joint infections (PJI) was introduced. The reported incidence of infections after prosthetic joint surgery ranges from 1 to 2%. Approximately 10-20% of the clinically infected prostheses remain culture negative, and are possibly false-negative. Based on literature we adapted our procedures for culturing prosthetic samples. We assessed which infections would have been missed using the old method instead of the new culture procedure.

Methods: Before 2010 we plated the orthopaedic specimens on a blood agar (5 days 36C), Levinthal agar (5 days 36C CO2), Anaerobe Agar (7 days 36C) and a TFB broth (1 day 36C plus subsequent sub culturing). With the new procedure we added a Thio broth and prolonged the incubation to 10 days for the Thio and TFB broth. The incubation times of the direct platings remained the same. During 2010 we recorded after how many days the cultures became positive, from which culture medium and in how many samples from each patient. From the patients with positive cultures we assessed if the longer incubation time was necessary for making the diagnosis of infection, using the microbiological definition that 2 or more samples positive with the same micro organism indicate the presence of PJI.

Results: There were positive cultures from 28 surgical procedures in 20 (2,7% from 750) patients. Prolonged culture was necessary for making a microbiological diagnosis of infection in 4 of the 28 procedures. In 6 procedures it was uncertain if the infection would have been missed using the old method. The new procedure had no additional value in 18 operations, i.e. the direct platings showed growth, most frequently after 1 or 2 days.

When 10-20% of the PJI would remain culture negative, approximately 2-4 patients with a clinically PJI would remain culture negative. The new procedure yielded a positive culture in 4 additional patients who would have been culture negative using the old culture procedure, thereby significantly increasing the yield of cultures in clinically infected prostheses. Especially in the 2nd or 3rd operations for debridement and changing the gentamicin beads the broth was more often positive than the direct plating.

Conclusions:

1. Prolonged incubation time is useful and adds a few microbiological confirmations of PJI.
2. This is especially true when cultures are taken in the second or third operation after revision, with prior use of antibiotics.

P123

Positive schistosomiasis serology in Eumycetoma patients in Sudan: cause or correlation?

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Introduction: Mycetoma is a distinct chronic granulomatous subcutaneous infectious disease endemic in many tropical and sub-tropical regions, the so-called mycetoma belt. Mycetoma can be caused by a variety of true fungi (eumycetoma) or by bacteria of the genus *Actinomyces* (actinomycetoma). Based on antibody measurements in earlier studies it was noted that although most people living in mycetoma endemic areas had antibodies against the causative agents, only few of them actually developed mycetoma. To date, it is still unknown why some people are predisposed towards mycetoma infection. This predisposition could be immunologically based or due to co-infections with other infectious agents. A large part of the mycetoma-belt overlaps with the endemic region for schistosomiasis. Schistosomiasis is endemic in 76 countries worldwide and besides malaria is the second important parasitic disease threatening public health. Sudan seems to have a high incidence of both infections. Co-infection with these infectious agents might influence the manifestations and outcome of either infection, especially since schistosomiasis has an immunosuppressive effect on the host. However, data regarding *Schistosoma*-mycetoma co-infections is lacking. In this study we determined if *Schistosoma*-mycetoma co-infections do occur in Sudan.

Methods: Serum samples were taken from 64 male patients (53 suffering from eumycetoma and 11 suffering from actinomycetoma) and 31 matched controls in the Sudan endemic area in 2008. Serum was stored at -20C until further use. To determine if mycetoma-patients had a serological response against *Schistosoma* spp., a *Schistosoma* egg antigen (SEA) ELISA and an indirect hemagglutination assay (IHA) with adult worm antigens were performed. Furthermore, the antibody response against various *Madurella mycetomatis* antigens was determined using luminex-technology.
Results: No difference in *Schistosoma* serology results between actinomycetoma patients and healthy controls was observed (p=1). However, eumycetoma patients had positive *Schistosoma* serology significantly more often than healthy controls (p=0.02). No correlation between *Schistosoma* antibody titers and the size of the mycetoma lesion was found. Although co-infection with *Schistosoma* is known to reduce the humoral immune response against antigens of other pathogens, the antibody responses against 4 different *M. mycetomatis* antigens was not reduced in *Schistosoma* co-infected individuals compared to mycetoma-only infected individuals.
Conclusion:

1. Positive *Schistosoma* serology is significantly more often detected in mycetoma patients compared with people living in the same mycetoma endemic area who did not develop mycetoma.
2. Eumycetoma is correlated with *Schistosoma* co-infection while actincomycetoma is not
3. *Schistosoma* co-infection is not associated with an increased mycetoma lesion size or a reduction in antibody response against mycetoma antigens.

P124

Bacterial cell wall component muramyl dipeptide synergizes with respiratory syncytial virus in cytokine production

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Introduction: Hospitalizations due to respiratory syncytial virus (RSV) have been associated with respiratory bacterial co-infections. Research into how a viral and bacterial co-infection can enhance each other can therefore give insight and potential targets for therapeutical intervention strategies. However, not much is known about the mechanism behind this synergy.
Muramyl dipeptide (MDP) is a component of the cell wall of both gram-positive and gram-negative bacteria and is recognized by NOD2, an intracellular pattern recognition receptor. NOD2 is a modulator of signals transmitted through Toll Like Receptor 3 (TLR3) and TLR4. Literature shows that RSV can be recognized by TLR3 (dsRNA), TLR4 (F-protein) and TLR7/8 (ssRNA). Our hypothesis is that RSV induced TLR activation is enhanced by NOD2 stimulation.
Methods: Human PBMCs were stimulated for 24hrs with RSV A2, purified TLR ligands and MDP. Subsequently cytokines were measured.
Results: Stimulation of human PBMCs with RSV A2 resulted in low cytokine responses (IL-6 420111 pg/ml, TNF 11820 pg/ml, IL-10 3617 pg/ml and IL-1beta 6425 pg/ml).
Stimulation with MDP gave a stronger cytokine response (IL-6 23 0009848 pg/ml, TNF 741246 pg/ml, IL-10 7448 pg/ml and IL-1beta 775409 pg/ml).
A combination of both stimuli resulted in high cytokine responses. Ratio's were calculated as followed; [RSV + MDP]/[RSV] + [MDP]. The combination of both stimuli resulted in a 2.00.28 fold increase in IL-6 production, a 3.81.07 fold increase in TNF production and a 2.20.30 fold increase in IL-1beta production. IL-10 did not show synergy. Stimulation of PBMCs with specific ligands for TLR3 (PolyI:C, structurally similar to dsRNA) and TLR 7 and 8 (ssRNA40 and R848, a mimetic of ssRNA) in combination with MDP did not show synergy. Viral ssRNA and dsRNA are therefore not the viral ligands that cause the synergy. This excludes TLR3, TLR7 and TLR8 as potential receptors involved in the crosstalk with NOD2.
Because purified F-protein is not available, dynasore was used to prove that TLR4 is the receptor involved in the synergy. Dynasore blocks the internalization of the TLR4 receptor and thereby the TRIF dependent pathway. Blocking with dynasore showed a 3.5 fold increase of the synergy in TNF production when MDP and RSV are combined. This suggests that the crosstalk between RSV and MDP is TRIF independent and therefore most likely NF-κB dependent. We hypothesize that TLR4 is not internalized and therefore constantly stimulated on the membrane what results in higher TNF production.
Conclusion: In this study we show a synergy in cytokine production by human PBMCs when stimulated with MDP and RSV. The synergy is not induced by TLR3, TLR7 and TLR8 and therefore most likely TLR4 dependent. Moreover, our results with dynasore support this assumption. The results with dynasore suggest that the synergy is NF-κB dependent. Ongoing experiments will have to show the influence of the synergy on viral infection of PBMCs.

P125

Detection of genes essential for growth of three important respiratory pathogens by genomic sequence footprinting

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Introduction: Respiratory tract infections are a leading cause of global mortality and morbidity. For instance, it has been estimated by the WHO that annually 4-5 million people die of bacterial pneumonia. Growth of respiratory pathogens such as *Streptococcus pneumoniae*, *Moraxella catarrhalis* and *Haemophilus influenzae* depends on a number of essential bacterial pathways, of which members are considered to form ideal candidate targets for drug design and/or vaccine development, however, detection of these essential genes by classical knockout studies is a time-consuming task. To rapidly screen multiple species for essential genes, a high-throughput method is required.
Methods: To identify microbial genes essential for growth of these three micro-organisms, we have applied the novel Genomic Sequence Footprinting (GSF) technology. GSF employs next generation sequencing innovations to generate footprints of bacterial transposon mutant libraries. Genes for which no mutants are detected in the knockout library are likely absolutely essential for growth of the micro-organism. To identify shared essential pathways in these bacterial species we have used statistical analysis, pathway analysis and functional category enrichment methods.
Results: In all three respiratory pathogens we observed that roughly 10% of all genes is essential, similar to what has been found in classical knockout studies in literature. Most of these genes encode functions involved in transcription, translation or replication. In accordance, we failed to delete these genes by classical knockout mutagenesis. The products of these genes are considered to play an essential role in biological processes of the bacterial cell, and consequently, are likely to be interesting candidate targets for drug design and/or vaccine development.
Conclusions:
1. High throughput screening of essential genes in bacterial pathogens is feasible using GSF.
2. Genes encoding orthologous proteins in all three species that were found to be essential, are considered to play an important role in critical processes in the cell.

P126

Identification of a putative operon involved in *Streptococcus pneumoniae* resistance to extracellular oxidative stress

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Streptococcus pneumoniae is a gram-positive, catalase-negative facultative anaerobe. It is a member of the normal human nasopharyngeal flora and can cause serious disease such as pneumonia, otitis media, meningitis, and bacteremia.
S. pneumoniae releases large amounts of hydrogen peroxide (H2O2) during aerobic growth, which is mainly produced by pyruvate oxidase (SpxB). Furthermore, the bacteria are exposed to oxidative stress produced by competing bacteria in the nasopharynx and by the host. However, how *S. pneumoniae*, an aerotolerant anaerobic bacterium that lacks catalase, protects itself against oxidative stress is still unclear.
Methods: Analysis of the genome identified a hypothetical open reading belonging to the thiol specific antioxidant (TlpA/TSA) family, located in a putative operon.
To determine the role of this protein in the survival of external oxidative stress, the gene was deleted using allelic replacement mutagenesis from the genome of D39 and R6; two commonly used laboratory-adapted strains, and three more recent clinically isolated strains Tigr4, G54 and 670-6B. Subsequently, the bacteria were exposed to 20 mM H2O2 and the amount of CFU's was determined using serial dilutions and the bacteria were harvested for further analysis.
Results: Interestingly, the survival rates of the more recently isolated wild type strains was higher (>1%) than that of the laboratory strains (<0.1%). Furthermore, in all strains the mutant had a lower survival rate of external oxidative stress than that of the wild type. Complementation analysis indicated that other genes in the operon also play a role in the protection against oxidative stress, The amount of the TlpA protein was determined to be under the influence of external oxidative stress.
From this data we conclude:
1. Laboratory adapted strains seem to be more sensitive to oxidative stress in comparison to more recent clinically isolated strains.
2. Deletion of this protein resulted in increased sensitivity to external H2O2 in the strains tested.
3. Complementation results shown that whole operon is necessary for resistance against oxidative stress.

Thus, we have identified a putative operon that is involved in the protection of *S. pneumoniae* against external peroxide stress.

P127

Pathways of *Streptococcus pneumoniae* invasion of and survival in eukaryotic host cells

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Introduction: The gram-positive bacteria *Streptococcus pneumoniae* is a commensal resident of the nasopharyngeal cavity. *S. pneumoniae* can however turn infectious and

invade other compartments of the body. This invasion usually goes via the lungs, and pneumococcal pneumonia has been a leading secondary infection and cause of death during influenza pandemics. Some other notable diseases caused by *S. pneumoniae* include meningitis, sepsis and otitis media.
The underlying host/pathogen interactions which govern the *S. pneumoniae* switch from extracellular commensal to invasive pathogen are still poorly understood. One proposed route of breaching cell barriers is via invasion and subsequent translocation of the host cells. In this work we have focused on revealing which cellular uptake mechanisms are involved in the *S. pneumoniae* invasion of the host cell. Furthermore we addressed the fate of internalized cells by determining the rate of survival inside the eukaryotic host cell. These are key processes, which ultimately may lead to the transversal of the endothelial barriers and possible further dissemination of the host.
Methods: To determine which mechanisms are involved in the *S. pneumoniae* invasion, and survival of the host cell, we used various chemical inhibitors to block discrete parts of the uptake and degradation systems. Subsequently, the invasive and intracellular survival properties of *S. pneumoniae* were assessed using Human Brain Microvascular Endothelial Cells (HBMEC).
Results: In this work we show that, *in vitro*, invasion of the host cells is facilitated mainly via clathrin mediated endocytosis, whereas caveolae mediated endocytosis only plays a minor part in invasion. Furthermore we demonstrate that the bulk of the intracellular *S. pneumoniae* is killed in the lysosome, or, to a lesser extent destined for recycling out of the host cell. A subset of the intracellular *S. pneumoniae* are however not killed or recycled, but, translocated out via the basal side of the endothelial host cells, and possibly allowing further dissemination of the host.
Conclusions:
1. *S. pneumoniae* mainly utilizes the clathrin mediated endocytosis uptake route for invasion of the endothelial host cells.
2. Internalized *S. pneumoniae* is mainly killed in the lysosome but a small subset avoids this fate and is translocated out via the basal side of the endothelial host cells.

P128

Exacerbations in COPD: the bug or the body?

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Introduction: Exacerbations, a characteristic feature of COPD, are associated with a decline in lung function leading to a decreased quality of life and higher morbidity and mortality among COPD patients. Bacterial infections are linked to the development of exacerbations. However, not all infected patients develop an exacerbation. Previously, it has been speculated that differences in bacterial load, location of the bacterial infection or the acquisition of a bacterial strain new to the immune system may explain differences in outcome once a COPD patient becomes infected. Here we tested the hypothesis that differences in virulence among *Haemophilus* strains of COPD patients account for differences in the outcome.
Material and methods: *Haemophilus* spp. were isolated from sputum samples of COPD patients GOLD III-IV either during the stable phase (SD-strains, n=5) or during acute exacerbations (AE-strains, n=5). *Haemophilus* spp. were identified with standard laboratory procedures. Difference in genotypes of *Haemophilus* spp. were investigated using Pulsed Field Gel Electrophoresis (PFGE). *Haemophilus* spp. with genetic distinct profiles were selected for further studies. Virulence of *Haemophilus* spp. was determined using an *in-vitro* cell culture model studying bacterial adherence to lung epithelium cells (A549), as well as IL-8 expression and release and ICAM-1 expression by these cells following exposure to different *Haemophilus* spp. A whole blood assay (WBA) was used to determine the differences in cytokine production (TNF-α, IL-6, IL-1β and IL-10) between different *Haemophilus* strains and to evaluate inter-individual differences. Blood was obtained from ten young healthy individuals.
Results:
1. The *in-vitro* study showed no significant differences in virulence in terms of adhesion, IL-8 expression and release and ICAM-1 expression in A549 cells between groups of *Haemophilus* spp. categorized as SD- or AE-strains.
2. The WBA showed highly similar inflammatory cytokine production between SD- and AE-strains. However, large variations in the cytokine production were observed individual volunteers when stimulation with a specific *Haemophilus* strain. These data suggest that important inter-individual differences in cytokine production exist between volunteers.

Conclusion: Our results show that it is unlikely that differences in virulence between *Haemophilus* spp. explain the different outcomes in COPD patients. Yet, the large inter- but not intra-individual differences in cytokine production suggest that the reactivity of the immune system is a more important predictor of the outcome of an infection than the virulence of different bacterial strains.

P129

Low prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in Dutch nursing homes

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Introduction: The goal of our study was to assess the prevalence of carriers of methicillin-resistant *Staphylococcus aureus* (MRSA) in Dutch nursing homes.

Methods: We randomly selected 88 nursing homes from the 718 nursing homes registered at kiesbeter.nl. Homes with fewer than 50 beds (n=94) and homes participating in the *Surveillance Netwerk Infectieziekten Verpleeghuizen* (n=7) were excluded. Twenty six homes, distributed all over the country, agreed to participate. Between April and November 2009 we sampled approximately 50% of the consenting residents from each nursing home. Two anterior nasal swabs (one from each nostril) were taken. One swab was analyzed at Bilthoven (RIVM) and one at Maastricht University Medical Centre.

Results: Bilthoven detected two carriers in two different nursing homes. Maastricht found the same two carriers and another one in a third home. The *spa*-types of the MRSA-isolates were t002, t037, and t740. Combining the estimates of both laboratories, the overall weighted prevalence of MRSA colonization in Dutch nursing homes was 0.33% (95% CI 0.14-0.74%).

Conclusion: The percentage of 0.33% MRSA colonization is still low and similar to the percentages found in earlier surveys in Dutch nursing homes (0.15% in 1992/1993 and 0.66% in 2002/2003) and the 0.09% (0.05-0.14%) prevalence which was recently reported for Dutch clinics.

P130

Evaluation of the Diasorin Liaison Parvovirus B19 chemiluminescence assay for the detection of Human Parvovirus B19 IgM and IgG antibodies

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Introduction: Human parvovirus B19 (PRV) is a significant pathogen in the immunocompromised host and those with underlying blood disorders, causing serious complications such as chronic anaemia, pure red cell aplasia and thrombocytopenia. Transplacental fetal infection during pregnancy can result in fetal death, fetal hydrops or congenital anemia. Therefore a rapid, sensitive and specific screening method for the detection PRV IgM and IgG antibodies is essential.

The new Diasorin PRV IgM and IgG chemiluminescence assay is designed for use on the Liaison platform. The assay is based on undenatured PRV antigen produced in a baculovirus expression system. In the present study we have evaluated the Diasorin Liaison PRV IgM and IgG assay with three enzyme immunoassays also based on PRV antigens produced by the baculovirus expression system.

Methods: Archived serum samples (n=110) were classified according to their serological profile as determined previously with the Biotrin (Biotrin International) PRV IgM and IgG assay. Samples were classified into 4 groups. Group 1; seronegative (IgM negative [neg]/ IgG neg; n=30), group 2: past infection (IgM neg/ IgG positive [pos]; n=20), group 3: acute infection (IgM pos/ IgG pos; n=30) and group 4: cross-reactive samples (EBV VCA IgM pos/IgG pos, EBNA IgG neg; n=10), rheumatoid factor positive (n=10) and parvovirus B19 IgM negative pregnant women (Biotrin); n=10). All samples were tested for parviovirus B19 IgM and IgG with the following assays: the Liaison (Diasorin), recomWell (Mikrogen), Novagnost (Siemens) and Novalisa (Novatec Immunodiagnostica).

As no standard reference test for the determination of PRV IgM or IgG is available, latent-class analysis was used on the whole data set to calculate sensitivity and specificity. Kappa-analysis was performed to determine the level of agreement between the Liaison and Biotrin assay. Mean interrun and intrarun coefficients of variation (CV) were calculated to assess the precision of the assays.

Results: The PRV IgM sensitivity for the Biotrin, Liason, recomWell, Novagnost and Novalisa assays were 98%, 97%, 100%, 98% and 100% respectively. The PRV IgM specifity was 97% for all assays. The PRV IgG sensitivity for the recomWell assay was 97% and PRV IgG sensitivities were 100% for all other assays. PRV IgG specificities were 100% for all assays. The level of agreement between the Liaison assay and the Biotrin assay for PRV IgM and IgG were 0.90 and 0.97, respectively. The mean intra assay mean inter assay CV's for PRV IgM and IgG were 2.13%, 7.15%, 3.5% and 1.84%, respectively.

Conclusion: The new Diasorin parvovirus B19 IgM and IgG antibody test for the Liaison platform showed a performance comparable to the Biotrin assay. The high sensitivity and specificity, the very good level of agreement with the Biotrin assay and the small intrarun and interrun CV's together with the added technological advantages of a fully automated platform, may be beneficial in the accurate and fast detection of parvovirus B19 antibodies.

P131

Experimental studies on the survival of *Coxiella burnetii*

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Introduction: The Q fever outbreak in the Netherlands between 2007 and 2009 is the largest well documented Q fever outbreak reported. Dairy goats and sheep are the main source of human cases, and it is hypothesized by the veterinary and public health community that humans were infected by inhalation of *Coxiella burnetii* contaminated aerosols. Contaminated aerosols could have been produced during abortions of dairy goats and sheep, but also by handling and transport of contaminated manure. To limit transmission of *C. burnetii* to the environment, control measures were implemented on handling and storage of manure from Q fever affected farms. Control measures included mandatory (plastic) covered storage of manure on farm premises for 90 to 150 days. To access the efficacy of manure storage duration, decimal reduction times (DRT) of *C. burnetii* under field storage conditions and laboratory studies were investigated.
Methods: Temperatures in the outer layer and inner core of stored manure were measured on two Q fever affected farms using calibrated temperature measurement equipment. In addition, the Nine Mile reference strain of *C. burnetii* was used in laboratory experiments. PBS, PBS+1,8% urea, and PBS+1,8% ammonia and manure extract was spiked with 105 *C. burnetii* organisms and treated with the following time-temperature combinations: 5, 10 and 15 s at 70 C and 72 C and 180, 360 and 540 s at 60 C and 65 C. Experiments were carried out in triplicate. Samples, before and after the treatments, were diluted and cultured on BGM cells to measure viability. Difference in viability before and after treatments indicates the reduction in viable *C. burnetii* induced by the treatments. Results of viability over time for each matrix were plotted against the four temperature treatments. A linear trend line was fitted, and the DRTs were calculated for each matrix at temperatures 40 C and 60 C.
Results: The temperature in the outer layer of the stored manure is over 60 C for 5 to 12 days. Core temperatures are over 40 C for 10 days, or more. The calculated DRTs in PBS, PBS+1,8% urea and PBS+1,8% ammonia at 40 C are 14381, 91833 and 36116 s, and at 60 C are 75,82, 179,06, and 129,93 s respectively. DRTs obtained using a manure extract will be presented at the meeting. Based on 10 storage days at a temperature of 40 C, a minimum of 9,4 log reduction can be reached. With 5 storage days at a temperature of 60 C, a minimum of 2413 log reductions of *C. burnetii* can be reached.

Conclusions:
1. The DRT of the Nine Mile reference strain of *C. burnetii* at 40 C and 60 C differs between the matrices in PBS and PBS+1,8% urea.
2. Apparently, urea and ammonia have a stabilizing effect on *C. burnetii*, resulting in a longer DRT compared to PBS.

P132

Maraviroc Inhibits Dual-R5 Viruses in a Dual/Mixed HIV-1 Infected Patient

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Introduction: HIV entry is a multistep process. After binding to the CD4 receptor the virus needs to bind to a co-receptor to further facilitate viral entry. The virus can bind to either the CCR5 co-receptor (R5-tropic virus) or the CXCR4 co-receptor (X4-tropic virus). The virus can also be dual-tropic in case it has the ability to utilize both co-receptors. Viral entry through the CCR5 co-receptor can be inhibited by maraviroc (MVC). It is the first licensed CCR5 co-receptor antagonist in clinical practice and is currently being used in HIV infected patients harbouring exclusively R5-tropic virus. It was recently demonstrated *in vitro* that some of the dual-tropic viruses are much more efficient in using the CCR5 co-receptor (dual-R5), whereas others use the CXCR4 co-receptor more efficiently (dual-X4). We hypothesize that in patients that are infected with a Dual-tropic/Mixed (R5 and X4-tropic viruses) (D/M) viral population, MVC may suppress viral replication of both R5 and dual-R5 tropic viruses.
Methods: A longitudinal analysis was performed during MVC add-on therapy in a D/M infected patient. Co-receptor tropism was determined phenotypically in the Trofile Assay, MT2 assay and genotypically by plasma env gp120-V3 analysis. In dept analysis of viral MT2 cultures was performed to obtain infectious virus clones from two time-points, pre and post MVC therapy. Tropism of these infectious clones was investigated in U-373-MAGI cells expressing $CD4^{+}CCR5^{+}$ or $CD4^{+}CXCR4^{+}$. Donor PBMCs and MAGI cell lines were used to determine MVC susceptibility. Viral replication kinetics were determined in donor PBMCs.
Results: In-dept clonal genotypic analysis revealed the presence of both R5-tropic variants and X4-tropic viruses before the start of MVC. One week later genotypic analyses revealed that MVC suppressed all R5-tropic variants and also part of the viruses classified as X4-tropic. All biological clones could replicate in both CCR5 and CXCR4 cell lines, indicating their dual-tropic nature. Phenotypic analysis of the pre-MVC biological clones demonstrated a preference for replication in the CCR5 cell line. In

contrast biological clones post MVC start had a preference for CXCR4 usage. Baseline biological clones were fully susceptible to MVC in donor PBMCs and in the CCR5 MAGI cell line. In contrast, during MVC therapy the dual-R5 tropic viruses were replaced by more X4-tropic viruses (dual-X4), which could not be fully inhibited by MVC.

Conclusion: These data indicate that some dual-tropic viruses have a preference to infect target cells via the CCR5 co-receptor and can be fully inhibited by MVC. Here we demonstrate the ability of MVC to inhibit viral replication of both R5 and dual-R5 tropic viruses in a D/M classified patient. During treatment with MVC dual-X4 tropic viruses may be selected that cannot or only partly be inhibited by MVC. Further research is warranted whether MVC in combination with an active backbone might be a useful treatment strategy in patients harboring dual-tropic virus.

P133

Impact of triplicate testing on genotypic tropism prediction in routine clinical practice

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Introduction: Maraviroc (MVC) is the first available antiretroviral drug targeting a human receptor. It binds to the CCR5 co-receptor thereby inhibiting replication of CCR5 using (R5-tropic) HIV-1 but not of CXCR4 using HIV-1 (X4-tropic). Guidelines state that MVC can be prescribed to patients infected with R5 tropic HIV-1 only. Therefore, tropism of the viral population needs to be assessed either phenotypically or genotypically. Preliminary data from clinical trials suggest that genotypic analysis in triplicate may increase detection of X4-tropic viruses. The objective of this study was to evaluate the impact of triplicate genotypic analysis on prediction of co-receptor usage in routine clinical practice.

Methods: Plasma or PBMC samples from antiretroviral therapy nave and experienced patients were collected in three European clinical centers. Viral-RNA was isolated from 200-500ul of plasma and proviral-DNA was isolated from 10E7 PBMCs. Gp120-V3 was amplified in a triplicate nested (RT)-PCR procedure and sequenced. Viral co-receptor usage was predicted using the Geno2Pheno$_{[coreceptor]}$ algorithm or PSSM$_{X4\text{-}R5}$. Prediction in Geno2Pheno$_{[coreceptor]}$ was performed with a cut-off of 10%, 20% False Positive Rate (FPR) or according to the current European guidelines on tropism testing.

Results: In total 266 V3 sequences were obtained from 101 samples. Genotypic X4 co-receptor usage was predicted for 28% of the sequences using 10% FPR and an additional 15% using 20% FPR. Triplicate genotypic analysis resulted in a discordance in detection and prediction of co-receptor usage in 7% (10% FPR) and 12% (20% FPR) of the viral RNA samples. In proviral DNA 14% of the samples were discordant using an FPR of 10% and 21% using 20% FPR. In 24 patients an X4 genotype was detected (24%) using a single amplification procedure with 10% FPR this increased to 28 predictions when the procedure was performed in triplicate. With a more conservative cut-off of 20% FPR the number of X4 predicted samples increased with 10 from 33 to 43. Reclassification from R5 to X4-prediction occurred in 4 samples based on 10% FPR, in 7 samples based on 20% FPR, and in 4 samples based on the European guidelines. However, 4 samples were also reclassified from X4 to R5-tropism when applying these guidelines.

Conclusion: Triplicate genotypic tropism testing did not significantly increase the number of X4-predicted samples in this relatively small dataset. However, 10-20% discordance was observed in single versus triplicate genotypic tropism testing using either viral RNA or proviral DNA. For the individual patient, additional detection of X4-variants due to triplicate testing might prove to be pivotal when CCR5-inhibitor therapy is applied since reclassification from R5 to X4-prediction renders patients ineligible for MVC therapy.

P134

Genetic markers for *Clostridium difficile* hypervirulent ribotypes 027 and 078

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Introduction: *Clostridium difficile* is an anaerobic bacillus that resides in the gut and has rapidly emerged as a leading cause of antibiotic associated diarrheal disease in humans. In the gut, *C. difficile* starts to produce toxins that cause intestinal damage, inflammation and clinical disease. In the past decade the incidence, complications and mortality of a *Clostridium difficile* infection have increased dramatically due to the emergence of hypervirulent strains PCR ribotype 027 (NAP01) and PCR ribotype 078 (NAP7/8). Typing of *C. difficile* pathogenic strains is essential for quick assessment of risk factors involved in development of clinical disease. In this study, using a bioinformatics approach, we identified unique loci in the PCR ribotypes 027 and 078 and used these markers to detect hypervirulent *C. difficile* strains.

Methods: Based on sequence comparisons between *C. difficile* strains we describe DNA regions that are unique for two major hypervirulent strains belonging to PCR ribotype groups 027 and 078. These unique DNA regions were used to develop type specific PCRs. To validate the type specific PCRs, we screened a large PCR ribotype strain collection (N=68) completed with diverse 027 and 078 isolates derived from different geographical locations and individual outbreaks. Comparative analyses were performed if other PCR ribotypes, than the hypervirulent ones, contained the identified genetic marker. In addition, clinical characteristics (severity of disease and 30-day mortality) of the hypervirulent ribotypes and other ribotypes with the genetic marker were compared to ribotypes without the genetic marker.
Results: Two unique DNA inserts were identified, one present in the genome ribotype 027 and the other present in the genome ribotype 078. We show that these genetic markers are stably present in the hypervirulent ribotypes; all ribotype 027 (N=24) and ribotype 078 (N=32) isolates tested positive in our PCR for the corresponding insert. In addition, we show that both inserts are also present in other, closely related ribotypes. All of these ribotypes were related to their hypervirulent counterpart (ribotype 027 or ribotype 078) by either marker mutations in their sequence, a similar toxin profile or resemblances in PCR ribotype banding patterns. Furthermore, we show that the ribotypes with the insert share some clinical characteristics with their hypervirulent counterpart. Type 027-like strains resemble type 027 in the presence of severe diarrhea (50% vs 43%) but not in mortality after 30 days (0% vs 22%). Type 078-like strains more often caused severe diarrhea and mortality compared to 078 strains (60% vs 51% and 36% vs 15%).
Conclusions:
1. The identified markers enable rapid and specific recognition of hypervirulent strains and other *C. difficile* ribotypes with similar characteristic features.
2. Moreover, loci unique for these strains will be very useful for routine typing of *C. difficile* because of its comprehensive power and ease and speed of use.

P135

The distribution of extended spectrum beta-lactamase (ESBL) genes in hospitalized and out-patients in the East of the Netherlands using a commercial DNA Microarray system

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Introduction: ESBL genes are distributed worldwide and their epidemiology is complex. The aim of this study was to describe the distribution of ESBL genes in isolates from three laboratories in the East of the Netherlands using a commercial DNA Microarray system (Check-ESBL, Check-Points, Wageningen). The three laboratories (Doetinchem, Ede, Velp) receive samples from patients admitted at 5 hospitals (Slingeland, Doetinchem; Gelderse Vallei, Ede; Rivierenland, Tiel; Rijnstate, Arnhem; Rijnstate Hospital Zevenaar, Zevenaar) and from patients in the surrounding community.
Methods: From February 2009 until March 2010 all gram-negative isolates from clinical and surveillance specimens which were screened ESBL positive with either VITEK2 or Phoenix were collected. Per patient only the first putative ESBL-positive isolate per species was further characterized with the Check-ESBL. Four patient categories were distinguished: i) patients admitted to an Intensive Care Unit (ICU), ii) non-ICU patients, iii) out-patients, admitted <1 year before collection of the isolate (<1), and iv) out-patients admitted >1 year prior to isolate collection or who had never been hospitalized before (>1). Statistical analysis was performed by the χ^2 test.
Results: Within 491 putative ESBL-positive isolates 247 (50.3%) contained ESBL genes as detected by the Check-ESBL assay. These 247 isolates originated from 236 patients. One hundred sixty-one (65.2%) of the isolates originated from urine, 28 (11.3%) from surveillance cultures (rectal/perineal swabs/faeces), 22 (8.9%) from respiratory specimens, 13 (5.3%) from blood cultures and 23 (9.3%) from other clinical specimens. The predominant species were *E. coli* (179), *E. cloacae* (29) and *K. pneumoniae* (26). A total of 116 isolates were from hospitalized patients (35 ICU, 81 non-ICU), 131 isolates were from out-patients (43 <1, 88 >1). The number of *E. cloacae* was significantly higher in hospitalized patients than in out-patients, 20.7% and 3.8%, respectively (P=0.001). With the Check-ESBL assay 274 ESBL genes were identified in the 247 isolates: 153 CTX-M-1 group, 67 CTX-M-9 group, 32 SHV, 14 TEM and 8 CTX-M-2 group. In *E. coli* and *K. pneumoniae* CTX-M-1 group ESBLs were predominant, 70.4% and 51.6%, respectively. In *E. cloacae* CTX-M-9 group ESBLs were predominant (57.9%). CTX-M-9 group ESBLs were significantly more prevalent in ICU patients (P=0.003). In hospitalized patients the number of SHV ESBLs was significantly higher than in out-patients (P <0.001). There was no significant difference in distribution of ESBL genes between the two outpatient groups.
Conclusions: 1) The Check-ESBL assay made it possible to describe the distribution of ESBL genes in our region. 2) The distribution of ESBL genes in our region is comparable

to earlier described distribution of ESBL genes. 3) The observation that the distribution of ESBL genes between the two out-patient groups is not significantly different, might reflect the loss of hospital-acquired ESBLs within 1 year after hospitalization, but needs further study.

P136

Life-cell imaging of aerobic bacteria; a tool to assess and model heterogeneous germination & outgrowth of *Bacillus subtilis* spores

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Introduction: Spores of various *Bacillus* spp. can remain in a dormant, stress resistant state for long periods. Their return to vegetative cells involves a rapid germination followed by a more extended outgrowth phase. Spore-forming bacteria are a special problem for the food industry as some of them are able to survive preservation processes. Spore germination & outgrowth progression is often very heterogeneous and therefore makes predictions of microbial stability of food products exceedingly difficult. Since outgrowing spores or vegetative cells are much easier to kill than dormant spores, it would be advantageous to rapidly and completely trigger germination of spores in foods and then inactivate all at the outgrowth stage with heat or another physical treatment. To do so with appropriate robustness one should have mechanistic detail of the cause of heterogeneity in spore germination and outgrowth. In order to monitor heterogeneity we made a novel cast for live imaging which allows the growth, germination & outgrowth of *Bacillus subtilis* cells and spores, respectively.

Methods: To quantitatively model the heterogeneity in spore germination and outgrowth we developed a single cell live imaging system. In order to check the efficiency of the setup, growth and division of B. subtilis 1A700 vegetative cells was checked at different concentrations of rich undefined media (TSB, LB), as well as a defined medium (MOPS). Phase-contrast images were recorded every 30 s for 4 hours and doubling times were calculated. We were able to monitor nine areas in one slide per time-point using a routine that steers the lens appropriately. Thus, maximally ~100 starting cells (or spores) could be examined per experiment.

Results: The data on growth and division of *B. subtilis* 1A700 vegetative cells on TSB, LB, and MOPS show that the setup is good for the growth and division of aerobic bacteria. The calculated generation times in our system are comparable to generation times obtained in shake flask cultures. Hence, the setup is suitable for heterogeneity measurements at the single spore level. Preliminary results show that also proper germination and outgrowth of spores is observed in our setup. To monitor where most heterogeneity ensues, recording of germination (phase bright to phase dark transition) and outgrowth times (formation of two cells) of individual spores is in progress.

Conclusions: We optimized the acquisition of live images which allows us to follow growth and division of *Bacillus subtilis* vegetative cells with growth rates similar to those obtained in well-aerated shake flask cultures.

Heterogeneity of *B. subtilis* spore behavior can be studied with single-cell analysis techniques that enable us to analyze individual spores during germination and outgrowth. Current challenges are to extend the observation time from 4 hours to 24 hours such that monitoring of spore outgrowth of damaged spores as well as under adverse conditions can be started.

P137

Monitoring strain diversity in metagenomics data using meta-MLST SNP profiling

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Introduction: Microbial consortia, or complex (undefined) mixtures of microbes, are ubiquitous in nature. They are found everywhere ranging from soil to gut and from biofilms to industrial fermentations.

Monitoring bacterial diversity in consortia from metagenomics sequence data, is typically done using e.g., 16S pyrosequencing techniques. These techniques mostly allow describing a bacterial consortium up to the genus level.

The assumption in many metagenomics studies and the subject of many published reviews is that knowing what microbial taxa are present also indicates their functionality in the community. Based on the limited knowledge about bacterial strains and their gene content, and thus functionality, the extrapolation of knowing what species is present to their functionality is therefore highly challengeable. In addition, in many environments, e.g. from acid mine drainage, human-controlled aquatic environments and dairy starter cultures, coexistence of multiple closely related strains is observed, of which the diversity is not only determined by their unique gene content, but also by plasmid content and phage sensitivity. Our understanding of a population's complexity will therefore largely depend on the ability to differentiate between genetically highly similar individuals.

Method: We describe a method that allows following the naturally occurring bacterial diversity at the strain level. It involves selecting 'core' genes in a set of isolated representative strains that are expected to be present in all bacterial strains present in the metagenome. Next, (combinations of) single nucleotide polymorphisms (SNPs) are determined that allow distinguishing (groups of) strains. These SNPs are subsequently used to categorize reads or contigs obtained from next generation sequence analysis of metagenomic samples into strains, similar to multi locus sequence typing (MLST).
Results: We apply this technique to follow strain level diversity of *Lactococcus lactis* in multiple-timepoint metagenomics data obtained during the cheese making process. The resulting strain types are mapped on metabolic pathways.
Conclusions: We show that 1) following highly similar individual strains directly from metagenome sequence data is feasible and 2) mapping of identified strain types on metabolic pathways gains insight in the metabolic potential of individual isolated strains vs. the metagenome.

P138

Need for cytomegalovirus polymerase chain reaction assay in patients with discordant cytomegalovirus IgM serology assays

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Introduction: AxSYM cytomegalovirus (CMV) IgM is known to be a very sensitive screening test for primary CMV infection, despite limited specificity. False positive CMV IgM results, as for other tests, have been described. Therefore, in clinical practice a positive AxSYM (Abbott) IgM result which is not confirmed by other serological assays, e.g. VIDAS (BioMrieux) CMV IgM assay, is not seldom considered to be false positive without serological evidence for primary and/or active cytomegalovirus infection.
Methods: In a retrospective study of patients hospitalized in 2005-2008, based on clinical suspicion, several AxSYM positive CMV IgM and VIDAS negative IgM patients were examined for CMV infection at Maastricht University Medical Centre. The patients identified with discordant CMV IgM assays were further investigated using CMV PCR in blood.
Results: Remarkably, out of 77 patients with AxSYM positive (mean: 0.969, range 0.503-4.185) and VIDAS negative (mean: 0.34, range: 0.02-0.67) CMV IgM, 6 patients (8%) were found to have an active CMV infection since they tested positive in the CMV-DNA PCR assay (range 118->100,000 copies/ml). Within this patient subgroup disease aetiologies were diverse and included congenital infection, pregnancy and immune suppression. In the literature, other researchers also reported positive AxSYM CMV IgM assays with negative IgM confirmation tests (VIDAS, Captia, Eurogenetics or IMx), which were evaluated as primary CMV infections since CMV IgG avidity indexes were low.
Conclusion:
1. The above mentioned results show that positive CMV IgM screenings assays which are interpreted as false positive due to, more specific but less sensitive, negative CMV IgM confirmation tests should be evaluated with care.
2. Moreover, additional CMV diagnostic tests (e.g. CMV-DNA PCR) are justified to prevent false negative conclusions in certain patient groups, especially pregnant women and immunocompromised patients.

P139

Influence of abiotic stress on survival and catabolic activity of *Desulfitobacterium hafniense* DCB-2

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The extensive uses of synthetic chemicals such as organohalides have resulted in substantial waste streams and environmental pollution at the local and global scales. Many of those waste streams consist of or contain toxic chemicals which affect human as well as environmental health and ecosystem functioning when treated improperly. Therefore, we are aiming to improve the rational use of bacterial catalytic activities for the treatment, removal and prevention of organohalide pollution.
The low G+C gram-positive genus *Desulfitobacterium* is a group of anaerobic bacteria that can use different aliphatic and aromatic organohalides as electron acceptors for their growth. Recently, the full genome sequences of *Desulfitobacterium hafniense* strains DCB-2 and Y51 have been elucidated, confirming their dedication to organohalide respiration as well as their versatility with respect to electron donors and alternative electron acceptors. In the present study, functional genomics approaches are being employed to understand influences of abiotic stresses on survival and catabolic activity of *D. hafniense* DCB-2.
Firstly, based on the available genome sequences, we constructed a pan-genomic microarray targeting the genomes of both *D. hafniense* strains DCB-2 and Y51. The array contains 21905 distinct 45-60 mer oligonucleotide probes that were designed by using PICKY software. Within this probe set, 2044 probes specifically target 848 genes that are unique to strain DCB-2; 2803 probes are specific to 1106 Y51-unique genes; 1927 oligonucleotides

target 1168 DCB-2 intergenic regions; 3479 probes were designed specific for 1774 Y51 intergenic regions. The remaining 11652 oligonucleotide probes were designed to detect genes and intergenic region shared by DCB-2 and Y51. In summary, we have one to three probes per target transcript and probes cover 97% of protein coding sequences and 80% of intergenic regions for both strains. Secondly, batch cultures of *D. hafniense* DCB-2 were exposed to the halo-aromatic electron acceptor 3-chloro-4-hydroxyphenylacetic acid at concentrations ranging from 10 to 60 mM, indicating that growth was impaired from concentrations above 10mM. Functional genomics approaches, including transcriptomics using the pangenomic microarray, as well as non-gel based proteomics and lipidomics, are currently being used to elucidate genome-wide responses to increasing concentrations of halogenated substrates, which, when encountered at elevated concentrations, might inhibit growth and activity of organohalide respiring bacteria, affecting the application of these dedicated degraders in strongly polluted environments.

P140

Characterisation of small intestinal *Streptococcus* and *Veillonella* populations

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Introduction: The human gastrointestinal tract is rich in nutrients from ingested food, which are used as energy source by the host and the resident microbiota. The small intestine is the first region where food components come in contact with the intestinal bacteria. Therefore, the small intestinal microbiota is expected to have an important influence on initial diet digestion and host physiology. However, little is known about the small intestinal microbiota, which is mainly due to sampling difficulties. Nonetheless, small intestinal samples can be collected from ileostomy subjects, which are individuals that had their colon surgically removed. At the end of surgery, the terminal ileum is connected to an abdominal stoma, which offers an opportunity for sampling of ileal contents. High throughput 16S ribosomal RNA profiling of stoma effluent revealed a bacterial community enriched in *Streptococcus* spp. and *Veillonella* spp. This study focuses on in-depth characterization of these bacterial populations and assessment of their diversity as well as aims to elucidate their functional properties and interactions.
Methods: Fresh ileostomy effluent was collected and maintained at 4C under anaerobic conditions. Ileostomy effluent was plated on Mitis Salivarius (MS) agar and Veillonella Selective Agar (VSA) for selective isolation of *Streptococcus* spp. and *Veillonella* spp., respectively. Following incubation at 37C, colonies from each plate were randomly picked and separately stored. Identification of the isolates employed 16S rRNA gene sequencing and subsequent classification. The 16S rRNA gene sequences were grouped into phylotypes based on a threshold of 98% sequence identity to assess the diversity among the bacterial isolates. The diversity at the strain level was assessed by employing amplified fragment length polymorphism (AFLP) analysis.
Results: A total of 120 isolates were obtained from MS agar of which 92 were identified as *Streptococcus*, while the remaining isolates were classified as *Enterococcus*. Out of 42 isolates obtained from VSA, 23 were assigned to the genus *Veillonella*. The remaining VSA isolates were identified as members of the genera *Enterococcus* (13), *Proteus* (4), and *Escherichia* (2). Grouping of the 16S rRNA gene sequences into phylotypes based on a threshold of 98% sequence identity revealed that for *Streptococcus* as well as *Enterococcus* 3 phylotypes could be identified. Isolates belonging to *Veillonella*, *Proteus*, and *Escherichia* were represented by a single phylotype. Furthermore, AFLP analysis demonstrated that for *Streptococcus* and *Enterococcus* 6 and 5 genomic lineages, respectively, could be distinguished.
Conclusion: *Streptococcus*, *Veillonella*, as well as *Enterococcus* isolates were successfully obtained. Phylogenetic analysis of the isolates revealed multiple phylotypes for *Streptococcus* and *Enterococcus* that could be further divided into multiple genomic lineages, which demonstrates the high diversity of the small intestinal microbiota. The genome sequences of representative isolates are currently determined and will be mined to elucidate the functional properties of these isolates as well as their potential microbial interactions that shape the small intestinal microbiota, with a special focus on *Streptococcus* spp. and *Veillonella* spp.

P141

Evaluation of the VITEK 2 ANC card for the identification of clinical isolates of anaerobic bacteria

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Objectives: The aim of this study is to compare the accuracy of the VITEK 2 ANC card (bioMrieux, Marcy l'Etoile, France) with 16S rRNA gene sequencing for identification of anaerobic species.
Methods: An evaluation of the VITEK 2 ANC card was performed with 301 anaerobic isolates, including 100 species that were not contained in the database. Each strain was identified by 16S rRNA gene sequencing, which was considered to be the reference method. Inocula were made

from subcultures grown for 24 to 48h on BBA agar. Cells were suspended in 0.45% NaCl, pH 5.7, and adjusted to a McFarland number 2.7 to 3.3 using a calibrated VITEK 2 DENSICHEK (bioMrieux, Marcy l'Etoile, France).

Results: Of the 301 isolates, 79.4% (239/301) and 60.1% (181/301) were correctly identified to the genus and species level respectively. Of the 201 isolates species which are included in the database, correct genus and species identification were obtained for 95.6% (192/201) and 90.0% (181/201). For the 100 isolates unclaimed at the species level, 47.0% (47/100) gave correct identification to the genus level and 16.0% (16/100) were accurately designated as not identified. Strains which were difficult to characterize to the species level using current phenotypic methods, were misidentified by VITEK 2 ANC card. For example, *Peptoniphilus harei* and *Peptoniphilus asaccharolyticus*, *Fusobacterium nucleatum* and *Fusobacterium naviforme*, *Prevotella nigrescens* and *Prevotella intermedia* cannot be easily distinguished from each other.

Conclusions: The VITEK 2 ANC card allows rapid identification of anaerobic bacteria within 6h. This system is an acceptable method for identification of those species which are included in the database.

P142

Development of carbapenem resistance during treatment caused by an IS element within the OmpK36 porin gene of a *Klebsiella pneumoniae* clinical isolate

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Introduction: Carbapenems are the drugs of choice for the treatment of serious infections caused by *Enterobacteriaceae*. Lately, the emergence of carbapenem resistance is a major worldwide concern. During treatment of a patient with meropenem increased carbapenem MICs were found. The aim of the study was to investigate the mechanism of acquired carbapenem resistance in a *Klebsiella pneumoniae* clinical isolate.

Methods: *K. pneumoniae* isolates were collected before and after therapy with meropenem. Detection of resistance genes and outer membrane proteins (Omp) was done by PCR. Longread PCR and sequencing was performed to identify possible insertion elements.

Results: PCR results showed the presence of TEM, SHV, DHA-1, and ArmA resistance genes. No KPC, NDM or MBL genes were found. The OmpK PCR was positive for OmpK35 and OmpK37, OmpK36 was missing. Long read PCR resulted in a PCR band of approximately 2100bp. Sequencing identified an IS26 element within the Ompk36 gene.

Conclusion: The combination of the OmpK36 porin loss due to insertion of IS26 and the presence of DHA-1 AmpC-lactamase is the most likely mechanism for acquired carbapenem resistance in this isolate.

P143

Emergence of multiple azole resistance mechanisms in *A. fumigatus* during azole therapy of a patient with aspergilloma

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Introduction: A 56-year old female with tumor necrosis factor (TNF)-receptor associated periodic syndrome was treated with itraconazole (ITZ) and subsequently posaconazole (POS) for bilateral pulmonary aspergilloma. During therapy sputum cultures remained positive for *A. fumigatus* and the isolates exhibited various resistant phenotypes. There was progression of the *Aspergillus* infection despite treatment with amphotericin B and caspofungin and the patient developed bacterial urosepsis and died. In this study the mechanisms responsible for the azole resistant phenotypes were investigated.

Methods: Minimum inhibitory concentration (MIC)-testing was performed according to the EUCAST reference method. The *cyp51A* gene and promoter region were sequenced and the isolates were genotyped by microsatellite typing. Cyp51A substitutions were introduced into a Cyp51A protein homology model to localize their positions in the protein. To investigate the impact of these amino acid substitutions on azole susceptibility, a new site-directed mutagenesis system was used to generate mutant *A. fumigatus* recombinants.

Results: Nine *A. fumigatus* isolates were cultured within a 10-month period. Microsatellite typing showed that all strains were isogenic. Only the first isolate exhibited a wild type phenotype, while various azole resistant phenotypes were observed in the remaining isolates. These corresponded with Cyp51A-substitutions at codons G54E, P216L, and F219I. G54 is a known hot-spot for substitutions conferring azole resistance, while P216L and F219I are unknown substitutions in Cyp51A. The homology model showed that both P216 and F219 were located close the opening of one of the two ligand access channels of the Cyp51A protein. *A. fumigatus* recombinants with a mutation leading to either the P216L or F219I substitution were constructed. The recombinant showed a phenotype similar to that of the patient isolates (ITZ MIC >16 mg/l; POS MIC 1-2 mg/l) confirming a role of Cyp51A P216L and F219I substitutions in azole resistance. After the patients' treatment was switched from ITZ to POS, the strain with the F219I

resistance mechanism showed a further increase of POS MIC and voriconazole resistance emerged. However, no additional mutations were observed in the *cyp51A* gene, indicating a non-*cyp51A* mediated resistance mechanism.
Conclusion: During azole therapy at least four resistance mechanisms developed within a 10 month period. Three of the mechanisms were associated with Cyp51A-substitutions. We show that two of these (P216L and F219I) are new amino acid substitutions resulting in azole resistance.

P144

Combined real-time PCR using different targets as a rapid screenings method for MRSA during outbreak

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A MRSA (ST064) outbreak of a nursing ward in a general hospital occurred in November 2009. According to the guidelines of the 'search and destroy' policy in the Netherlands samples were taken from healthcare workers and patients who were in close proximity of the index patient. These samples were cultured and found MRSA positive for 3 healthcare workers after 6 days. Because of the spread of MRSA the outbreak management team decided to further use of PCR as a rapid screenings method.
On the Laboratory of Infectious Diseases a real-time-PCR is used for the separate detection of a sequence specific for *Staphylococcus aureus* and the mecA-gene. Disadvantage of this method is the occurrence of inconclusive MRSA results due to the uncertain origin of the mecA-gene caused by MRSA or by MRSE. Therefore conventional culture is used for confirmation.
For the screening of healthcare workers an additional real-time-PCR was used based on the conjunction area of the SCCmec and Orf-X as described by Huletsky et al. and modified by Lieuwe Roorda.
Aim of this study is to show that combined real-time-PCR using different targets shows less inconclusive MRSA results what enables the ability of health care workers to soon participation in the working process sooner.
Methods: DNA extraction was performed on a Brain Heart Infusion broth which was incubated for 18-24h at 35C using a bacteria DNA III extraction kit (Roche) on a MagNApure. Real-time-PCR was performed on a ABI PRISM7500 (Applied Biosystems) using oligo's for *S. aureus* and mecA as described by E. van Hannen (Nieuwegein, the Netherlands) and for the conjunction area of SCCmec and Orf-X.as modified by Lieuwe Roorda (Rotterdam, the Netherlands).
Results: A total of 318 samples, obtained from 158 health care workers, were tested for *S. aureus*, mecA gene and SSCmec-OrfX area. In 40 (12.5%) samples, from 33 (20.8%) healthcare workers, a positive result for *S. aureus* and mecA was found. Since the presence of MRSA could not be excluded it is necessary to wait for the final result of the culture. With addition of a real-time PCR for the SSCmec-OrfX area only 10 (3.1%), from 9 (5.7%) healthcare workers remained MRSA PCR positive. Of these samples 7 appeared MRSA culture negative, 2 were MRSA culture positive and in 1 sample culturing was not continued.
Conclusion: Combining real-time-PCR on different targets decreases the number of inconclusive MRSA results, and increases the ability of health care workers to soon participation in the working process sooner.

P145

Zebrafish Embryo Model for the Pathogenesis of Biomaterial Associated Infection

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Introduction: Biomaterials are widely used in modern medicine, and biomaterial associated infection (BAI) remains an important problem, with an infection rate of still 1-5% of the millions of implants applied per year. The main causative agent of these infections is *Staphylococcus*, mainly *Staphylococcus epidermidis*, a normally commensal human skin bacterium. There is a great need for an *in vivo* screening model which can contribute to the understanding of the pathogenesis of BAI and can be used for large scale screening of biomaterials. Their high embryo yield and transparency makes them highly suitable for assessment of immune responses and infection, which can be followed in real time by fluorescent microscopy.
Methods: The model was developed using zebrafish embryos from a wild type (WT) zebrafish line and a transgenic mpo::eGPF (Green Fluorescent Protein) line with GFP-expressing neutrophils. Infections without biomaterials were established using WT *S. epidermidis* strain O-47 in embryos expressing GFP, and O-47 expressing GFP in WT zebrafish embryos. The bacteria were injected in the yolk or in the embryo tissue. The infection susceptibility was also explored for *S. aureus* strain RN4220 and the same strain expressing mCherry (a red fluorescent protein) or GFP. As a model for biomaterial, we used polystyrene red fluorescent beads (10m in diameter). To study BAI the polystyrene beads and *S. epidermidis* were injected simultaneously in the tissue above the yolk extension.

Results: The zebrafish embryos were highly susceptible to *S. aureus* infection with a high embryo mortality rate. *S. epidermidis* caused less severe but persistent infection. Infection susceptibility was higher with yolk injection compared to tissue injection. Implanting of the polystyrene beads was done at several time points, the optimal time point to achieve subsequent infection being three days post fertilization (dpf). The presence of the polystyrene beads caused a increase in the susceptibility to infection.

Conclusions: 1. Zebrafish embryos are susceptible to infection caused by *S. aureus* and *S. epidermidis*. 2. As in other animal models and in human patients, *S. aureus* shows higher virulence than *S. epidermidis*. 3. The susceptibility to *S. epidermidis* infection increases when a foreign material is present, which is in line with the general principle of enhancement of susceptibility to infection associated with the use of medical devices/biomaterials. 4. Therefore the zebrafish embryos can be used as a model for BAI, creating a promising tool for studying the pathogenesis of BAI, and screening biomaterials for induction of immune responses and for susceptibility to infection.

P146

Strain-specific contribution of branched-chain amino acid transport genes to prolonged pneumococcal colonization

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Introduction: Nutritional balance is essential for colonization and virulence of many bacteria, among which the respiratory pathogen *Streptococcus pneumoniae*. ATP-binding cassette (ABC) transporters play a central role in this process, being involved in import and export of a variety of substrates, such as amino acids, sugars, and anions. The pneumococcal genome encodes over 70 ABC transporters, typically consisting of membrane-associated ATP-binding proteins, membrane-spanning permeases, and a substrate-binding protein that confers the substrate specificity. Some are known to be important for full virulence, such as the cation transporters PsaA, PiuA, and PiaA. Branched-chain amino acids (BCAAs) are among the most abundant amino acids in proteins, and are used by pneumococci as co-factor for the nutritional regulator CodY. The pneumococcal ABC transporter for BCAAs is encoded by the *livJHMGF* operon. Recently, a *livHMGF* mutant was shown to have reduced virulence in mouse models of pneumonia and septicemia, but only when in competition with its wild-type. Since colonization is the first step toward pneumococcal disease, we investigated the contribution of the Liv ABC transporter to pneumococcal colonization. In addition, we examined SP0626 (*brnQ*; encoding a BCAA carrier protein), SP0629 (encoding a hypothetical protein likely to be co-transcribed with SP0626), SP0826 (*ilvE*, a BCAA aminotransferase), and the transcriptional regulator SP0743, located upstream of *livJHMGF*.

Methods: Single knock-out mutants of the selected individual genes and the whole *liv* operon were generated in two different strains of *S. pneumoniae* (D39 and TIGR4), and tested with the wild-types in a murine single infection model of colonization. Mice were lightly anesthetized and infected intranasally with a 10 l-inoculum (110^6 CFU). At predetermined times after infection (t=0.5, 24, 48, 96, 192h), groups of mice were sacrificed by cervical dislocation and bacteria were recovered from the nasopharynx by flushing each nare with PBS.

Results: No differences in *in vitro* growth between the wild-type strains and their mutants were detected. In D39, the single *livJ* mutant and the *liv* operon mutant showed a similar phenotype during murine colonization, attenuation at 192h, while the *livH* mutant displayed colonization levels comparable to wild-type. In TIGR4, an attenuated colonization phenotype was only observed for the *livJ* mutant, and then only at 24 and 96h post-infection. Significant bacterial reductions were observed 48-192h post-infection upon deletion of *brnQ* in D39 only, while no attenuation was found for SP0629 in either strain background. Mice infected with the *ilvE* mutant had significantly lower bacterial load in the nasopharynx at 24, 96-192h post-infection in D39, while attenuation was only detected 192h post-infection in TIGR4. Finally, major significant reductions in bacterial load of the nasopharynx of mice infected with the SP0743 mutant were observed throughout the course of infection in both strains.

Conclusions:

1. Both the BCAA ABC transporter LivJHMGF and the BCAA carrier protein BrnQ contribute to prolonged pneumococcal colonization, albeit mainly in the D39 strain.
2. The putative transcriptional regulator SP0743 is required for full pneumococcal colonization, but further experiments are required to assess which genes it regulates.

P147

Mycoplasma hominis cultured from cerebrospinal fluid after subarachnoid hemorrhage

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Introduction: Urogenital colonization with *Mycoplasma hominis* is common in sexually active adolescent females. Extra-genitourinary infections caused by this species

are described in the literature. However, it is still a rare pathogen cultured from cerebrospinal fluid.

Methods: A 48 years old woman was admitted because of a subarachnoid hemorrhage. Fever (39C) was noted six days after craniotomy. On the ninth hospital day, blood cultures became positive with *Staphylococcus aureus*. Flucloxacilline intravenous and a combination of intravenous and intrathecal vancomycine were administered. This infection responded, however, 3 days later, she again developed high fever in spite of the antibiotics. After 6 days of incubation, small colonies were detected on blood agar from CSF taken on day 9, 16 and 17. There were no bacteria present on the gram-stain. No identification could be obtained by using MALDI-TOF MS. No growth was detected in seven CSF cultures before day 9 and 18 cultures after day 17.

Result: *M. hominis* was detected from CSF by using 16S rDNA gene amplification. Moxifloxacine (400 mg daily) was given intravenous for two weeks. Clinical conditions improved with negative repeat CSF cultures.

Conclusion: Amplification of 16S rDNA for M. hominis in CSF should be included in diagnostic workup of patients after subarachnoid hemorrhage. Clinicians should consider this rarely recognised pathogen in the differential by those not responding to standard therapy with negative results in routine bacterial cultures.

P148

Evaluation the new Roche 4800 system for molecular detection of *Chlamydia trachomatis* and *Neisseria gonnorhoea* in rectal swabs and (self)collected vaginal swabs

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Introduction: *Chlamydia trachomatis* (CT) and *Neisseria gonnorhoea* (NG) are the most prevalent sexual transmitted diseases worldwide. For molecular detection standard samples like cervical swabs and urines types are well known for molecular diagnostics in most laboratories. New developments create the opportunity to include more difficult samples types like rectal swabs and (self collected) vaginal swabs, with regard to inhibition, sensitivity and specificity. This study compares the performance of the new Cobas 4800 with the Abbott M200 for the detection of CT and NG in these difficult sample types.

Methods: In a multicenter study 1100 co-collected swabs were tested (900 vaginal; 200 rectal). Sample co-collection was approved by the medical ethical board and informed consent was obtained from all patients included in the study for co-collection. The M2000 system was used a routinesystem in the collecting centers (Streeklab Groningen & Elisabeth ziekenhuis Tilburg) and the co-collected samples were subsequently blinde and snet to the VU University to be tested in the Cobas 4800 system. All swabs were collected on site in their corresponding transport buffer. After testing the results were compared and discrepant results were tested additionally with an independent real time PCR method.

Results: In the 900 vaginal swabs 10% (n= 90) were positive for CT and 0.5% (n=4) for NG and in the 200 rectal swabs 11% CT positives (n=22) an 6% NG positives (n=12) were identified. In the vaginal samples 9 discrepant results between the Cobas 4800 and M2000 system were identified. Five with borderline values and 4 with clear different results (1 NG, 3CT). Two M2000 positive results (Ct 35.9; 32.3) were available for home brew analysis and could not be confirmed. In the rectal samples 8 discrepant results were found including 3 clear differences (2 NG; 1 CT). The CT discrepant sample was positive in the Cobas 4800 system (Cp 33.1) and negative in the M2000 system. For NG 2 samples were negative in the Cobas 4800 system but positive (Ct 23.8; 32.0) in the M2000 system. All 3 clear discrepant results were available for home brew analysis and confirmed the Cobas 4800 results.

Conclusion: Both samples types showed a high concordance between the two systems (κ 0.95 for CT; κ 0.93 for NG). For detection of vaginal swabs some small differences were found both for CT and NG but these were equally spread between the two systems. There is no difference between self collected and clinician taken vaginal swabs. All clear rectal swabs discrepancies confirmed the Cobas 4800 results. This remarkable difference has to be further studied. In general, (self collected) vaginal swabs and rectal swabs show reliable results for routine detection of CT and NG.

P149

Antibiotic susceptibility testing methods: the fastest way to the clinician

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Introduction: Rapid identification (ID) and antibiotic susceptibility testing (AST) lead to early administration of appropriate antibiotics thereby improving the prognosis of patients with bloodstream infections. However, for AST, very few rapid techniques are available. We aimed to compare three methods for rapid AST with respect to their results and potential for use in clinical practise. The first method was inoculating the BD Phoenix system with bacteria

harvested directly from blood cultures. Furthermore, we developed a new, more rapid method for AST combining culture and PCR techniques (RAMAST), which requires a shorter incubation time. PCR on resistance genes has the potential for even more rapid AST, since it does not require previous culture. Therefore, we designed a PCR-assay for three resistance genes in *Staphylococcus* species.

Methods: For both the direct Phoenix method and RAMAST, bacteria were harvested directly from positive blood cultures using Serum Separator Tubes.

For the direct Phoenix method, these bacteria, from 81 positive blood cultures, were used to inoculate the BD Phoenix system according to manufacturers' guidelines.

For RAMAST, of 19 blood cultures containing *Staphylococcus aureus*, a suspension containing 5 x 10^5 CFU/ml was made in broth. This suspension was incubated with a panel of antibiotics for 6 hours. Afterwards, growth (a resistant strain) or absence of growth (a susceptible strain) was determined by using a quantitative 16S SYBR Green assay.

For the PCR-assay, 133 blood cultures were diluted, centrifuged and the bacterial pellet was resuspended in sterile water. Using a SYBR Green based PCR assay, genotypic antibiotic resistance was determined for gentamicin (*aacaph* gene), oxacillin (*mecA* gene) and penicillin (*BlaZ* gene).

Results were compared with those of the conventional Phoenix method. In case of discrepancies between the two methods, microbroth dilution was performed as golden standard.

Results: The direct Phoenix method showed an agreement with the conventional methods of 95.4%, with 1.1% false-intermediate results, 3.1% false-resistant results and 0.4% false-susceptible results. All antibiotics showed an agreement of >90%, except for thrimethoprim-sulfamethoxazole and erythromycin. Starting from positive blood cultures, time to results was on average 13.5 hours.

RAMAST showed an agreement for *S. aureus* of 97.9% with 2 false-resistant results for amoxicillin. Time to results was 9 hours.

The PCR-assay showed an agreement of 89% for penicillin with 8 false-resistant and 6 false-susceptible results. Agreement for oxacillin was 98% with 2 false-resistant and 1 false-susceptible results. Agreement for gentamicin was 94%, with 6 false-resistant and 1 false-susceptible results. Time to results was approximately 2.5 hours.

Conclusion: All three methods are easy to perform and show promising results. The direct Phoenix method requires only minimal changes in laboratory routine. RAMAST is more laborious, but offers results within one working day. The PCR-assay is the most rapid method, but needs to be improved and more resistance genes will be added. The assay can potentially be adjusted for AST directly on whole blood, enabling even more rapid AST.

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Biodegradation of Sodium Dodecyl Sulfate (SDS) in newly isolated denitrifying bacteria

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Surfactants are compounds widely used in the formulation of several products designed for cleaning and solubilization purposes. After use these compounds end up in waste water treatment plants or in the environment. Although aerobic degradation of several surfactants is well described, many seem to be persistent under anaerobic conditions. Nitrate is a common water contaminant that when produced in excess can be easily transported to groundwater supplies. Since nitrate and surfactants can both be found in wastewater treatment systems and in nature, the biodegradation of surfactants coupled to nitrate reduction can occur. Considering that this is a scarcely documented subject, the objective of this study was to investigate the biodegradation of anionic surfactants under anoxic conditions. Sodium dodecyl sulfate (SDS) is used in cosmetics and personal hygiene products and was selected as model anionic surfactant. An enrichment of SDS-degrading denitrifying bacteria was performed. SDS was added as sole electron donor to a final concentration of 0.4mM. Sodium nitrate was added (4.3mM) as electron acceptor and activated sludge was used as inoculum. Two pure cultures, strains SN1 and SN2, were obtained from the enriched culture and identified using molecular techniques. Biodegradation performance of both strains was evaluated by measuring biomass growth, SDS mineralization and denitrification. Biomass growth was followed by optical density at 600nm. SDS degradation was indirectly determined by analyzing the dissolved organic carbon (DOC) content in the liquid medium. Nitrate and nitrite concentrations were analyzed using ion chromatography. N_2 and CO_2 production were determined using gas chromatography. The physiological characterization of the strains was performed to investigate the optimal growth conditions and the use of different electron donors and acceptors. SN1 was identified as 99% similar to *Pseudomonas stutzeri* (CCUG 11256^T) and SN2 was found to be 98% similar to *Pseudomonas nitroreducens* (DSM 14399^T). SN1 and SN2 completely mineralized SDS to CO_2 in less than 14 hours, while NO_3^- was reduced to NO_2^- and finally to N_2. The biomass growth was fast considering the doubling time of approximate 2 hours for both strains. SN1 and SN2 could use NO_3^-, NO_2^- and O_2 as electron acceptors, showing a similar pattern in the use of most of the tested carbon sources. Optimal growth of SN1 was found at pH values above 8, while SN2 grows better at a pH value of about 7.5. The optimal temperature was 30C for

both strains. Our research provided insight into alternative anaerobic metabolic pathways used by bacteria to degrade anionic surfactants in wastewater treatment systems and contaminated natural sites.

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Metagenomic analysis of nasopharyngeal microflora; diversity, variability and seasonal effects

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Introduction: Disturbance of a balanced nasopharyngeal (NP) microbiome might result in symptomatic respiratory and/or invasive infections, which occur primarily in fall/ winter and in young children. It is unknown whether seasonal infection patterns are associated with concomitant changes in NP microflora.

Methods: We characterized the NP microflora of 96 healthy children of 18 months ofageby barcoded pyrosequencing of the V5-V6 hypervariable region of the 16S-rRNA gene, and compared microflora over seasons.

Results: The 1.100.000 sequences generated represented 13 taxonomic phyla and 243 species-level phyla types (OTUs). The 5 predominant phyla were *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, *Actinobacteria* and *Fusobacteria*, however, showing high inter-individual variability and no core microbiome. Microbial flora profiles varied strongly with season, with in fall/winter a predominance of *Proteobacteria* (relative abundance (% of all sequences): 75% versus 51% in spring) and *Fusobacteria* (absolute abundance (% of children): 14% versus 2% in spring), and lower *Bacteroidetes* (absolute abundance:54% versus 91% in spring), and *Firmicutes*, which was mainly caused by the absence of (*Brevi*)*Bacillus*, and Lactobacillus species (absolute abundance: 10% versus 96% in spring). The observed seasonal shift could not be attributed to recent antibiotics or viral co-infection.

Conclusion: The NP microflora is highly diverse and shifts with season, underlining a potential role in seasonal infection patterns.

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Steady state population processes and distribution of habitable sites in the leaf environment

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Most commonly, carrying capacity of the phyllosphere is described as the maximal load of colony forming units that the phyllosphere can maintain. That underlying processes of growth and death exist has been assumed but thus far never been measured. We used the recently developed reproductive success bioreporter (or repsuc, reads backwards CUSPER) to determine growth at maximal load at a single-cell level and estimate the number of growth conducive sites in the phyllosphere. The concept of CUSPER is based on the dilution of pre-formed stable green fluorescent protein (GFP) from dividing bacteria, which inversely relates GFP concentration to reproductive success. Bean leaves were inoculated with different densities of CUSPER-bioreporter cells to saturate the environment to different levels and increase the effect of the imposed phyllosphere heterogeneity on colonizing bacteria. Bacteria were recovered after reaching carrying capacity, measured by CFU counts, and single-cell GFP content was measured for CUSPER analysis. Apparently bacteria were still growing after reaching carrying capacity. Growth dynamics showed large differences in the contribution of individual colonizer cells to the final population, some cells divided more successfully than others. Growth at a single-cell level and stable CFU counts on a population level imply that cells became incapable to grow on agar plates or died. CUSPER analysis further revealed that between 0.5-3% of initial colonizer cells were exceptionally successful. This suggests that these cells colonized sites that offered high amounts of resources, revealing localized hotspots of growth in the phyllosphere.

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Exploring the oral microbiota of children at various developmental stages of their dentition in the relation to their oral health

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An understanding of the relation of commensal microflora to health is essential in preventing disease. Here we studied the oral microbial flora of children (N=74, aged 3-18 years) in natural transition from deciduous to

permanent dentition and related the microbial profiles to their oral health status. The microbial composition of saliva was assessed by barcoded pyrosequencing of the V5-V6 hypervariable regions of the 16S rRNA, as well as by using phylogenetic microarrays.

Pyrosequencing reads (126174 reads, 1045 unique sequences) represented 8 phyla and 113 higher taxa in saliva samples. Four phyla – *Firmicutes*, *Bacteriodetes*, *Proteobacteria* and *Actinobacteria* – predominated in all groups. Deciduous dentition harbored higher proportion of *Proteobacteria* (*Gammaproteobacteria*, *Moraxellaceae*) than *Bacteroidetes*, while in all other groups *Bacteroidetes* were at least as abundant as *Proteobacteria*. *Bacteroidetes* (mainly genus *Prevotella*), *Veillonellaceae* family, *Spirochaetes* and candidate division TM7 increased with the increasing age, reflecting maturation of the microbiome driven by biological changes with age. Microarray analysis enabled further analysis of the individual salivary microbial flora.

Of 350 microarray probes, 156 gave a positive signal with, on average, 77 (range 48-93) probes per individual sample. Caries-free oral status significantly associated with the higher signal of the probes targeting *Porphyromonas catoniae* and *Neisseria flavescens*. The potential role of these microorganisms as oral health markers should be assessed in large-scale clinical studies. The combination of both, open-ended and targeted molecular approaches provides us with information that will increase our understanding of the interplay between the human host and its microbiome.

P155

Whole-genome sequencing of strains of the *Mycobacterium tuberculosis* Beijing genotype family

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The Beijing genotype family is an epidemiologically important sub-group of *Mycobacterium tuberculosis*. It has been suggested that the high frequency of Beijing isolates in some areas could be explained by selective advantages. The emerging and most frequently isolated 'Typical Beijing' lineage may have the ability to circumvent BCG-induced immunity.

To investigate the phylogeny of the Beijing genotype of *M. tuberculosis,* the genome of six Beijing strains from three different countries was sequenced with next-generation sequencing. The phylogeny of these strains was established using single nucleotide polymorphisms (SNPs). The three Typical Beijing strains clustered very tightly in the Beijing phylogeny suggesting that Typical Beijing strains represent a monophyletic lineage and result from recent diversification. Typing of a selection of *M. tuberculosis* strains with a subset of the SNPs and comparison of the IS*6110* restriction fragment length polymorphism (RFLP) patterns of these strains to a database of 1522 Beijing RFLP patterns revealed that about 80% of all Beijing strains belong to the Typical Beijing subclone, which indicates clonal expansion.

To identify the genomic changes that are characteristic for all Typical Beijing strains and to reconstruct their most recent common ancestor, the presence of SNPs were assayed in other Beijing strains. Furthermore we developed a robust typing scheme using single nucleotide polymorphisms (SNPs) and regions of difference (RDs) and applied it to 259 Bejijng strains originating from 45 countries. Our results suggest that Beijing strains spread globally on multiple occasions and that the TB epidemic caused by the Beijing genotype is at least partially driven by modern migration patterns. We identified 53 SNPs that define the minimal set of polymorphisms for all Typical Beijing strains. Nonsynonymous polymorphisms in genes coding for the regulatory network were overrepresented in this set of mutations. We hypothesize that alterations in the response to environmental signals may have enabled Typical Beijing strains to circumvent BCG-induced immunity.

P156

The advantages of the Sigma-Transtube® open cell polyurethane foam swabs in microbiological transport systems with improved cellular flow dynamics and reduced sample entrapment

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Microbiological transport systems have always been a key point of research in diagnostic development. Bacterial entrapment within microbiological transport systems pre-inoculation phase decreases sensitivity.

Device manufacturers aim to improve cellular flow dynamics; to which one such advent is the use of polyurethane foam (as used in the Medical Wire S-Transwab). Molecular and automated advances in microbiological diagnostics are fast becoming the leading gold standard for detection of microorganisms. Such transport devices are also designed to be compatible with automated inoculation systems (automated screw top removal and 100l direct liquid extraction onto media).

Research in the use of polyurethane foam provides recognition that high sample absorption and then 100% release are the essential goals for swab design. The reduction of bacterial entrapment with the use of

polyurethane greatly increases the sensitivity of detection of pathological bacteria (especially infection control concerns, such as MRSA), even in high level mixed bacterial species environments. The retention of micro-organisms in the liquid phase allows greater sensitivity with culture media detection (swab elution), molecular-based techniques, and automated inoculation techniques compared to conventional swab system methods.
The working definition of this research study is to show the enhanced sensitivity of microbiological transport systems using polyurethane foam, over traditional fibre-based conventional systems.
A number of polyurethane-based transport systems and fibre-based transport systems are used with a recognised validation standard method (swab elution) to compare the level of sample entrapment in the devices. The swab elution method is used to reflect the performance characteristics of each transport system, and is not intended to necessarily reflect the concentration of microorganisms in clinical specimens. Swab elution is used to assess the sample entrapment of the device, and does not reflect the roll-based method used in most clinical laboratories. The organism used is *Staphylococcus aureus* ATCC 25923. At 0.5 McFarland standard is used with an adopted Mile's and Misra type methodology. A spectrophotometer is used to determine the initial inoculum concentration (absorbance at 625nm shall be 0.08 to 0.10). Initial inoculum concentration is verified by serial dilutions and plating to non-selective media. Samples are done in sets of 3 with duplicate cultures, to which the average will be used to determine the concentrations of organisms yielded by the transport device.
The results showed a greater level of reduced bacterial entrapment in the S-Transwab polyurethane foam based transport device compared to the fibre-based transport device. The data shows a greater percentage of CFU/ml where yielded by the S-Transwab device compared with that of the fibre-based device. Fibre-based swabs show reduced sensitivity by increased sample entrapment.
The use of polyurethane swabs in molecular based techniques and automated inoculation techniques (with direct liquid extraction/greater retention of organisms in the liquid phase) should be the way forward to enhancing the efficiency and accuracy of microbiological diagnostics. Greater sensitivity of the S-Transwab polyurethane will ensure enhanced detection of microorganisms, and synergistically improve infection control and diagnostic prevalence with POCT and other molecular advances.

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Detection of viral respiratory infection using the Genomica CLART® PneumoVir hybridization array system

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Introduction: Viral respiratory infections are an important cause of morbidity and mortality. Recently the diagnosis of viral infection has been shifting away from virus culture to molecular diagnosis mainly based on PCR. To be able to test for a growing number of viral agents many commercial multiplex systems have been developed. In this study the Genomica CLART PneumoVir system has been tested for diagnosis of viral respiratory infection. PneumoVir tests for 11 viral respiratory pathogens in one test, includes sub-typing and can be performed in batches of 1 to 96 samples.
Methods: The PneumoVir system was compared to conventional virus culture techniques and a thoroughly validated in-house multiplex real-time PCR. 129 Samples were tested with all 3 techniques, and an additional 258 samples were tested with the PneumoVir and real-time PCR. All samples that were submitted for diagnosing respiratory viruses in the winter of 2009/2010. Apart from the availability of sufficient sample material for all tests, there were no further selection criteria. Both molecular tests were performed on the same nucleic acid isolate, prepared using a MagNA Pure LC extractor.
Results: When compared to virus culture, both molecular techniques exhibited a significant increase in sensitivity. Using culture, a viral agent was detected in 30% of the samples whereas PneumoVir reported a virus in 47% and real-time PC in 52% of all samples. Notable is the high specificity of virus culture. Only in one case the culture result was not supported by another test. The overall agreement between the two molecular tests was very good (86%). However, when comparing the tests per virus, there are larger differences. Most notable is the apparent lower sensitivity (50%) of the PneumoVir test for rhinovirus. Although many samples were found positive by the real-time PCR, and negative by PneumoVir, there seems to be no correlation to the concentration of the virus. These findings suggest this is not a sensitivity issue, but suggets some of the over 100 rhinovirus genotypes are not detected by the PneumoVir system. Discordant samples will be sequenced to provide more data on this issue.
Conclusion:

1. The Genomica CLART PneumoVir system is more sensitive then virus culture.
2. The PneumoVir test performance is comparable to multiplex real-time PCR.
3. The PneumoVir test has relatively long hands-on time, and because PCR tubes are opened during the process,

should be handled carefully in a lab where other molecular tests are performed.

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Correct identification of ESBL in *Enterobacteriaceae* isolated from hospitalized patients: Towards molecular detection methods?

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Correct detection of Extended-Spectrum Beta-Lactamases (ESBL) is crucial for infection control and choice of antimicrobial therapy. The current national guideline in the Netherlands recommends the use of phenotypical tests, which are sometimes difficult to interpret. We performed a study to determine the number of false-positive results using phenotypic tests according to the national guideline in a large teaching hospital.

Methods: All patients that had been isolated in The Amphia Hospital because of the detection of ESBL from 2006 until 2010 were included in the survey. The strains had been stored at -80 °C. The presence of ESBL had been confirmed according to the current national guideline for the detection of ESBL, using E-test (BioMrieux, Marcy l'Etoile, France). All strains were tested using a new commercial genotypical method, i.e. the ESBL microarray (Check-Points, Wageningen, the Netherlands) which uses specific amplification of commonly known ESBL-genes followed by visualization and interpretation using dedicated hardware and software. If the microarray did not confirm the presence of ESBL genes, this was considered a false positive phenotypic result. Also, all strains were tested using the Double disk confirmation test (DDCT).

Results: In total 177 patients were included. The mean duration of isolation was 15 days and the total number of isolation days was 2.573. Among the 177 patients, 22 could not be confirmed with the array. This results in a positive predictive value (PPV) of the phenotypic assay of 88%. The 22 patients with a negative result using the microarray were isolated for a total of 223 days, which could have been avoided. When using the DDCT in addition to the E-test there where 9 false positive results. Most ESBL positive strains were *E. coli* (147/177) carrying blaCTX-M-1 (90/177).

Conclusion: Using the E-test a significant number of patients are isolated while there is no ESBL present. Therefore, a genotypic confirmation is preferred. Although this increases the cost for the laboratory, the avoidance of isolation days saves cost for the wards. A cost-effectiveness evaluation of the genotypic assay is needed to determine the preferred diagnostic strategy.

The DDCT identified 13 of the 22 false positives and may be a more reliable method than E-test. Since the DDCT was only performed on the strains that were already selected by E-test we cannot determine the PPV. A prospective head to head comparison of the DDCT with E-test is needed to determine which phenotypic test is to be preferred.

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Understanding the complex regulation of heterogeneic sunA expression in *Bacillus subtilis*

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Introduction: To fend off competitors, *Bacillus subtilis* strain 168 produces the antimicrobial peptide Sublancin 168. This defense mechanism is expressed heterogeneously within the cell population. Sublancin 168 has bactericidal activity against gram-positive organisms, such as *Staphylococci*, *Streptococci* and *Bacilli* (including *Bacillus subtilis* itself when it is lacking in the immunity gene, *sunI*). Several key transcription factors are known to be involved in the control of expression of *sunA*: Spo0A-P, AbrB, Abh and Rok. The complex control of transcription of *sunA* is partly understood and our challenge lies in understanding the origin of heterogeneity in P*sunA* expression.

Methods: The *sunA* promoter was linked to a GFP reporter gene as described by Botella et al. The promoter-GFP fusion was analyzed by fluorimetric experiments in 96 well plates in diluted LB medium (25%), which allows high-throughput measurement of promoter activity of wild type and mutant strains in real time. To analyze GFP expression on a single-cell level, a microscopy setup has been configured to monitor live cells during their growth. Bacterial cells were immobilized on a small strip of agar that is then sealed under a coverslip and aerated by two large air compartments (5). Time-lapse movies were recorded and analyzed using the open-source ImageJ program and growth curves were calculated using Microsoft Excel. Growth inhibition assays were performed to confirm the presence of functional Sublancin 168.

Results: We show that heterogeneity in P*sunA* activity is regulated via the transition state regulator AbrB. Rok is an important transcriptional regulator, and has a strong inhibitory effect on *sunA* promoter activity. Rok has no effect on *sunA* transcription heterogeneity. Spo0A-P can increase P*sunA* expression more than an *abrB* knockout strain can in combination with a mutation in *rok*.

Discussion: Heterogeneity in *sunA* promoter activity has been shown previously, but has not until now been extensively studied. It is advantageous to the cell that the

SP prophage tempers Sublancin production to match the energy need of the cell. To enable this tempering, *sunA* expression heterogeneity is regulated via AbrB and the intensity of heterogeneity is mediated via Rok. Spo0A-P affects *sunA* expression via AbrB and an additional, as yet unidentified pathway.

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Novel approaches for analysing gut microbes and dietary polyphenols

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Polyphenols, ubiquitously present in the food we consume, may modify gut microbial composition and/or activity, and moreover, be converted by the colonic microbiota to bioactive compounds that influence host health. The polyphenol content of fruit and vegetables and derived products is implicated in some of the health benefits bestowed on their consumption. Elucidating the mechanisms behind polyphenol metabolism is an important step in understanding their health effects. Yet, this is no trivial assignment due to the diversity encountered in both polyphenols as well as gut microbial composition, which is further confounded by the interactions with the host. To study the conversion of polyphenols and its impact on host metabolism a human intervention using a bolus dose of grape/wine, tea polyphenol or placebo in a crossover design with 20 subjects was performed. During 48 hours after the intervention blood and urine were collected for metabolite analysis using NMR and GC-MS. Prior to the start of the first intervention period a faecal sample was collected and analysed for microbial diversity using the Human intestinal tract chip (HIT-Chip) and quantitative PCR. Data analysis using the subject as their own control was applied to separate the effect of the intervention from the variation between individuals and group mean values. Subjects showed inter-individual variation in the appearance of microbe related metabolites in urine and could, based on this variation, be divided into fast and slow producers. HIT-Chip analysis (GI-Health b.v.) showed inter-individual variation in gut microbial composition. Appearance of all metabolites was correlated with the ranked abundance of all microbes showing, amongst others, a correlation between two valerolactones and species related to *Clostridium leptum*. In conclusion general profiling approaches can be used to identify microbes involved in or linked to conversion of polyphenols and are particularly useful when conversion pathways are diverse and not yet fully known. Here we showed that the microbiota are largely responsible for the observed inter individual variation in polyphenolic metabolites However further work is required to get a better mechanistic understanding of polyphenol conversion down to the level of genes/proteins involved rather then on the level of species or microbial group. Understanding polyphenol-gut microbiota interactions and gut microbial bioconversion capacity will facilitate studies on bioavailability of polyphenols in the host, provide more insight on health effects of polyphenols, and potentially open avenues for modulation of polyphenol bioactivity for host health.

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A transcriptional control mechanism linking carbohydrate metabolism and virulence in *Streptococcus suis*

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Background: *Streptococcus suis* is a major porcine pathogen worldwide that causes severe disease in pigs and humans. It mainly colonizes the upper respiratory tract of healthy animals. During airway colonization of the host, streptococcal pathogens often use complex carbohydrates as carbon source. In these pathogens, polysaccharide metabolism plays important roles during nasopharynx colonization and invasive disease. Tight transcriptional control of gene expression in response to nutritional and environmental changes is therefore fundamental to optimize the performance of pathogens during host infection.

We studied the cell-surface amylopullulanase (ApuA), a bifunctional carbohydrate degrading enzyme that also enables *S. suis* to adhere to porcine tracheal epithelial cells. Upstream of apuA gene lies a putative transcriptional regulator belonging to the LacI-GalR family of transcription repressors that we have designated as ApuR. Our working hypothesis is that ApuR regulates the expression of S. suis apuA and the downstream phosphotransferase genes (sgaT) that might be involved in uptake of specific sugars liberated by the activity of ApuA. We therefore investigate the role of ApuR that, through the

regulation of the apuA operon, may control nutrient acquisition and adhesion of the pathogen during host colonization.

Method: The ApuA enzyme has been characterized by the construction of a apuA::spc mutant via insertion of a spectinomycin cassette into the pullulanase domain. The resulting mutant has been tested versus the wild type in adhesion assays using a porcine tracheal cell line (NPTr) and for conditional growth on minimal media supplemented with different carbon sources: pullulan (the natural ApuA substrate), maltotriose or glucose.

To study the activity of ApuR, a recombinant His-tagged ApuR was cloned into expression vector pTrcHis in *E. coli* and purified by affinity chromatography. The recombinant protein was used in Electrophoresis Mobility Shift Assays (EMSA) to investigate which region(s) of the apuA promoter is specifically bound by ApuR. To identify the regulon of apuR, we performed Agilent microarray gene expression analysis of bacteria grown in different carbohydrate sources.

Results: In the *in vitro* adhesion assays, apuA::spc mutant had less capacity to adhere to NPTr cells compared to wild type and reduced growth in minimal media with pullulan as carbon source. The EMSA results showed that an addition of recombinant ApuR protein retained two of the three apuA promoter fragments. These fragments might contain the predicted binding site. Using DNA microarray analysis we found that under the tested conditions, apuR controls expression of apuA and sgaT genes via repression.

Conclusion: We demonstrated that:

1. ApuA promotes adhesion to porcine epithelium *in vitro*, highlighting a link between carbohydrate utilization and the ability of *S. suis* to colonize and infect the host.
2. ApuR negatively regulates the apuA operon via specific binding to its own promoter.
3. Microarray analysis indicated that expression of apuR and apuA operon were correlated depending on the carbon source.

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Characterization of biofilm-deposit complexes associated with Microbiologically Influenced Corrosion (MIC) in European harbours

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A study performed in 1990-1993 estimated a minimum of 12-13% of European harbours affected by a particularly aggressive localized MIC phenomenon, called Accelerated Low Water Corrosion (ALWC) occurring at or below the low water zone of steel waterfront structures. ALWC is well recognized to affect harbours worldwide, causing drastic reductions of the service life of steel structures due to unusual high corrosion rates. Prevention and mitigation solutions exist, but are costly, while ALWC prediction has so far not been possible due to the lack of understanding of abiotic and biotic factors and of the mechanisms involved. Presence and potential implication of sulfate-reducing and sulfur-oxidizing bacteria in ALWC has been reported in previous studies. However, the mechanisms and characteristics of the biofilm-deposit complex involved in ALWC remain unclear.

In this study, a detailed characterization of ALWC biofilm-deposit complexes retrieved from European harbours is performed to define bio-factors associated with ALWC in the perspective to develop molecular diagnostics and predictive tools.

Several samples from zones affected by accelerated and normal corrosion were taken from European harbours affected by ALWC since 10 to 20 years. These samples were studied using different analytical methods: (1) cultivation targeting for the first time almost all MIC associated-bacterial metabolic groups (sulfate-reducing bacteria (SRB), thiosulfate-reducing bacteria (TRB), sulfur-oxidizing bacteria (SOB), iron-oxidizing bacteria (IOB), iron-reducing bacteria (IRB), manganese-reducing bacteria (MnRB) and marine aerobic heterotrophic bacteria; (2) 16S rDNA and dsrB PCR-DGGE fingerprinting methods, (3) microscopic methods (e.g. SEM) and (4) analytical methods to determine chemical composition (i.e. XRD, XRFS, and Raman spectrometry).

All targeted bacterial groups could be enriched from samples originating from ALWC and from the reference sites confirming that the presence of specific metabolic groups is not a specific criterion for characterizing ALWC biofilm, but the presence of IOB in ALWC samples might indicate a potential implication of this metabolic group, which was not considered before.

Analysis of the different layers of biofilm-deposit complexes indicated a clear dominance and distribution of active and specific SRB and SOB populations along with the presence of an oxygen gradient within the ALWC deposits. Iron sulfide detected with SRB in the inner layers of ALWC samples confirmed the occurrence of SRB activities and their likely contribution to the ALWC process. Based on these results, the synergetic effect of different bacterial metabolic groups and specific metabolic properties along with biomineralization processes occurring in the case of ALWC could be proposed.

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Varicella screening in pregnancy?

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Aim: Defining the cost-effectiveness of different strategies of varicella screening in pregnancy.
Background: A primo infection of varicella zoster virus (VZV) during pregnancy can cause an asymptomatic intra-uterine infection of the fetus (8-12%) and can give severe complications in preganant women, like varicella pneumonia. A small number of the fetuses with an intra-uterine VZV infection will develop congenital varicella syndrome (CVS). The highest risk (2%, 95% CI 0,8-1,4%) for getting CVS is between 13 and 20 weeks amenorrhoea. There is no report of CVS after 24 weeks amenorrhoea.
A positive history for VZV gives a seropositivity of almost 100% and from people with a negative or uncertain history for VZV 86,6% is seropositive. Maternal age and residential area are not different in seropositive and seronegative pregnant women. There is however a positive relationship of the rate of seropositivity with the increasing number of previous children. Counselling of seronegative pregnant women to prevent a VZV infection in pregnancies can decrease the risk of infection. Risk contacts are face-to-face contacts of more than 5 minutes, a stay of more than 1 hour in the same room and family contacts with an infected person.
Applying varicella zoster immunoglobulines (VZIG) within the first 20 weeks of pregnancy will prevent the development of CVS and after 20 weeks of pregnancy it will mitigate the severeness of complications for the mother. NB. Varicella vaccination in not available in the Netherlands at this moment.
Results: The cost-effectiveness of three models are studied: no screening, selective serotest and test-all. In the selective serotest, only the women with negative or uncertain history for varicella are tested. This model is more cost-effective in comparison to no screening. The cost reduction is caused by a decrease of direct medical costs and indirect costs. The costs of the test-all strategy are three times higher in comparison to the selective serotest, which is caused by the enormous screening costs and is clearly not cost-effective.
Conclusion: For women with a negative or uncertain history of varicella routine serum testing is recommended and post partum vaccination is a cost-effective strategy and can prevent 35-43% of varicella case during pregnancy.

P165

Development of a rapid strategy to determine the toxin gene content in *Staphylococcus aureus*

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Introduction: Many *Staphylococcus aureus* strains produce one or more toxins including staphylococcal enterotoxins (sea-d), staphylococcal exfoliative toxins A an B (eta, etb) and toxic shock syndrome toxin 1 (TSST-1). Various clinical pictures, such as staphylococcal food poisoning and serious skin infections, can be found when a patient is infected with a *S. aureus* strain which produces one or more of these toxins. For surveillance purposes, Dutch hospitals send these strains to the National Institute for Public Health and the Environment (RIVM) to determine the toxin genes. Previously the determination of the toxin-content relied on 8 single PCRs, one of which acts like an internal control. In the newly developed PCR only one multiplex PCR is performed targeting the same targets, a target which acts as a internal control is the coagulase (coag) gene.
Methods: A subset of 192 isolates were selected. The selection was based on all toxin positive *S. aureus* strains that were sent to the RIVM in the period 2002-2010 and 7 control strains (n=199). All strains harbored one or more of the above mentioned toxin genes. For molecular characterization and validation the 8 single PCR and newly developed multiplex PCR are performed on all the strains. The single PCRs were visualized by gel electrophoresis and the multiplex PCR, whose amplification products were generated with labeled primers, was separated by capillary electrophoresis on an automated sequencer.
Results and conclusion: Among the 199 strains tested, 57 (28.6%) were positive for sea, 32 (16.1%) isolates were positive for the TSST-1 gene (tst), 25 (12.6%) were found to be seb positive and 30 (15.1%) contained the gene for sed. Eta and etb revealed a positive result for 31 (15.6%) and 20 (10.1%), respectively . These results were found for both PCR methods. Different results were found for sec . With the single pcr method 31(15.6%) isolates were positive and in the multiplex method 36(18.1%) isolates where positive for sec. The 5 discrepant positive strains were sequenced and revealed an 100% homogeneity with the sec-gene. We conclude that the new multiplex primer set described is more sensitive and specific in detecting the toxin genes of *S. aureus*.

P166

Validation of a newly developed genus PCR for direct detection of *Mycobacteria* in clinical samples

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Introduction: Non-tuberculous mycobacteria (NTM) can cause a wide variety of infections, mainly in immune-compromised and, in increasing incidence, also in immune-competent patients. Molecular detection of NTM is often based on 16S-rDNA PCR. However, specificity of these tests is poor due to cross-reacting species present

in clinical samples and due to contamination in reagents, used for DNA isolation and PCR. The 16S-23S Internal Transcribed Spacer (ITS) contains sequence variations to differentiate mycobacterial species but also has conserved regions. Based on these regions a *Mycobacterium* genus-specific PCR (gMyb PCR) was developed and validated.

Methods: The gMyb primers and probe were designed based on alignments of conserved ITS sequences from 18 mycobacteria species, regularly diagnosed by our laboratory. The primer-probe set constitutes of 6 different forward primers, 1 reversed primer en 1 probe. Sensitivity and specificity were validated on genomic DNA samples of 14 mycobacterial strains and 5 gram-positive bacterial strains. Furthermore, a prospective study on 623 clinical samples and retrospectively on 34 samples was carried out. The gMyb PCR was compared to our currently used 16S real-time (rt) PCR (Savelkoul, pers. comm.) for detection mycobacteria, and further evaluated by reversed-line blot used for confirmation of the 16S rtPCR, and culture results.

Results: All mycobacterial strains were detected by both gMyb and 16S rtPCR. Gram-positive bacterial strains (input 1ng DNA) did not react in the gMyb PCR in contrast to the 16S rtPCR which cross-reacted with 4 out of 5 tested gram-positive bacterial strains. In negative extraction controls sometimes false-positive signals, with high Ct-values, were observed. For the 16S rtPCR, this was seen more frequently.

Prospective comparison of the gMyb PCR with the 16S rtPCR, confirmed by reversed-line blot and culture results, showed that all positive samples were detected. No additional positive samples were found. The number of samples that cross-reacted in the 16S rtPCR was 131 out of 623 samples (21%; a cut-off value of Ct=35 was routinely used) and for the gMyb PCR this was 84 out of 623 samples (13%; no cut-off used). Compared to culture results 4 direct clinical samples were missed in both the gMyb PCR as well as in the 16S rtPCR, indicating reduced extraction efficiency or sampling error for these specific samples. Also, retrospective analysis showed that gMyb PCR accurately detected the *Mycobacterium* genus in clinical samples.

Conclusion: The gMyb PCR is more specific than the 16S rtPCR, as reflected by decreased number of false-positives, although this number is still high. In addition, negative extraction controls gave false-positive gMyb signals, probably due to contamination of DNA isolation reagents by environmental *Mycobacteria*. Further evaluation shows that application of a cut-off value of Ct=35 for the gMyb reaction, preserves accurate diagnosis and leads to substantial reduction of the number of false-positives (22/623 samples: 3.5%). In combination with rtPCR detection of *M. tuberculosis*, the new gMyb real-time PCR offers a reliable method for screening of clinical samples for presence of the broad spectrum of micro-organisms belonging the *Mycobacterium* genus.

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Immunoglobulin subclass switching due to exposure to *Staphylococcus aureus*

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Staphylococcus aureus (*S. aureus*) is equipped with a vast number of virulence factors but, little is known about their role in pathogenesis. Insight in the possible relation between a specific infection and the expression of certain virulence factors is generally lacking. It is known that immunoglobulin subclass switching can be indicative for location and duration of antigen exposure. To study this for *S. aureus* we developed a dedicated bead-based flow-cytometry technique (xMap, Luminex) that allows us to simultaneously quantify the humoral immune response against up to 40 relevant *S. aureus* virulence factors per test. With this system we studied the anti *S. aureus* immunoglobulin titers (IgG, IgG1, IgG2, IgG3, IgG4, IgA and IgA1) in serum of 10 adult persistent *S. aureus* carriers, 10 adult non-carriers and 10 one year old children. Furthermore we included 10 patients with bacteraemia, 10 with skin infection-, 10 with respiratory infection and 10 with a joint infection.

Antigens can be divided into 3 groups, those that primarily evoke an IgG response (SEB, SEM and SSL9), an IgA response (Efb) and those that do both. The majority of the antigens can be found in this group, which in itself can be subdivided into two groups: first, surface proteins, SCIN and CHIPS tend to invoke more IgA than IgG, second the classical toxins invoke more IgG than IgA. Interestingly, in persistent carriers in of the antigens the ratio IgA/IgG was higher than for non carriers, indicating IgA induction due to colonization.

All antigens invoke an IgG1-, a limited group an IgG2- and almost no IgG3-response. In both patients and healthy volunteers an IgG4 response was found against proteins such as TSST-1, SCIN, CHIPS, LukPV-S, LukPV-F, EtA, SSL1 and SSL9. Although in several children IgG4 was found against these antigens, in most of them no IgG4 was detected. Since IgG4 class switching takes place only after several months of continuous exposure, this indicates either during colonization and or during (formal) infections men are exposed for a prolonged time to these virulence factors.

P168

A multicentre quality control study on molecular diagnostics of gastroenteritis in clinical faeces samples

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Introduction: Currently, no proficiency panels are available for molecular detection of bacteria and parasites in clinical samples causing gastroenteritis. In order to evaluate the performance of medical microbiology laboratories, in August 2009 a quality control panel containing clinical faeces samples was organised. The study was an initiative of the gastroenteritis study-group in the Netherlands. To better understand the differences in performance of the participating laboratories, in January 2010 an additional control panel with purified genomic DNA was distributed.
Methods: The faeces panel consisted of 11 duplicated and randomized faeces samples (total 22). The samples contained, in varying loads, none, one or several of the following bacteria and/or parasites: *Salmonella enterica, Campylobacter jejuni, Giardia lamblia* and/or *Cryptosporidium parvum*. The performances of the laboratories were evaluated anonymously in combination with a questionnaire on the molecular methods used. The DNA panel contained dilution series of purified genomic DNA of aforementioned organisms.
Results: Of twenty laboratories participating, 9 tested both for bacteria and parasites, 3 bacteria only and 8 parasites only. Four out of 12 labs, reporting bacterial targets, submitted results corresponding with the expected outcome. For the parasite targets this was 9 out of 17 labs. Two false-positives were submitted by 2 different labs. However, incorrect results were mainly false-negatives and were on samples with low target-loads. Average ΔCt-values of high-positive samples were significantly higher for labs that missed low-positives. On the DNA panel the score of the laboratories missing bacterial targets in the faeces was good, indicating these labs had problems primarily with pre-treatment and DNA isolation. In contrast, laboratories with false-negative results for parasite targets mainly displayed inefficient PCR reactions. Analyses, on the molecular methods used, revealed that laboratories with false-negatives added higher percentage of the sample to the extraction procedure than those with correct results (33% vs. 18%; $p=0.065$). Furthermore, laboratories that missed *G. lamblia* combined PCR-mastermixes and PCR-platforms derived from different suppliers. The same laboratories pre-treated samples by heating (80-100C) in lysis buffer whereas flawless laboratories pre-treated by vigorously shaking in lysis buffer.
Conclusion: Twenty laboratories participated in this first quality control study for molecular detection of gastroenteritis. Non-corresponding results were mainly false-negatives due to decreased sensitivity (caused by insufficient pre-treatment and DNA isolation and/or PCR). In order to improve lab performance we recommend the following: For laboratories with false-negative results for bacterial targets, to optimize the amount of sample input and/or to improve other steps during DNA isolation procedure. Laboratories missing parasite targets are advised to avoid excessive heating during sample pre-treatment and importantly, to optimize PCR reactions.

P169

Assessment of laboratory capacity for response to outbreaks: What do we need?

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Introduction: The Centre for Infectious Disease control (CIb) of the National Institute for Public Health and the Environment (RIVM) is responsible for supporting the public response aimed at reducing the burden of infectious diseases. This is done through surveillance, provision of diagnostic services, coordination of the response to outbreaks, and applied research, in partnership with regional health services (GGD) and medical microbiology and research laboratories. The Laboratory for Infectious Diseases and Perinatal Screening (LIS) has an important role in this mission, but the role of the laboratory is not always clearly defined.
Methods: To assess which laboratory functions are most frequently needed and to increase preparedness, an overview was made of the signals from the reports of the 'early warning committee' (Signaleringsoverleg) at RIVM from 2006 until 2010. The early warning committee is a weekly meeting of representatives from different units of the CIb and of the Food and Consumers Safety Authority (VWA). Infectious disease outbreaks, threats and remarkable events are discussed and a weekly report is sent to subscribers. For a considerable part of the signals, follow-up information was provided, which was considered as a part of the original signal.
Results: In total 625 signals were reported of which 282 involved events in the Netherlands. Of those signals, 126 concerned bacterial infections, which were further analysed. The source of the bacterial signals was surveillance (24%), GGD (22%), notification (14%), reports from a medical microbiological laboratory (MML; 14%), VWA (8%) and a range of other sources (17%). A wide variety of pathogens were mentioned; *Salmonella* species, *Mycobacterium tuberculosis* and *Legionella pneumophila* were the most common. In all, 60% of signals involved 20 different bacteria subject to notification. For 16 of these bacteria, either pathogen surveillance or other designated reference laboratory activities have been defined, either at RIVM or elsewhere. The remaining signals involved 25 different bacteria, including 13 pathogens for which some form of pathogen surveillance or other laboratory program

exists. The most common transmission routes were respiratory (30%) and through food (28%). Five signals, of which one concerned a clear outbreak, involved more than 1,000 patients.
For most reported outbreaks and elevations primary diagnostics are available in many MMLs, and the size of outbreaks was limited in most cases. The role of LIS mostly involved advanced laboratory procedures, such as confirmation of diagnosis or typing to delineate the size of the outbreak or help in source finding, and helping in discussions on case definitions.
Conclusion: Based on this evaluation, laboratory capacity to respond to infectious disease signals is adequate for the majority of notifiable diseases, but limited for a range of 'rare' pathogens that combined have encompassed 1 in 4 signals discussed in the weekly meeting. As the Q-fever outbreak has shown, it is unpredictable which of the pathogens listed could potentially give rise to more widespread outbreaks for which enhanced capacity and interlaboratory coordination is needed. This points at the need for generic preparedness to ensure rapid diagnosis and strain characterisation to support source-finding activities.

P170

Deep sequencing whole transcriptome exploration of the alternative σE regulon in *Neisseria meningitidis*

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Introduction: Bacteria live in an ever-changing environment and must alter protein expression promptly to adapt to these changes and survive. Bacteria have developed sets of specific response genes that are regulated by a subset of the alternative s^{70}-like sigma factors in order to respond to a changing environment. Recently we have described the existence of a σ^E regulon in *N. meningitidis*, and identified its anti-s factor MseR. Deletion of *mseR* resulted in over-expression of the σ^E operon, increasing the expression of *sE* and of *msrA/msrB*. In order to unravel the complete σ^E regulon in meningococci, we sequenced the complete RNA transcriptional content of wild type meningococci and compared it with that of a *mseR* mutant.
Methods: Total enriched RNA of wildtype *N. meningitidis* strain H44/76 and *mseR* mutant cells harvested at mid-log was used for sequencing using the SOLiD Next-Generation Sequencing system (Applied Biosystems) basically as described in the SOLiD Whole Transcriptome Analysis Kit and the SOLiD v3 Plus system's instructions. All resulting reads are strand-specific and were mapped using the BWA algorithm to the annotated chromosome of H44/76 that we sequenced by shotgun 454 Titanium (Roche) pyrosequencing according the the manufacturer's instructions. Technical replicates were performed for both wt and Δ*mseR*. Differential expression of all genes and intergenic regions (IGRs) for both their sense and antisense expression was assessed using DEseq.
Results: From a total of over 300 million reads (between 25 and 50 nucleotides in length), almost ~78% could be confidently mapped to the H44/76 chromosome. In H44/76Δ*mseR* cells both the σ^E operon and *msrA/msrB* were 16.7-fold ($p<0.0001$) and 80.5-fold ($p<0.0001$) upregulated respectively, confirming previous proteomic and microarray results. We also identified genes hitherto not associated with the σ^E regulon in meningococci. The expression of the putative iron-regulated integral membrane protein gnmh_2040 was shown to be 2.8-fold ($p<0.0001$) upregulated, whilst downregulation was observed of the cupper containing nitrite reductase *aniA* (-3.6-fold; $p<0.0001$) and the vaccine candidate Neisserial surface protein A *nspA* (-2.6-fold; $p<0.0001$). Analysis of differential expression in IGRs showed enhanced transcription (16.3-fold, $p<0.0001$) of a non-coding RNA molecule located between genes encoding gnmh_2157 and *dnaE* in H44/76Δ*mseR* cells, identifying a σ^E dependent small non-coding RNA (sRNA).
Conclusions: We have developed a method suitable for analyzing differential expression of all transcripts in bacteria using deep sequencing Whole Transcriptome Analysis. Using this method we unraveled the complete σ^E regulon in the obligate human pathogen *N. meningitidis*. Our results confirm previous proteomic and microarray results and extend on these with the discovery of novel σ^E regulated coding and non-coding transcripts.

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Arcobacter butzleri-associated diarrhoea with an erythromycin resistant strain

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Background: *Arcobacters* are aerotolerant *Campylobacter*-like organisms. Two of the five *Arcobacter* species have been considered as human pathogens. *Arcobacter butzleri* and, more rarely, *Arcobacter cryaerophilus* are associated with watery diarrhoea. Due to refinements in isolation and identification methods, *A. butzleri* has emerged to be a relevant pathogen. This species ranks fourth for *Campylobacteraceae* isolation and appears to have the same pathogenic potential as the other species in the genus. The highest incidence of *A. butzleri* infections is found non-industrialized countries, mostly due to untreated water. In Europe only a small part of bacterial gastro-intestinal

infections is caused by this micro-organism, the most important source of human contamination may be food. Of the *A. butzleri* strains described in literature, 21.3% is resistant to erythromycin and 3.3% to ciprofloxacin. We report a case of human diarrhoea caused by a erythromycin resistant strain.

Case: A56 year-old patient who had received a kidney transplant in January 2010 for kidney failure due to advanced type II diabetes was seen as outpatient with diarrhoea. In April 2010 he has been admitted because of BK-virus nephropathy for which he received cidofovir. Immune-modulating medication at discharge consisted of tacrolimus 2mg twice daily and prednisolone 10mg once daily. The patient visited Morocco for 18 days in the summer of 2010 and developed watery diarrhea afterwards, without involvement of blood or mucus. After two days, treatment with ciprofloxacin was started and the diarrhoea resolved 7 days later. No recurrent disease developed.

Laboratory findings: Selective *Campylobacter* culture media revealed no growth. A 5% sheep blood agar plate with cellulose-acetate filter (pores of 0.65m) showed growth of oxidase-positive and catalase-negative curved gram-negative rods, suspected for *Campylobacter* species. Biochemical tests, MALDI-TOF MS and 23S rRNA gene sequencing identified the bacterium as *A. butzleri*. Disc diffusion showed a zone of 0 mm for nalidixic acid. Susceptibility testing with E-tests showed the following MICs: cefuroxime 64 mg/L, erythromycin 256 mg/L, tetracycline 4 mg/L, tigecycline 3 mg/L, ciprofloxacin 0.5 mg/L, gentamicin 2 mg/L and meropenem 2 mg/L. Regarding erythromycin resistance, 23S rRNA gene sequencing did not show the A2058G, A2059G or T2182C mutation that is known to result in clarithromycin resistance. From 2000-2010 only two other arcobacter strains (*A. butzleri* and *A. nitrofigilis*) were isolated from fecal samples submitted for microbiological examination to the Leiden University Medical Center. One *A. butzleri* (2004) showed intermediate resistance to erythromycin (disc diffusion zone 25mm) and susceptibility to ciprofloxacin (zone 32 mm). One *A. nitrofigilis* (2003) was susceptible to both erythromycin (32 mm) and ciprofloxacin (38 mm).

Conclusion: *A. butzleri* is an uncommon cause of diarrhoea in Europe. Treatment of humane diarrhoea caused by *A. butzleri* can be complicated by acquired resistance to erythromycin. The mechanism of resistance has not yet been revealed.

P172

Mass spectrometric and transcriptional analysis of adhesin-like wall proteins in *Candida glabrata* biofilms

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Background: An important initial step in *Candida glabrata* biofilm development is the adherence to a surface. Adherence is mediated through the cell wall by adhesion-like wall proteins (Awp's). We anticipated Awp's to play a role in biofilms. Expression of genes encoding Awp's may be influenced by different environmental growth conditions. In response, the cell wall can vary its Awp composition, allowing adaptations to new environments. This study shows mass spectrometric and transcriptional analysis of Awp's in *C. glabrata* biofilms grown in two different types of media: YPD and SdmYg.

Methods: Biofilms and free-floating planktonic cells (control) were cultured *in vitro*. Awp's were identified by mass spectrometry. Awp gene expression levels were quantified by Real-Time PCR.

Results: Epa6, Awp2, Awp4 and three novel Awp's; Awp5, Awp6 and Awp7 were identified in biofilms. Primers were designed for five known adhesin genes (*EPA1, EPA3, EPA6, EPA7* and *EPA22*) and seven AWP genes (previously identified *AWP1, AWP2, AWP3* and *AWP4* and newly identified *AWP5, AWP6* and *AWP7*). In both media tested, significant up-regulation of most EPA and AWP genes was observed in biofilms when compared with planktonic cells. Expression of *AWP2* in YPD grown biofilms and *AWP4* in SdmYg grown biofilms, was significantly down-regulated when compared with planktonic cells. A trend was observed for EPA gene expression to be more pronounced in biofilms grown in SdmYg medium compared to YPD medium. Similar, more abundant AWP gene expression was observed in biofilms when grown in YPD medium compared to SdmYg medium.

Conclusions: Elevated expression levels of adhesin genes in biofilm cells support the idea that adhesins are required in the cell wall to mediate the actual attachment of the cell to a surface, whereas for planktonic cells this is probably less important. The ability to regulate the expression of several different adhesins in response to different environmental growth conditions supports the adaptive nature of the cell wall, which may help *C. glabrata* to survive, colonize, adhere to and infect different niches of the human body.

P173

Utilizing genomic data to find and produce novel active lantibiotics

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To fulfill the demand of new antibiotic compounds especially against multi-antibiotic resistant bacteria, we

are screening all publicly available genomic data in an automated fashion to identify lantibiotic synthesis gene clusters. For this purpose we developed a dedicated tool Bagel2. Lantibiotics are ribosomally synthesized, post translationally modified small proteins which are antibacterial. The enzymatic modification of lantibiotics is essential for their antibacterial activity and improves their stability significantly. The identified lantibiotic coding genes from diverse organisms are expressed together with the modification machinery of a known lantibiotic (Nisin) in *Lactococcus lactis*. Subsequently, the produced and modified lantibiotic is tested for activity against a set of indicator strains. The first results show that the software predictions using Bagel2 are correct and that the modification is working as we have 'woken up' the first active lantibiotic.
This novel lantibiotic from *Corynebacterium lipophiloflavum* DSM 44291 could be produced when fused to the leader of nisin, processed by factor Xa, yielding an active lantibiotic, that was shown to contain several thioether rings and dehydrated residues.

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Interactions of an *Acinetobacter baumannii* and an *Acinetobacter junii* strain with human epidermal skin constructs

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Background: *Acinetobacters* have emerged as important nosocomial pathogens. *Acinetobacter baumannii* and to a lesser extent its closely related unnamed species 3 and 13TU are clinically the most relevant species. In particular, *A. baumannii* comprises strains that are multidrug resistant and are associated with outbreaks of colonization and infection worldwide. Several other *Acinetobacter* species, including *A. junii* and *A. lwoffii* can be found as colonizers of the human skin and are infrequently associated with infection.
The aim of the present study was to determine the skin's innate defense response against colonization by an *A. baumannii* and an *A. junii* strain using a human epidermal skin construct.
Methods: Air-exposed human epidermal skin constructs were generated as described previously. Skin constructs were exposed to 105 colony forming units (CFU) of *A. baumannii* type strain RUH3023T and A. junii RUH2228T. The bacterial suspension was aspirated after 1 h, requiring the adherent bacteria to obtain all nutrients and growth factors from the skin constructs. Bacterial counts and biofilm formation were determined up to 72 h after exposure. Immunocytochemistry was used to assess the effects of bacterial presence on keratinocyte proliferation, differentiation and stress. Innate immune response by the human skin upon colonization by each strain was assessed by real-time PCR analysis and ELISAs.
Results: Both *A. baumannii* RUH3023T and *A. junii* RUH2228T colonized the epidermis up to a density of approximately 108 CFU after 12 h and persisted up to 72 h, but did not invade the epidermis. *A. baumannii* RUH3023T formed biofilm on the epidermis after 48 h, whereas *A. junii* RUH2228T did not.Bacterial colonization did not affect keratinocyte differentiation and proliferation nor did it induce stress. In response to colonization with *A. baumannii* type strain RUH3023T, Toll-like receptor 2 and the chemokine interleukin-8 were upregulated compared with non-exposed skin. These genes were also upregulated in response to colonization with *A. junii* RUH2228T but to a lower extent. Interestingly, the antimicrobial peptide human beta defensin 2 was up-regulated in response to *A. baumannii* colonization but not after *A. junii* colonization.
Conclusion: Although bacterial colonization of human skin constructs by *A. baumannii* RUH3023T and *A. junii* RUH2228T were similar, the inflammatory and antimicrobial responses of the constructs to these bacteria differed, which might provide some insight into the differences in pathogenicity of these strains.

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Multidrug-resistant *Acinetobacter baumannii* in German veterinary clinics

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Introduction: Members of the genus *Acinetobacter* comprise well-known nosocomial pathogens responsible for infections and epidemic spread among critically ill human patients including multitrauma patients. The main three clinically relevant species, *A. baumannii*, A. genomic species (gen. sp.) 3 and A. gen. sp. 13TU, and one environmental species, *A. calcoaceticus*, form the so-called *A. calcoaceticus-A. baumannii* (Acb) complex whose representatives cannot be distinguished well by phenotypic attributes. Over recent years, an increase in the number of multidrug-resistant *Acinetobacter* isolates from animal specimens was recognized by the microbiology department of the Giessen Veterinary Faculty. The aim of this study was to investigate the species and strain diversity of these organisms and to compare them with a large set of human *Acinetobacter* strains of the Leiden University Medical Center collection.

Methods: From 2000 through 2008, 137 *Acinetobacter* isolates were phenotypically identified as belonging to the Acb complex. Of these, 56 were selected for further investigation. The organisms were characterized by three genotypic methods including amplified ribosomal restriction analysis (ARDRA), macrorestriction analysis (PFGE) and AFLPTM genomic fingerprinting.
Results: Using ARDRA, 52 isolates were identified to *A. baumannii*, three to A. gen. sp. 3 while one strain with a unique profile remained unclassified. With PFGE, three main clusters of strains with highly similar profiles and six unique types were distinguished. These findings were confirmed by AFLP analysis. Moreover, by comparison to reference strains included in the Leiden AFLP database, 19 isolates were identified as belonging to the European clones that are notorious for their association with outbreaks worldwide.
Conclusion: The study indicates persistence and spread of genotypically related strains within and among the German veterinary clinics. The occurrence of the European clones I-III might indicate that, like in human medicine, *A. baumannii* is an upcoming opportunistic pathogen in veterinary medicine. It also raises the question, whether the organisms can spread from animals to humans or whether the animals have acquired the organisms from humans.

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Taxonomy of the *Acinetobacter calcoaceticus-Acinetobacter baumannii* complex with the proposals of *Acinetobacter pittii* sp. nov. (formerly genomic species 3) and *Acinetobacter nosocomialis* sp. nov. (formely genomic species 13tu)

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Introduction: The *Acinetobacter calcoaceticus-Acinetobacter baumannii* (ACB) complex includes *A. calcoaceticus, A. baumannii,* and four genomic species with provisional designations, i.e. gen. sp. 3, gen. sp. 13TU, gen. sp. 'Close to 13TU' and gen. sp. 'Between 1 and 3'. Numerous studies have shown that gen. sp. 3 and 13TU are medically important groups commonly isolated from hospitalized patients. In the present study, we investigated intra- and inter-species diversity of the ACB complex with the aim to propose formal species names for gen. sp. 3 and 13TU.
Methods: A total of 80 strains of the ACB complex were studied, which were allocated to *A. baumannii* (n=25), *A. calcoaceticus* (n=11), gen. sp. 3 (n=20), gen. sp. 13TU (n=20), gen. sp. 'Close to 3TU' (n=2) or gen. sp. 'Between 1 and 3' (n=2). The strains were selected to cover the breadth of the known intra-species diversity. All strains were investigated by AFLP, amplified rDNA restriction analysis and by 45 nutritional or physiological tests. Subsets of the strains were studied by 16S rRNA gene (n=21) and *rpoB* sequence analyses (n=64), multilocus sequence analysis (MLSA) (n=29) or had been classified previously by DNA-DNA reassociation (n=34). In addition, 190 strains representing the other *Acinetobacter* species were included for comparative analysis.
Results: Using AFLP, the species of the ACB complex formed well-separated clusters at the species cut-off level of 50% similarity. The results of 16S rDNA and *rpoB* analyses, and MLSA supported the monophyly and genomic distinctness of the ACB complex and each of its individual species. The only exception was *A. baumannii* which clustered separately from the other ACB species using 16S rRNA sequence analysis. Phenotypic analysis corroborated both the metabolic versatility of the ACB complex and its phenotypic distinctness from the other *Acinetobacter* species. Although no phenotypic features were identified that could unambiguously differentiate between the ACB complex species, uneven distribution of some properties or their combinations among the species was found. While the growth at 44 °C and inability to assimilate malonate, L-tartrate and citraconate were distinguishing features for most gen. sp. 13TU strains, the vast majority of the gen. sp. 3 strains could be separated from the other species by the ability to assimilate L-tartrate and to grow at 41 °C but not at 44 °C.
Conclusion: Considering the genomic distinctness and medical relevance of gen. sp. 3 and 13TU, the names *Acinetobacter pittii* sp. nov. and *Acinetobacter nosocomialis* sp. nov. are proposed for these genomic species, respectively. The type strain of *A. pittii* sp. nov. is LMG 1035^T (= ATCC 19004^T) and that of *A. nosocomialis* sp. nov. is LMG 10619^T (= CCM 7791^T).
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Identity and ecophysiology of bacteria producing GDGT membrane lipids

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Branched glycerol dialkyl glycerol tetraether (GDGTs) membrane lipids have been found in soils, peat bogs and coastal sediments, and they are used as paleoenvironmental

proxies. GDGTs are synthesized by yet unidentified soil bacteria, and members of the *Acidobacteria* were proposed as candidates for branched GDGTs synthesis1. Aim of the study was to identify bacteria able to synthesize GDGT's, using an integrated cultivation- and biomolecular approach. Soil samples were taken along a temperature gradient adjacent to the Tt watershed (Southwest of France), which were previously shown to contain largely different amounts of GDGT's. Enrichment cultivation was performed at different conditions with respect to pH, temperature and carbon source. For phylogenetic comparison of microbiota in the Tt watershed field samples and enrichment cultures, 16S rRNA gene clone library analysis, DGGE fingerprinting of 16S rRNA gene fragments, as well as barcoded pyrosequencing analysis of PCR-amplified 16S rRNA gene fragments were used.

Clone library analysis showed that samples with high concentration of branched GDGTs were dominated by members of *Acidobacteria* subdivision 1, whereas samples with low GDGT concentration were dominated by members of *Acidobacteria* subdivision 6. Peterse et al., suggested that *Acidobacteria* subdivision 1 may be producers of branched GDGTs.

In an enrichment culture obtained from a high-GDGT Tt watershed sample, branched GDGTs were observed in the 'living' fraction of the microbial biomass. Presently, different strategies are applied to enrich and isolate the bacteria that produce GDGT lipids from the enrichment culture.

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Isolation of novel affinity proteins against *Clostridium difficile* products by lactobacillus surface display

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Background: Antibody therapy is still a rapidly growing market. A putative target for therapy is *Clostridium difficile* infection (CDI). The disease is caused by several toxins, notable TcdA and TcdB, which cause serious damage to the epithelial layer of the intestine. Bacteria can offer a cost effective means of production, but bacteria are not capable of functionally expressing full sized antibodies. Some applications, however, require only the antigen binding site of the antibody, which can be functionally expressed by bacteria. Established methods for the isolation of functional variable domains from naïve libraries such as phage display technologies are often laborious and time consuming. Moreover, isolated fragments sometimes loose their binding characteristics when sub-cloned and expressed in bacteria. Direct expression of a library from which affinity proteins are to be isolated on the surface of bacteria can greatly reduce the time needed to isolate positive binders, while no sub-cloning is needed.

Aims: We aimed to develop a library in *Lactobacillus* based on a human protein with folding characteristics similar to those of antibody variable domains. The second aim was to isolate affinity proteins against TcdA, TcdB and TcdC, a major regulator protein of *Clostridium difficile*.

Methods: Following establishment of adequate expression of the scaffold in *Lactobacillus*, the sequence corresponding to the main complementarity determining region CDR3 of classical antibodies was randomized with variable loop sizes. Randomization of the loop sequences of these Engineered Loop Containing Affinity Molecules (ELCAM's) was done using a technology that avoids the incorporation of stop codons. Following several optimization steps an ELCAM library was generated with a variability of $>10^9$. These ELCAM's can be used for therapeutic as well as research applications.

Results: *Lactobacilli* were chosen for the expression of ELCAM's for several reasons. First of all lactobacilli are gram-positive bacteria allowing good surface expression by coupling of the scaffold to a PrtP anchor for cell wall anchoring. Moreover, they are Generally Regarded as Safe (GRAS) Organisms and can be taken orally in large quantities without deleterious effects, making them the bacterium of choice for therapeutic gastro-intestinal applications. Finally culturing of *Lactobacilli* does not require complex media, does not require aeration and can easily be scaled up.

Using Magnetic Activated Cell Sorting (MACS) several TcdC specific ELCAM's could be selected, differing in length as well as sequence of the CDR3 region. Two clones shared some sequence similarities, suggesting that they recognize the same epitope.

Preliminary results with single chain antibodies (scFv) directed against TcdA and TcdB showed that lactobacilli, expressing anti toxin proteins at their surface, can potentially be used for treatment of CDI. We anticipate that morbidity and mortality can be further reduced by using toxin neutralizing ELCAM's.

Conclusion: A library has been generated with large diversity that can be stably expressed at the surface of lactobacilli. The library has been validated by sequencing of individual clones and by isolation of multiple ELCAM's against a target of choice. The anti TcdC ELCAM's are currently being used for elucidation of regulatory functions of TcdC.

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Genome-wide identification of genes required for non-typable *Haemophilus influenzae* R2866 serum resistance

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Non-typeable *Haemophilus influenzae* (NTHi) is a leading cause of otitis media in children. Moreover, this gram-negative bacterial respiratory tract pathogen can cause invasive inflammatory diseases such as meningitis and sepsis. In general, NTHi is killed by complement mediated lysis, but various clinical isolates of NTHi show resistance to complement mediated lysis.
We applied genomic array footprinting in the search for genes accounting for serum resistance of the NTHi 2866 strain. A library of 30.000 independent transposon mutants was exposed to active and heat-inactivated serum. Mutants that failed to survive in either conditions were identified by comparing microarray hybridization patterns of mutant-specific DNA probes. GAF analysis revealed that 26 genes are required for survival in active serum, whereas disruption of 7 genes enhanced survival. Importantly, the *lgtC* gene for lipooligosaccharide synthesis was among the essential genes identified. This gene was previously described to be involved in serum resistance of NTHi R2866. Furthermore, we identified 8 other lipooligosaccharide synthesis genes and several novel genes essential in serum resistance.
In conclusion, lipooligosaccharide production appeared to be the most prominent serum-resistance mechanism of NTHi R2866. However, additional mechanisms that prevent complement mediated lysis exist, which are currently under investigation.

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Transfer of Conjugative transposons from *Clostridium difficile*

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Background: *Clostridium difficile* strain 630 was shown to contain many mobile genetic elements of which five were putative and one proven conjugative transposons. Several of these elements contain hypothetical genes or putative ABC transporters in their accessory modules, which may affect the adaptation of the host cell to its environment. As all but one of these conjugative transposons do not contain an obvious antibiotic resistance gene, the study of transfer of these elements has been hampered by the lack of a selective marker.
Methods: PCR was used to detect excised circular molecules of these elements. The ClosTron mutagenesis system was used to mark the elements with an antibiotic resistance gene. Target genes were chosen in the predicted accessory module of the elements to minimize effects on transfer. Filter-mating assays were performed on these recombinant elements using either *C. difficile* or *B. subtilis* as recipient, and transfer frequencies calculated using the number of transconjugant cells per donor cell.
Results: Excision from the host genome into a circular intermediate was detected for all but one of the elements and this was not affected by insertion of the ClosTron. Conjugative transfer of CTn*1*, CTn*2*, CTn*4*, CTn5 and CTn7 was shown into recipient strain *C. difficile* CD37. Intergenic conjugative transfer of CTn5 was also shown into *B. subtilis.*
Conclusion: The putative conjugative transposons of strain 630 show conjugative transfer into *C. difficile* some into and *B. subtilis* hosts. Use of the ClosTron system to mark genes in order to investigate transfer of mobile genetic elements in *C. difficile* is a novel application of this mutagenesis system. The genes selected as targets for mutation are not essential for conjugative transfer, strengthening the hypothesis these have accessory functions such as host adaptation to the environment. This work confirms the hypothesis that *C. difficile* has a high number of conjugative elements in its genome.

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Characterization of spores of *rodZ* (*ymfM*) and *yhcA* mutants in *Bacillus subtilis* and their germination and outgrowth behavior under weak organic acid stress

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Introduction: Bacterial spores are a metabolically ‘sleeping state’ of the organism and can survive extreme environmental stress for prolonged periods. Such survival is a major problem to microbial food stability and the food industry as spores are commonly present in the ingredients of many food products. Germination & outgrowth of spores can subsequently lead to unwanted vegetative growth causing spoilage and disease in man. In wild-type *Bacillus subtilis* cells grown under optimal conditions information on the molecular processes involved in germination & outgrowth progression shows that among the germination & outgrowth specific genes also some stress specific genes are, transiently, expressed. What a possible functional role of this could be during germination & outgrowth is unclear. Here, we characterize two of these identified genes: *yhcA* and *rodZ*, formerly *ymfM*. Unknown gene *yhcA* encodes a possible multidrug efflux facilitator and is also responsive to weak organic acid stress in vegetative cells. RodZ has been recently identified to be involved in cell wall synthesis and possibly has a role in membrane synthesis as well. Vegetative cells of a *rodZ* mutant strain are highly sensitive to weak organic acid stress.
Methods: Spores of wild-type and mutant strains were generated using a standardized protocol. Sporulation yield and (wet) heat resistance of the generated spores were determined at 98 and 105°C. Germination & outgrowth

of spores was followed by measuring the optical density at 600 nm in a microtiter plate reader under rigorous shaking at 37°C in buffered TSB medium of pH 7.4 in the presence of sorbic acid (0-125 mM). Germination was triggered by heat activation (30 min at 70°C) and the addition of asparagine, glucose, fructose, and KCl (AGFK).
Results: After harvesting the different spore crops more than 99% consisted out of spores. The total amount of spores was also not different between the wild-type and mutant strains. In addition, the heat resistance of the spores did not show significant differences between the wild-type and mutant strains. Germination & outgrowth of the *rodZ* mutant was slower when compared to the wild type under optimal conditions. Sensitivity of the *rodZ* mutant towards sorbic acid was only observed during late-outgrowth and the following vegetative growth. The *yhcA* mutant did not seem to change the germination and outgrowth behavior. However, the following vegetative growth was more reduced in the mutant strain than in the wild-type under stressed conditions.
Conclusions: The *rodZ* gene plays a substantial role during germination and outgrowth of *B. subtilis* spores optimal conditions. Inactivation of the *yhcA* gene did not seem to affect the germination & outgrowth behavior. This approach helps us to understand the molecular mechanisms that are important for germination & outgrowth under (mild) stress conditions, such as weak organic acid stress and/or heat treatments. With this insight we will be able to identify new targets that help to prevent the germination and outgrowth of spoilage *Bacilli* in food-products using milder preservation techniques that allow optimal food quality at assured microbial food safety.

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16S rDNA sequence analysis as a supplement to traditional blood culturing for improved detection of fastidious growing micro-organisms

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Objectives: Bloodstream infections may be life-threatening, especially in immunocompromised patients with pathogenic bacteria as the most common causative agent. The blood culture is the "golden standard" for microbiological diagnosis of these pathogens. However, there is no independent "gold-standard" test with which to evaluate the blood culture. Although blood culturing is a sensitive detection method it has limitations due to antibiotic use or fastidious growing micro-organisms making detection and identification difficult. In our laboratory blood culturing is supplemented with 16S rDNA sequence analysis in case of a culture-positive result without bacterial growth or fastidious growth or in case the blood culture remains negative and yet strong clinical suspicion of bacteremia persists.
Methods: From January 2008 till September 2010, a total of 329 blood cultures, performed using the BaCT/ALERT 3D (bioMérieux), were requested for additional 16S rDNA sequence analysis. Of these, 140 yielded fastidious growths after blood culturing was detected culture- positive and 189 yielded no growth or remained negative in BaCT/Alert. DNA isolation on blood cultures was performed using EasyMAG (bioMérieux) and for bacterial isolates PrepMan Ultra (Applied biosystems) was used. A 1500 bp amplification of the 16S rDNA gene was carried out, of which 500 bp were sequenced and compared to the BiBi^{LE} database for identification.
Results: The 140 fastidious growing samples yielded 99 different species belonging to 51 genera, of which the most prevalent species were; *Fusobacterium nucleatum* (n=5), *Streptococcus mitis* (n=5), *Streptococcus thermophilus* (n=4), *Streptococcus sanguinis* (n=4). The most prevalent genera were: *Streptocossus* spp. (n=31), *Clostridium* spp. (n=7), *Actinomyces* spp. (n=6), *Acinetobacter* spp. (n=6) and *Bacillus* spp. (n=5).
In 57 of the 189 blood culture-negative samples 16S rDNA could be detected and additional sequence analysis was performed. Thirty-six different species were identified belonging to 21 genera of which the most prevalent species were; *Capnocytophaga canimorsus* (n=5), *Streptococcus pneumoniae* (n=4), *Aggregatibacter aphrophilus* (n=4), *Fusobacterium necrophorum* (n=4) and *Fusobacterium necrophorum* (n=4). The most prevalent genera were: *Streptocossus* spp. (n=13), *Fusobacterium* spp. (n=8) and *Capnocytophaga* spp. (n=5).
Conclusion: False-negative blood culture results are under diagnosed particularly among patients with bacteremia due to fastidious growing bacteria such as *Capnocytophaga canimorsus* or autolytic *Streptococcus pneumoniae* and we therefore recommend in case of clinical suspicion of bactereamia additional 16S rDNA sequential analysis for improved microbiological diagnosis.

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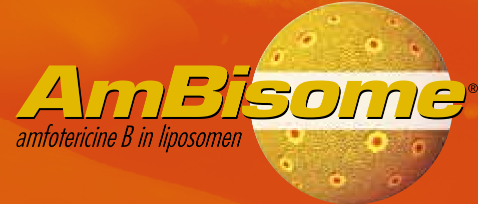

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*AmBisome is geregistreerd voor de behandeling van ernstige systemische mycosen, veroorzaakt door Candida albicans of Aspergillus spp.

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“Constant op zoek naar totaaloplossingen, dat vereist werkelijk partnership. Samen werken aan efficiëntie op het laboratorium en duidelijke uitslagen voor de patiënt. Het zeer brede portfolio, de aanwezige kennis van ons vak, de goede trainingen en servicefaciliteiten stellen ons in staat het begrip ‘totaaloplossingen’ in de praktijk waar te maken.”

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NAAM VAN HET GENEESMIDDEL AmBisome® KWALITATIEVE EN KWANTITATIEVE SAMENSTELLING Amfotericine B 50 mg in liposomen. FARMACEUTISCHE VORM Poeder voor infusievloeistof. KLINISCHE GEGEVENS **Therapeutische indicaties** Behandeling van ernstige systemische mycosen, veroorzaakt door Candida albicans of Aspergillus spp. bij patiënten bij wie het gebruik van conventioneel amfotericine B om redenen van ernstig nierfunctieverlies gecontraïndiceerd is. Empirische behandeling van vermoede schimmelinfecties bij patiënten met neutropenie. AmBisome is ook met succes toegepast bij de behandeling van viscerale leishmaniasis. Bij immuun-gecompromitteerde patiënten (zoals met HIV besmette patiënten) kwamen, evenals na andere vormen van behandeling van viscerale leishmaniasis bij deze patiënten, frequent recidieven voor. Voor het geëigende gebruik van anti-schimmelmiddelen moeten nationale en/of lokale richtlijnen in acht genomen worden. **Contra-indicaties** AmBisome is gecontraïndiceerd bij patiënten met een bekende overgevoeligheid voor één van de componenten, behalve in levensbedreigende situaties wanneer de toestand alleen verbeterd kan worden door behandeling met AmBisome. **Bijzondere waarschuwingen en bijzondere voorzorgen bij gebruik.** AmBisome mag alleen intraveneus worden toegediend en mag alleen aan patiënten worden gegeven in het ziekenhuis onder supervisie van medisch geschoold personeel. Patiënten met preëxistente nierinsufficiëntie dienen gedurende de behandeling onder oplettend toezicht te staan. Het middel moet gereserveerd worden voor de behandeling van patiënten met progressieve, mogelijk levensbedreigende schimmelinfecties die worden veroorzaakt door gevoelige organismen. Er zijn zeldzame meldingen gedaan van anafylaxie in samenhang met de toediening van AmBisome. Als een ernstige anafylactische reactie optreedt, moet de infusie onmiddellijk worden afgebroken en de patiënt dient geen verdere infusen met AmBisome te ontvangen. Ernstige reacties samenhangend met de infusie kunnen optreden bij toediening van amfotericine B-bevattende producten, inclusief AmBisome (zie Volledige Productinformatie paragraaf 4.8, "Bijwerkingen"). Hoewel reacties bij de infusie over het algemeen niet ernstig zijn, moet worden overwogen om voorzorgsmaatregelen voor de preventie of behandeling van deze reacties te treffen bij patiënten die met AmBisome behandeld worden. Lagere infuussnelheden of routinematige toediening van difenhydramine, paracetamol, pethidine en/of hydrocortison zijn waardevol gebleken voor preventie of behandeling van dergelijke reacties. AmBisome kan nefrotoxische effecten hebben. Echter, bij gelijke dosering per kg lichaamsgewicht wordt het in het algemeen beter verdragen dan niet-liposomaal amfotericine B. De nierfunctie dient tijdens de behandeling met amfotericine B-bevattende producten frequent (dagelijks) te worden gemeten. Het wordt eveneens aanbevolen om de leverfunctie en het serumelektrolytgehalte (met name magnesium en kalium) te meten en de normale bloedtellingen uit te voeren. De uitslag van de laboratoriumtesten kan een leidraad zijn voor dosisaanpassingen. Bij progressieve stoornissen in lever- of nierfunctie dient dosisverlaging te worden overwogen; als ook daarna de afwijkingen progressief zijn, dient nogmaals het mogelijk nut van doorgaande behandeling te worden afgewogen tegen de bijwerkingen. Wanneer de medicatie wordt onderbroken voor een periode van meer dan zeven dagen, dient men de behandeling opnieuw te starten met de laagste dosering, welke geleidelijk kan worden verhoogd zoals is beschreven onder "Dosering en wijze van toediening". Dit geneesmiddel mag niet gebruikt worden voor de behandeling van banale niet-systemische of onduidelijke schimmelinfecties die alleen maar positieve huid of serologische tests vertonen. Bij de behandeling van diabetes patiënten dient men er rekening mee te houden dat elke flacon AmBisome 900 mg saccharose bevat. Er zijn geen gegevens bekend omtrent de affiniteit van het intacte liposoom voor dialyse-membranen; om een eventueel verlies van activiteit te vermijden dient bij dialysepatiënten alleen met de toediening van AmBisome gestart te worden nadat de nierdialyse beëindigd is. Er zijn geen gegevens bekend over behandeling van hemodialyse- of peritoneaaldialyse-patiënten met AmBisome. AmBisome moet niet gemengd of tegelijkertijd toegediend worden met andere geneesmiddelen. Zie ook 6.2, "Gevallen van onverenigbaarheid". **Bijwerkingen** Over het algemeen is de incidentie van bijwerkingen die bij behandeling met conventioneel amfotericine B kunnen optreden hoog. In samengevoegde gegevens uit gerandomiseerd, gecontroleerd klinisch onderzoek waarin AmBisome werd vergeleken met conventioneel amfotericine B in meer dan 1000 patiënten, waren de voor AmBisome gerapporteerde bijwerkingen beduidend minder ernstig en minder frequent dan die voor amfotericine B. Koorts en rillingen/rigor zijn de meest voorkomende met de infusie samenhangende bijwerkingen van de eerste AmBisome dosis indien geen premedicatie ter voorkoming ervan wordt gebruikt. Minder vaak voorkomende met de infusie samenhangende reacties kunnen bestaan uit één of meer van de volgende symptomen: rugpijn en/of beklemming of pijn van de borst, dyspnoea, bronchospasmen, flushing, tachycardie, hypotensie. Deze effecten verdwenen snel nadat de infusie werd gestopt. Ze komen niet altijd bij iedere volgende dosis voor, of wanneer de infusiesnelheid wordt verlaagd (2 uur). Zie ook "Speciale waarschuwingen en bijzondere voorzorgen" voor de preventie en behandeling van deze reacties. Er zijn geïsoleerde gevallen van leverfalen (soms met fatale afloop) gemeld na gebruik van AmBisome. Een causaal verband met AmBisome kon nog niet worden vastgesteld. Behalve zeldzame post-marketing rapporten van anaphylactoïde reacties zijn af en toe minder ernstige op allergische reacties gelijkende bijwerkingen gerapporteerd bij AmBisome infusie, inclusief zeer zeldzame meldingen van angioedeem. Men dient bedacht te zijn op bekende bijwerkingen van amfotericine B, onder andere hemolyse, renale tubulaire acidose, bot- of spierpijn en anorexie. **Houder van de vergunning** voor het in de handel brengen Gilead Sciences International Limited, Granta Park, Abington, Cambridge CB21 6GT Verenigd Koninkrijk NUMMER(S) VAN DE VERGUNNING VOOR HET IN DE HANDEL BRENGEN In het register ingeschreven onder RVG : 15610. Uitsluitend recept (UR) Datum: januari 2010

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VERKORTE PRODUCTINFORMATIE
CANCIDAS® 50 mg poeder voor concentraat voor oplossing voor intraveneuze infusie.
CANCIDAS® 70 mg poeder voor concentraat voor oplossing voor intraveneuze infusie.

Samenstelling
CANCIDAS 50 mg bevat 50 mg caspofungin (als acetaat).
CANCIDAS 70 mg bevat 70 mg caspofungin (als acetaat).

Indicaties
- Behandeling van invasieve candidiasis bij volwassen patiënten of kinderen.
- Behandeling van invasieve aspergillose bij volwassen patiënten of kinderen die niet reageren op amfotericine B, toedieningsvormen van amfotericine B met lipiden en/of itraconazol of deze niet verdragen.
- Empirische therapie voor vermoede schimmelinfecties (zoals *Candida* of *Aspergillus*) bij volwassen patiënten of kinderen met koorts en neutropenie.

Contra-indicaties
Overgevoeligheid voor het actieve bestanddeel of één van de hulpstoffen.

Waarschuwingen en voorzorgen
De werkzaamheid van caspofungine tegen de minder vaak voorkomende niet-*Candida*-gisten en niet-*Aspergillus*-schimmels is niet vastgesteld.
Bij gelijktijdig gebruik van CANCIDAS met ciclosporine werden geen ernstige bijwerkingen aan de lever opgemerkt. Sommige gezonde volwassen vrijwilligers die ciclosporine samen met caspofungine kregen, vertoonden een voorbijgaande verhoging van het alaninetransaminase (ALT) en aspartaattransaminase (AST) van minder dan of gelijk aan 3 maal de bovenste waarde van het normale bereik (ULN), die bij stopzetting van de behandeling verdween. CANCIDAS kan gebruikt worden bij patiënten die ciclosporine krijgen als de mogelijke voordelen opwegen tegen de potentiële risico's. Zorgvuldige controle van de leverenzymen moet worden overwogen als CANCIDAS en ciclosporine gelijktijdig worden gebruikt.
Bij een matige leverfunctiestoornis wordt een verlaging van de dagelijkse dosis naar 35 mg aanbevolen. Er is geen klinische ervaring met ernstige leverinsufficiëntie of bij kinderen met elke mate van leverinsufficiëntie. Te verwachten valt dat de blootstelling hoger is dan bij matige leverinsufficiëntie; bij deze patiënten moet CANCIDAS voorzichtig worden toegepast.
De gegevens over de veiligheid van een behandeling die langer duurt dan 4 weken zijn beperkt.

Bijwerkingen
Volwassen patiënten
Flebitis was in alle patiëntpopulaties een vaak gemelde lokale bijwerking op de injectieplaats. Andere locale reacties waren erytheem, pijn/gevoeligheid, jeuk, afscheiding, en een brandend gevoel.
De gemelde klinische en laboratoriumafwijkingen bij alle met CANCIDAS behandelde volwassenen waren over het algemeen licht en maakten zelden stopzetting noodzakelijk.
De volgende bijwerkingen zijn gemeld:
[Zeer vaak (≥1/10), Vaak (≥1/100, <1/10), Soms (≥1/1.000, <1/100)]
Vaak: verlaagd hemoglobine, verlaagd hematocriet, verminderd aantal leukocyten, hypokaliëmie, hoofdpijn, flebitis, dyspnoe, misselijkheid, diarree, braken, verhoogde leverwaarden (AST, ALT, alkalische fosfatase, direct en totaal bilirubine), uitslag, pruritus, erytheem, hyperhidrose, artralgie, koorts, rillingen, pruritus op infusieplaats.
Soms: anemie, trombocytopenie, coagulopathie, leukopenie, verhoogd aantal eosinofielen, verminderd aantal trombocyten, verhoogd aantal trombocyten, verminderd aantal lymfocyten, verhoogd aantal leukocyten, verminderd aantal neutrofielen, vochtophoping, hypomagnesiëmie, anorexia, gestoorde elektrolytenbalans, hyperglykemie, hypocalciëmie, metabole acidose, angst, desoriëntatie, slapeloosheid, duizeligheid, dysgeusie, paresthesie, slaperigheid, tremoren, hypo-esthesie, oculaire icterus, wazig zien, oedeem van het ooglid, verhoogde traanvorming, palpitaties, tachycardie, aritmieën, atriumfibrilleren, hartfalen, tromboflebitis, flushing, opvliegers, hypertensie, hypotensie, verstopte neus, faryngolaryngeale pijn, tachypnoe, bronchospasmen, hoest, paroxysmale dyspnoe 's nachts, hypoxie, rhonchi, wheezing, buikpijn, pijn in de bovenbuik, droge mond, dyspepsie, last van de maag, opgezwollen buik, ascites, constipatie, dysfagie, winderigheid, cholestase, hepatomegalie, hyperbilirubinemie, geelzucht, gestoorde leverfunctie, hepatotoxiciteit, leveraandoening, erythema multiforme, maculaire uitslag, maculopapulaire uitslag, pruritische uitslag, urticaria, allergische dermatitis, gegeneraliseerde pruritus, erythemateuze uitslag, gegeneraliseerde uitslag, morbilliforme uitslag, huidlaesie, rugpijn, pijn in extremiteiten, botpijn, spierzwakte, myalgie, nierfalen, acuut nierfalen, pijn, pijn rond catheter, vermoeidheid, koud gevoel, warm gevoel, erytheem op infusieplaats, verharding op infusieplaats, pijn op infusieplaats, zwelling op infusieplaats, flebitis op injectieplaats, perifeer oedeem, gevoeligheid, ongemak op de borst, pijn op de borst, aangezichtsoedeem, gevoel van andere lichaamstemperatuur, verharding, extravasatie op infusieplaats, irritatie op infusieplaats, flebitis op infusieplaats, uitslag op infusieplaats, urticaria op infusieplaats, erytheem op injectieplaats, oedeem op injectieplaats, pijn op injectieplaats, zwelling op injectieplaats, malaise, oedeem.
Onderzoeken*:*
Vaak: verlaagd kalium in bloed, verlaagd bloedalbumine.
Soms: verhoogd bloedcreatinine, positief voor rode bloedcellen in urine, verlaagd totaal eiwit, eiwit in urine, verlengde protrombinetijd, verkorte protrombinetijd, verlaagd natrium in bloed, verhoogd natrium in het bloed, verlaagd calcium in bloed, verhoogd calcium in bloed, verlaagd chloride in bloed, verhoogd glucose in bloed, verlaagd magnesium in bloed, verlaagd fosfor in bloed, verhoogd fosfor in bloed, verhoogd ureum in bloed, verhoogd gamma-glutamyltransferase, verlengde geactiveerde partiële tromboplastinetijd, verlaagd bicarbonaat in bloed, verhoogd chloride in bloed, verhoogd kalium in bloed, verhoogde bloeddruk, verlaagd urinezuur in bloed, bloed in urine, afwijkende ademgeluiden, verlaagd kooldioxide, verhoogde concentratie immunosuppressivum, verhoogde INR, cylinders in urinesediment, positief op witte bloedcellen in urine, en verhoogd pH van urine.
Kinderen
Het algehele veiligheidsprofiel van CANCIDAS bij kinderen is over het algemeen vergelijkbaar met dat bij volwassenen.
Zeer vaak: koorts.
Vaak: verhoogd aantal eosinofielen, hoofdpijn, tachycardie, flushing, hypotensie, verhoogde leverenzymen (AST, ALT), uitslag, pruritus, rillingen, pijn op de injectieplaats.
Onderzoeken:
Vaak: verlaagd kalium, hypomagnesiëmie, verhoogd glucose, verlaagd fosfor en verhoogd fosfor.
Post-marketingervaring
Sinds de introductie van het product zijn de volgende bijwerkingen gemeld:
leverfunctiestoornis, zwelling en perifeer oedeem, hypercalciëmie.

Farmacotherapeutische groep
Antimycotica voor systemisch gebruik, ATC-code: J 02 AX 04

Afleverstatus
UR

Verpakking
CANCIDAS 50 mg is beschikbaar in een verpakking met 1 injectieflacon.
CANCIDAS 70 mg is beschikbaar in een verpakking met 1 injectieflacon.

Vergoeding
CANCIDAS wordt volledig vergoed.
Raadpleeg de volledige productinformatie (SPC) voor meer informatie over CANCIDAS.

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Tel.: 0800-9999000, email medicalinfo.nl@merck.com
www.msd.nl, www.univadis.nl

Maart 2010

Verkorte productinformatie Mycamine® 50 mg/100 mg (januari 2011) **Samenstelling:** Mycamine® 50 mg/100 mg poeder voor oplossing voor infusie (in natriumvorm). De toe te dienen hoeveelheid na reconstitutie is 10 mg/ml en 20 mg/ml, resp. (in natriumvorm). **Farmacotherapeutische groep:** Overige antimycotica voor systemisch gebruik, ATC-code: J02AX05. **Therapeutische indicaties:** ***Volwassenen, adolescenten ≥ 16 jaar en ouderen:*** Behandeling van invasieve candidiasis; Behandeling van oesofageale candidiasis bij patiënten voor wie intraveneuze therapie geschikt is; Profylaxe van Candida infectie bij patiënten die allogene hematopoiëtische stamceltransplantatie ondergaan of van wie wordt verwacht dat ze aan neutropenie lijden gedurende 10 dagen of langer. ***Kinderen (inclusief neonaten) en adolescenten < 16 jaar:*** Behandeling van invasieve candidiasis; Profylaxe van Candida infectie bij patiënten die allogene hematopoiëtische stamceltransplantatie ondergaan of van wie wordt verwacht dat ze aan neutropenie lijden gedurende 10 dagen of langer. Bij de beslissing Mycamine te gebruiken dient rekening gehouden te worden met het potentiële risico voor de ontwikkeling van levertumoren. Mycamine dient daarom uitsluitend te worden gebruikt als andere antifungale middelen niet in aanmerking komen. **Dosering en wijze van toediening:** Behandeling van invasieve candidiasis: 100 mg/dag, 2 mg/kg/dag bij een lichaamsgewicht < 40 kg. Als de patiënt in onvoldoende mate reageert, bv. indien de kweken positief blijven of de klinische toestand niet verbetert, dan mag de dosis worden verhoogd tot 200 mg/dag bij patiënten met een lichaamsgewicht > 40 kg of tot 4 mg/kg/dag bij patiënten met een lichaamsgewicht ≤ 40 kg. Profylaxe van *Candida* infectie: 50 mg/dag, 1 mg/kg/dag bij een lichaamsgewicht < 40 kg. Behandeling van oesofageale candidiasis: 150 mg/dag, 3 mg/kg/dag bij een lichaamsgewicht < 40 kg. **Contra-indicaties:** Overgevoeligheid voor het werkzame bestanddeel of voor één van de hulpstoffen. **Waarschuwingen en voorzorgen bij gebruik:** De ontwikkeling van foci van veranderde hepatocyten (FAH) en hepatocellulaire tumoren werd bij ratten waargenomen na een behandelperiode van 3 maanden of langer. De leverfunctie dient zorgvuldig te worden gecontroleerd tijdens behandeling met micafungine. Om het risico op adaptieve regeneratie en mogelijk daaropvolgende levertumorvorming te minimaliseren, wordt vroegtijdig staken aanbevolen indien significante en persisterende verhoging van ALT/AST optreedt. De micafungine behandeling dient uitgevoerd te worden na een zorgvuldige risico/voordelen bepaling, met name bij patiënten met ernstige leverfunctiestoornissen of chronische leverziekten die preneoplastische aandoeningen vertegenwoordigen, of bij het tegelijkertijd ondergaan van een behandeling met hepatotoxische en/of genotoxische eigenschappen. Er zijn onvoldoende gegevens beschikbaar over de farmacokinetiek van micafungine bij patiënten met ernstige leverfunctiestoornis. Er kunnen anafylactische/anafylactoïde reacties optreden, waarna de infusie met micafungine moet worden stopgezet en de juiste behandeling moet worden ingesteld. In zeldzame gevallen is er hemolyse gerapporteerd. In dit geval dient nauwlettend te worden gevolgd of er geen verslechtering optreedt en er dient een risico/baten analyse gedaan te worden van voortzetting van de therapie. Patiënten dienen nauwlettend te worden gecontroleerd op verslechtering van de nierfunctie. Patiënten met zeldzame galactose intolerantie, Lapp lactasedeficiëntie of glucose-galactose malabsorptie dienen dit middel niet te gebruiken. **Interacties:** Patiënten die Mycamine in combinatie met sirolimus, nifedipine of itraconazol ontvangen, dienen te worden gecontroleerd op toxiciteit van sirolimus, nifedipine of itraconazol. Gelijktijdige toediening van micafungine met amfotericine B-desoxycholaat is alleen toegestaan wanneer de voordelen duidelijk opwegen tegen de risico's, met een scherpe controle op mogelijke toxiciteit van amfotericine B-desoxycholaat. **Bijwerkingen:** De volgende bijwerkingen deden zich vaak (≥ 1/100 tot < 1/10) voor: leukopenie, neutropenie, anemie, hypokaliëmie, hypomagnesiëmie, hypocalciëmie, hoofdpijn, flebitis, misselijkheid, braken, diarree, buikpijn, verhoogd bloedalkaline-fosfatase, verhoogd aspartaataminotransferase, verhoogd alanineaminotransferase, verhoogd bilirubine in het bloed, afwijkende leverfunctietest, uitslag, pyrexie, koude rillingen. Naast bovengenoemde bijwerkingen zijn bij kinderen tevens vaak thrombocytopenie, tachycardie, hypertensie, hypotensie, hyperbilirubinemie, hepatomegalie, acuut nierfalen en verhoogd bloedureum gemeld. In de volledige SPC tekst worden de soms en zelden voorkomende bijwerkingen gemeld. **Afleverstatus:** UR. **Overige productinformatie:** Astellas Pharma B.V. Elisabethhof 19, 2353 EW Leiderdorp. Tel.: 071-5455854 Fax: 071-5455850.

Referenties: 1. number of patient days calculated from Kg sold (Source: IMS Midas Kg sales 12/02 to 10/10) /Average daily dose over 14 days recommended treatment (Source:product SPC's). Patient treatment length assumed to be 14 days. Data on file (Source: Report to EMA 10/10)
2. SmPC Mycamine 25042008

MYC2011-729