Metagenomic detection of antimicrobial resistant respiratory pathogens

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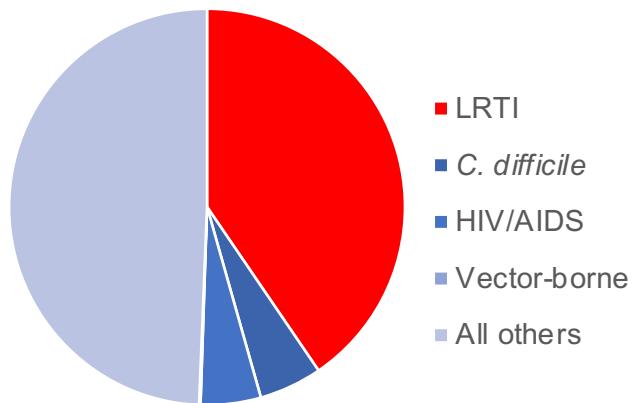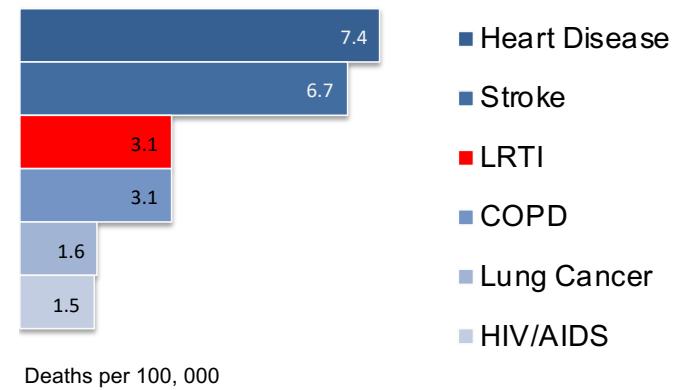
Lower respiratory tract infections (LRTI) are a leading cause of global mortality

Infectious Disease Mortality in the USA

- LRTI
- *C. difficile*
- HIV/AIDS
- Vector-borne
- All others
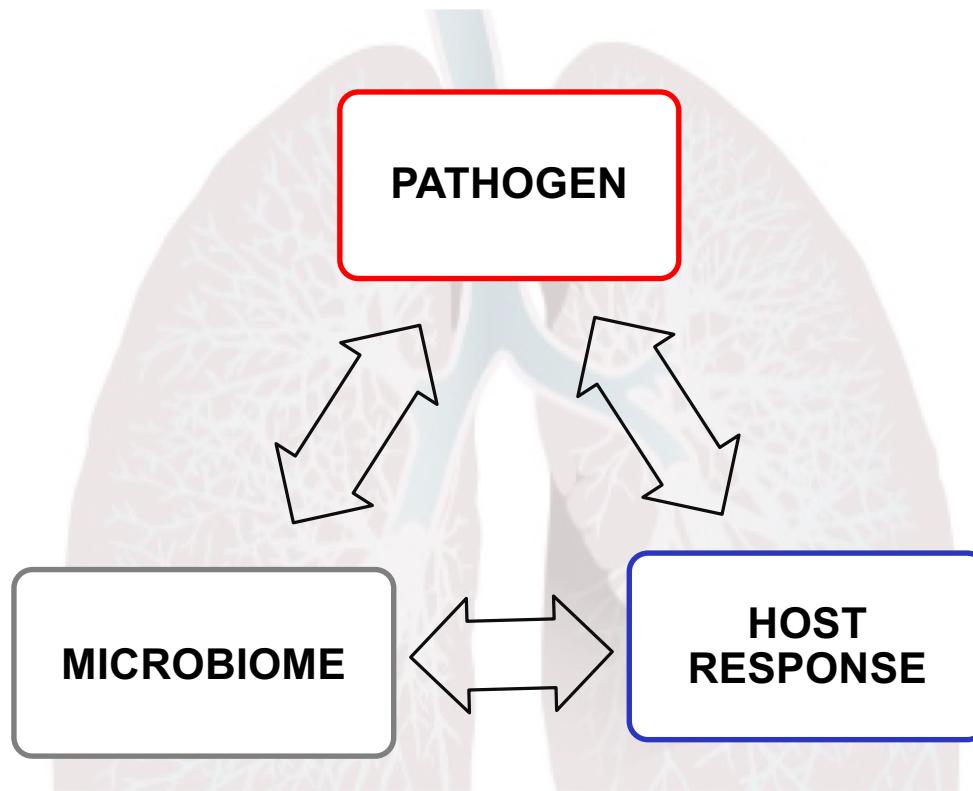
Leading Causes of Death Worldwide

	Deaths per 100,000	
Heart Disease	7.4	
Stroke	6.7	
LRTI	3.1	
COPD	3.1	
Lung Cancer	1.6	
HIV/AIDS	1.5	

Deaths per 100, 000
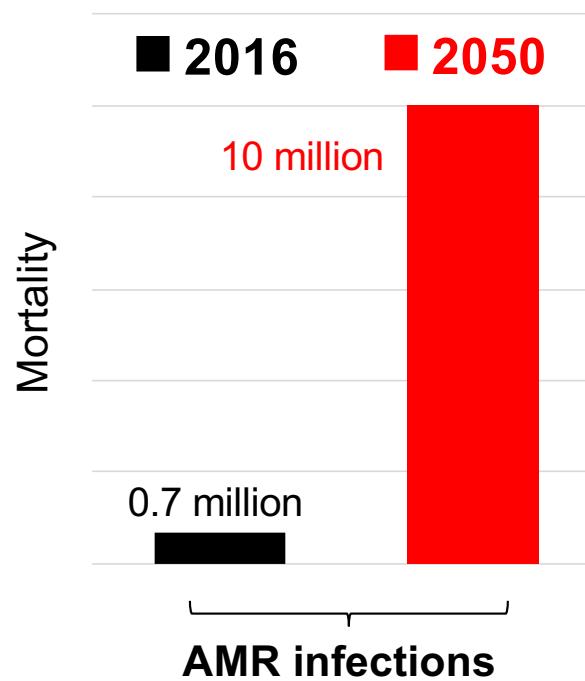
Hansen et al. JAMA. 2016.

World Health Organization. 2017.

A need for better LRTI diagnostics

Mortality from Antimicrobial Resistant Infections

O'Neil, AMR report, 2016. Clin Infect Dis. 2017 Jan 1; 64(1): 106–107.

Current paradigm of empiric treatment for LRTI contributes to the AMR problem
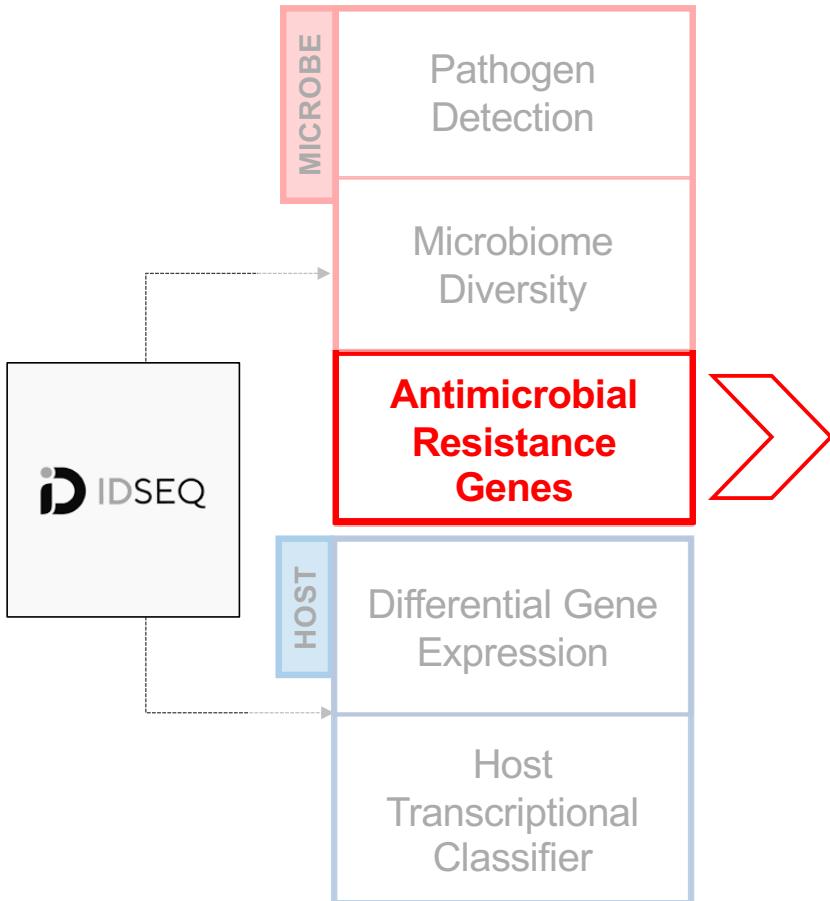
- **Antibiotic <u>over</u>treatment (**40-67% cases**)**
  - Selection for AMR pathogens
  - Microbiome disruption: *C. difficile* infection
  - Adverse drug reactions/allergies
  - Increased morbidity and mortality

- **Antibiotic <u>under</u>treatment**
  - Infection progression and/or transmission

Vaughn et al. Ann Intern Med. 2019.

# Metagenomics: a key tool to combat AMR

- **Early detection**
  - Effective treatment of resistant microbes to avoid treatment failure
  - Targeted therapy to improve antimicrobial stewardship

- **Surveillance**
  - Global transmission patterns
  - Regional distribution of resistant pathogens
  - Emergence of novel resistance mechanisms

mNGS for AMR pathogen detection
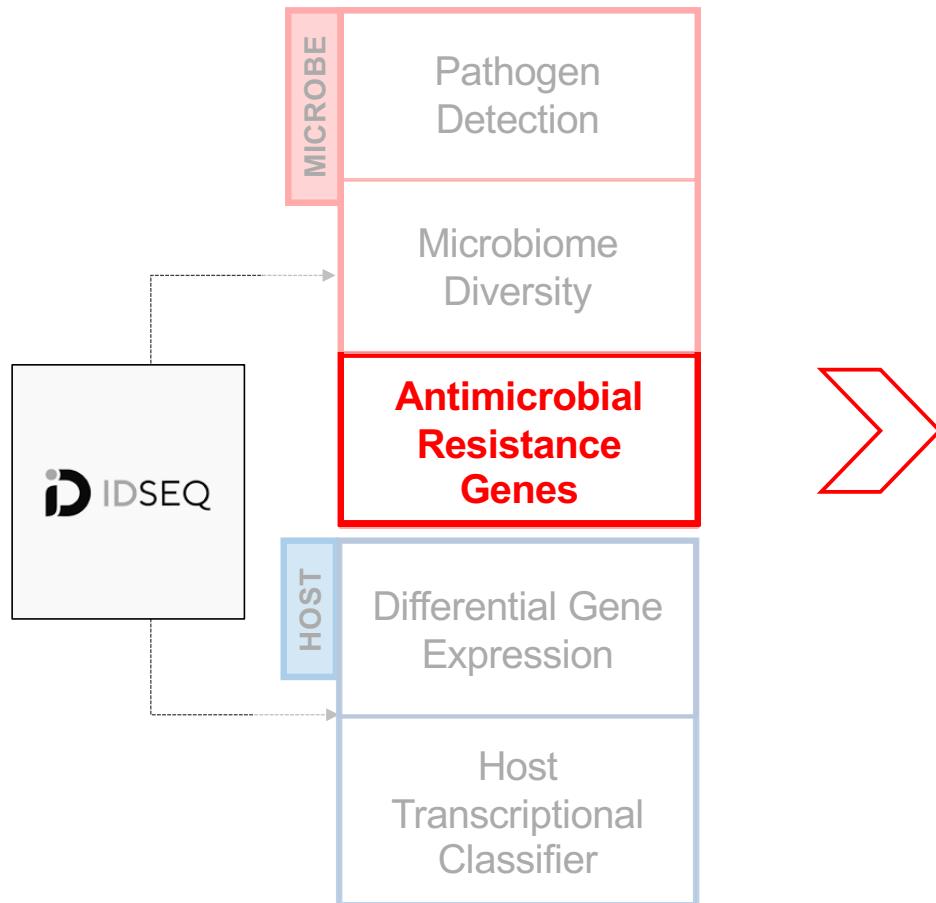
Phenotypic Testing:

- **Resistant** (red)
- **Susceptible** (blue)
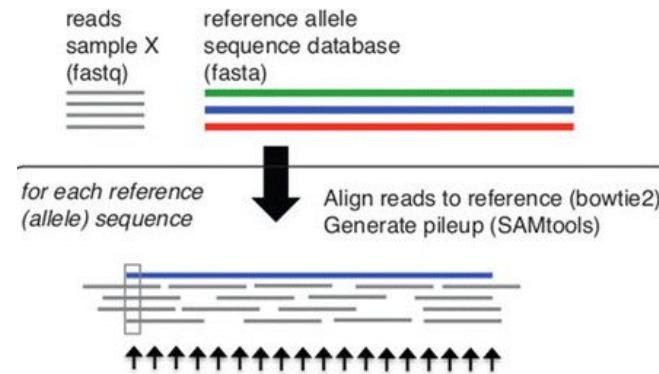
ID	Organism	Pen/Amp	Vancomycin	Methicillin	Clindamycin	TMP/SMX	Ceftriaxone
225	*Staphylococcus aureus*	Resistant	Susceptible	Resistant	Resistant	Susceptible	n/a
288	*Staphylococcus aureus*	Resistant	Susceptible	Resistant	Resistant	Susceptible	n/a
409	*Staphylococcus aureus*	Resistant	Susceptible	Resistant	Resistant	Susceptible	n/a
212	*Staphylococcus aureus*	Resistant	Susceptible	Susceptible	Resistant	Susceptible	n/a
230	*Staphylococcus aureus*	Resistant	Susceptible	Susceptible	Resistant	Susceptible	n/a
239	*Staphylococcus aureus*	Resistant	Susceptible	Resistant	Resistant	Susceptible	n/a
289	*Staphylococcus aureus*	Resistant	Susceptible	Susceptible	Susceptible	Susceptible	n/a
304	*Staphylococcus aureus*	Resistant	Susceptible	Susceptible	Susceptible	Susceptible	n/a
311	*Staphylococcus aureus*	Resistant	Susceptible	Susceptible	Susceptible	Susceptible	n/a
314	*Staphylococcus aureus*	Resistant	Susceptible	Susceptible	Susceptible	Susceptible	n/a
386	*Staphylococcus aureus*	Resistant	Susceptible	Susceptible	Susceptible	Susceptible	n/a
407	*Staphylococcus aureus*	Resistant	Susceptible	Susceptible	Susceptible	Susceptible	n/a
277	*Enterococcus faecium*	Resistant	Resistant	n/a	Resistant	n/a	n/a
311	*Streptococcus pneumoniae*	Susceptible	Susceptible	n/a	n/a	n/a	Susceptible
334	*Streptococcus pneumoniae*	Susceptible	Susceptible	n/a	n/a	n/a	Susceptible
337	*Streptococcus pneumoniae*	Resistant	Susceptible	n/a	n/a	n/a	Resistant
399	*Streptococcus pneumoniae*	Susceptible	Susceptible	n/a	n/a	n/a	Susceptible
463	*Streptococcus pneumoniae*	Susceptible	Susceptible	n/a	n/a	n/a	Susceptible
213	*Escherichia coli*	n/a	n/a	n/a	n/a	Susceptible	Susceptible
213	*Klebsiella pneumoniae*	n/a	n/a	n/a	n/a	Susceptible	Susceptible
232	*Klebsiella pneumoniae*	Resistant	n/a	n/a	n/a	Susceptible	Resistant
251	*Klebsiella pneumoniae*	n/a	n/a	n/a	n/a	Susceptible	Susceptible
239	*Pseudomonas aeruginosa*	Resistant	n/a	n/a	n/a	Susceptible	Resistant
268	*Pseudomonas aeruginosa*	Resistant	n/a	n/a	n/a	Susceptible	Resistant
268	*Stenotrophomonas*	Resistant	n/a	n/a	n/a	Susceptible	Resistant
257	*Enterobacter aerogenes*	Resistant	n/a	n/a	n/a	Susceptible	Resistant
298	*Enterobacter cloacae*	Resistant	n/a	n/a	n/a	Susceptible	Resistant
343	*Burkholderia cepaciae*	n/a	n/a	n/a	n/a	Susceptible	Susceptible
350	*Serratia marcescans*	Resistant	n/a	n/a	n/a	Susceptible	Resistant
382	*Serratia marcescans*	Resistant	n/a	n/a	n/a	Susceptible	Resistant
485	*Heamophilus influenzae*	Resistant	n/a	n/a	n/a	Susceptible	Susceptible

mNGS methods for AMR pathogen detection

Rules-based AMR model:

- SRST2 + custom AMR gene database

- 5% minimum allele coverage
- SNP detection: CARD RGI tool
- Phenotypic resistance prediction:
  - CARD ontology
  - 2 MD adjudication

Wright, G et al. Nucl. Acid Res. 2017.
KA Holt et al. Genome Medicine. 2014.

MICROBE

Pathogen Detection

Microbiome Diversity

Antimicrobial Resistance Genes

HOST

Differential Gene Expression

Host Transcriptional Classifier

IDSEQ

mNGS vs culture for AMR prediction

S. aureus only (n = 12)

	Sensitivity	Specificity	Accuracy
DNA-Seq	52%	92%	80%
RNA-Seq	40%	95%	77%
combined	83%	95%	91%

All (n = 31)

	Sensitivity	Specificity	Accuracy
DNA-Seq	52%	92%	80%
RNA-Seq	69%	96%	88%
combined	88%	92%	91%

A 'false-positive' case

2/25 56 y/o man admitted for venous thrombosis

2/27 New fever, hypotension, hypoxia
- Rx: aztreonam, vancomycin, azithromycin

2/28 Transferred to ICU, respiratory Cx obtained → **mNGS:**
- Enrolled in study, sample for mNGS collected

3/02 Cx grows *Klebsiella*
- Vancomycin, azithromycin stopped

3/04 Improved, discharged from ICU

3/07 new hypoxemic respiratory failure
Re-admitted to ICU, Rx vancomycin, TA Cx obtained

3/13 TA Cx finalize: *MRSA*

mNGS:
-*K. pneumoniae*

AMR genes:
-*SHV-190*
-*mecA*
-*ermA/C*

Staph. aureus
Susceptibility testing:

Clindamycin	Resistant
Nafcillin	Resistant
Penicillin G	Resistant
Bactrim	Susceptible
Vancomycin	Susceptible
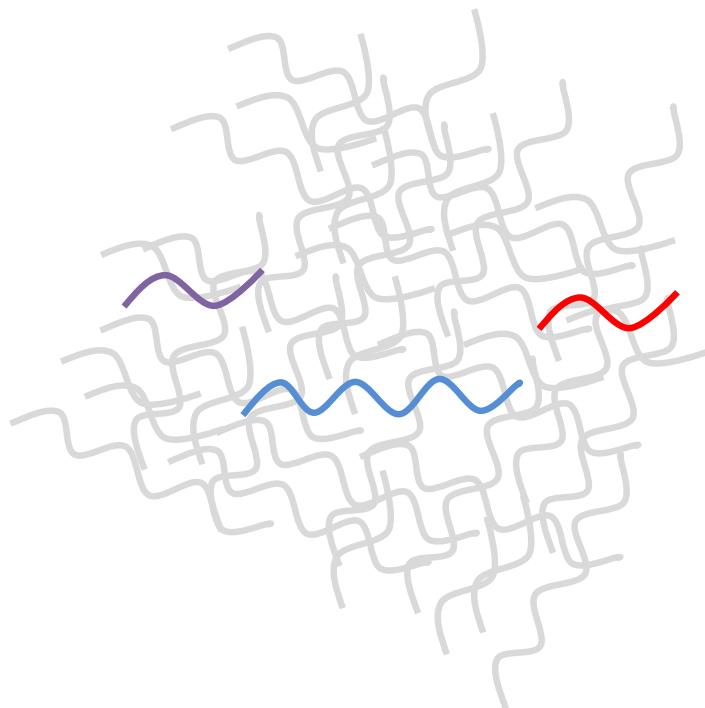
Clindamycin	Resistant
Erythromycin	Resistant

mNGS vs culture for AMR prediction

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DNA-Seq	52%	92%	80%
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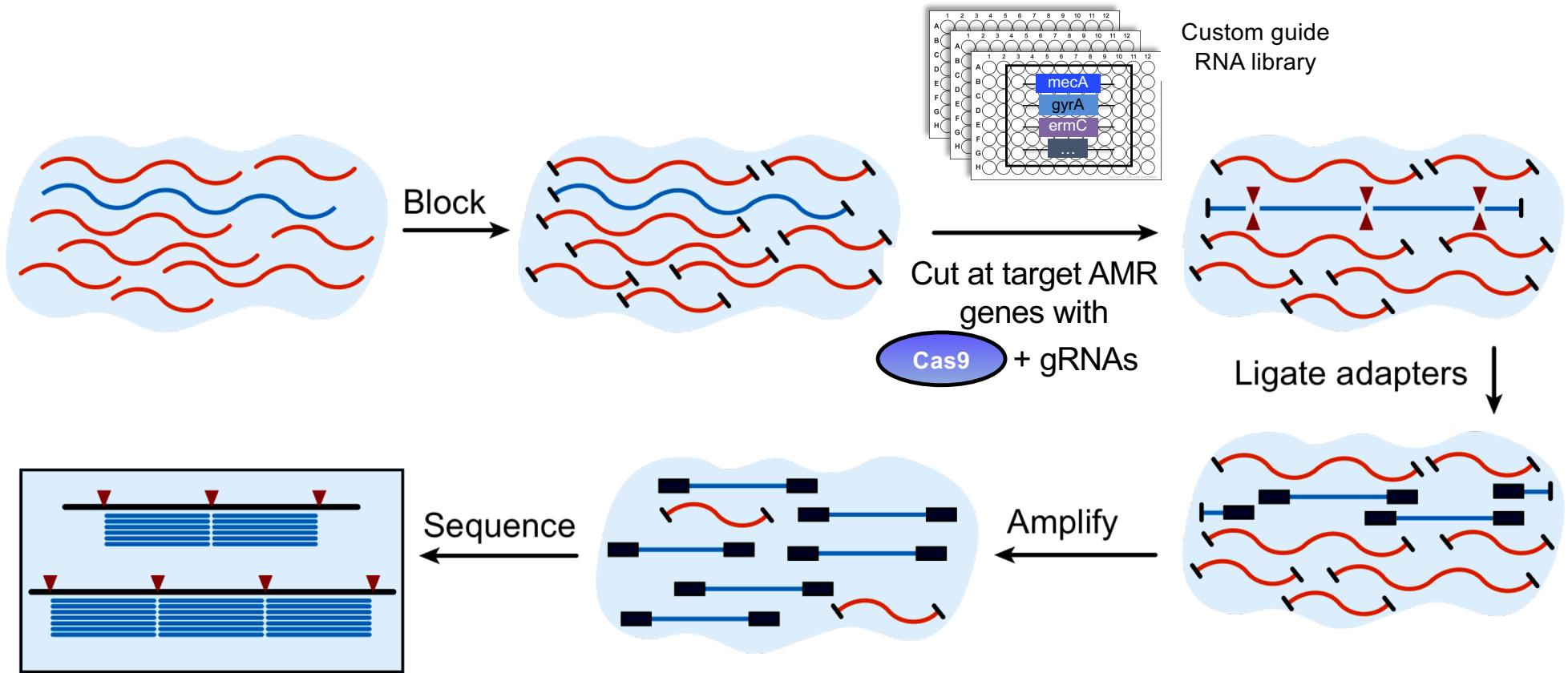
All (n = 31)

|	Sensitivity	Specificity	Accuracy
DNA-Seq	52%	92%	80%
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# Resistance gene sequences are rare, ~ 1 in 10^8
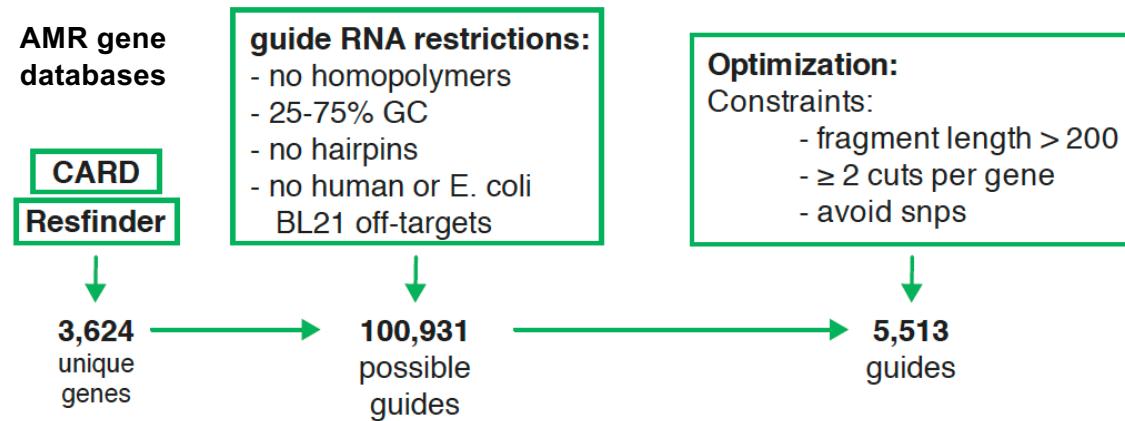
FLASH: Finding Low Abundance Sequences by Hybridization
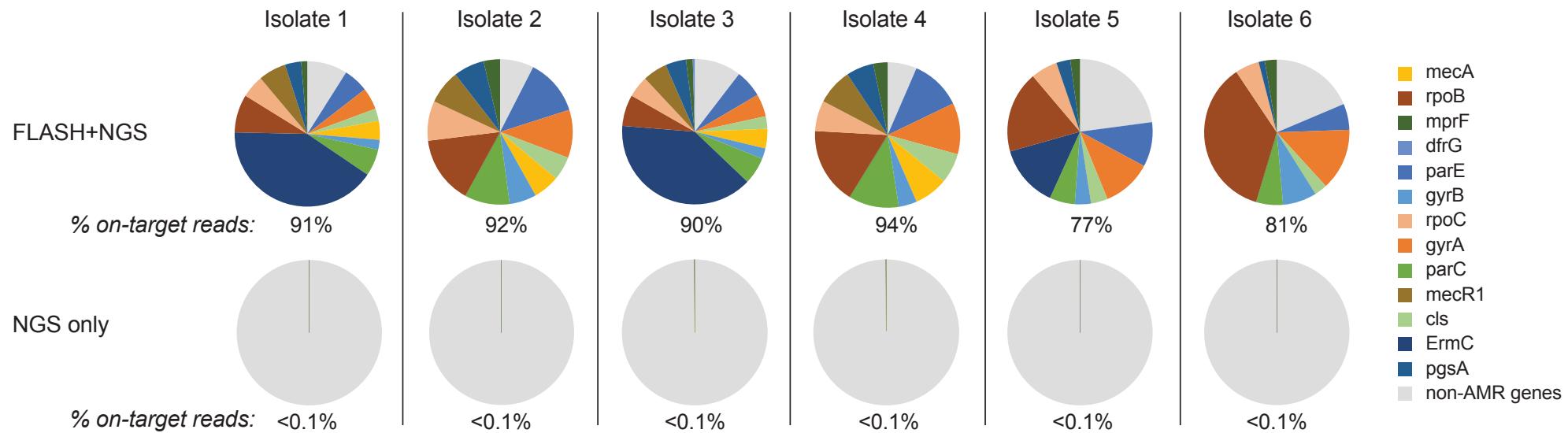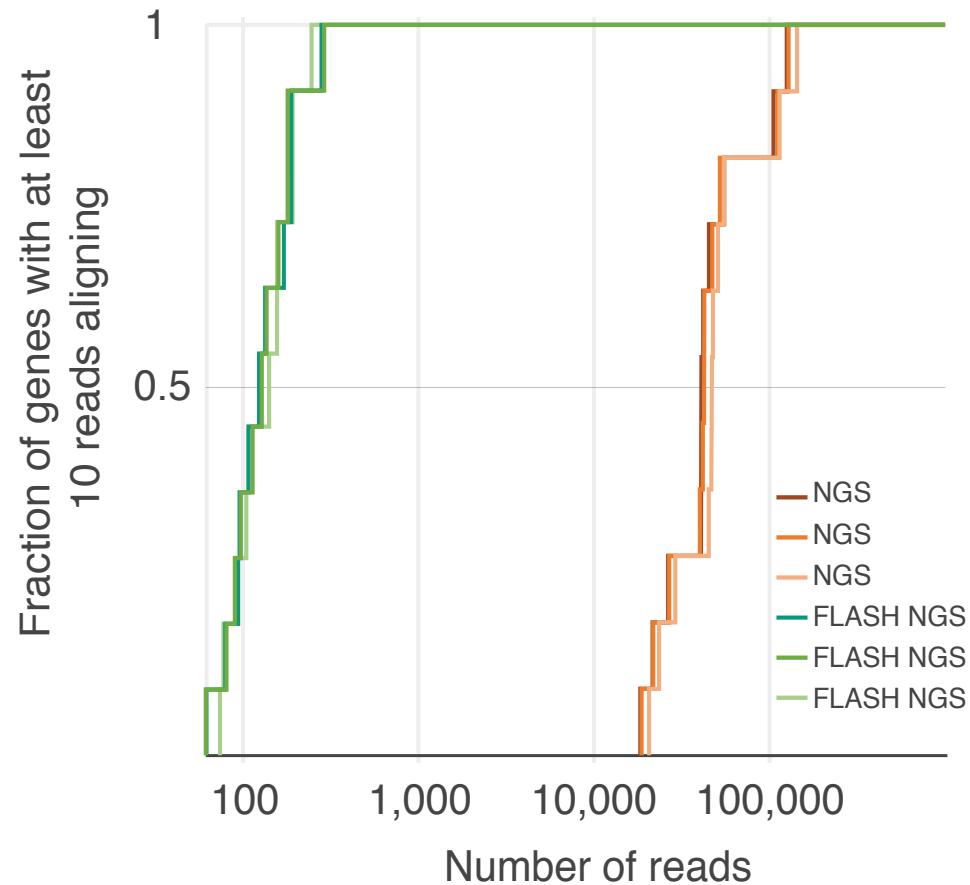
FLASH CRISPR/Cas9 guide RNA design

AMR gene databases

CARD

Resfinder

3,624 unique genes

guide RNA restrictions:
- no homopolymers
- 25-75% GC
- no hairpins
- no human or E. coli BL21 off-targets

100,931 possible guides

Optimization:
Constraints:
- fragment length > 200
- ≥ 2 cuts per gene
- avoid snps

5,513 guides

Quan, Langelier, Crawford et al. 2019. Nucleic Acids Research.

FLASH of clinical *Staphylococcus* isolates

Quan, Langelier, Crawford et al. 2019. Nucleic Acids Research.
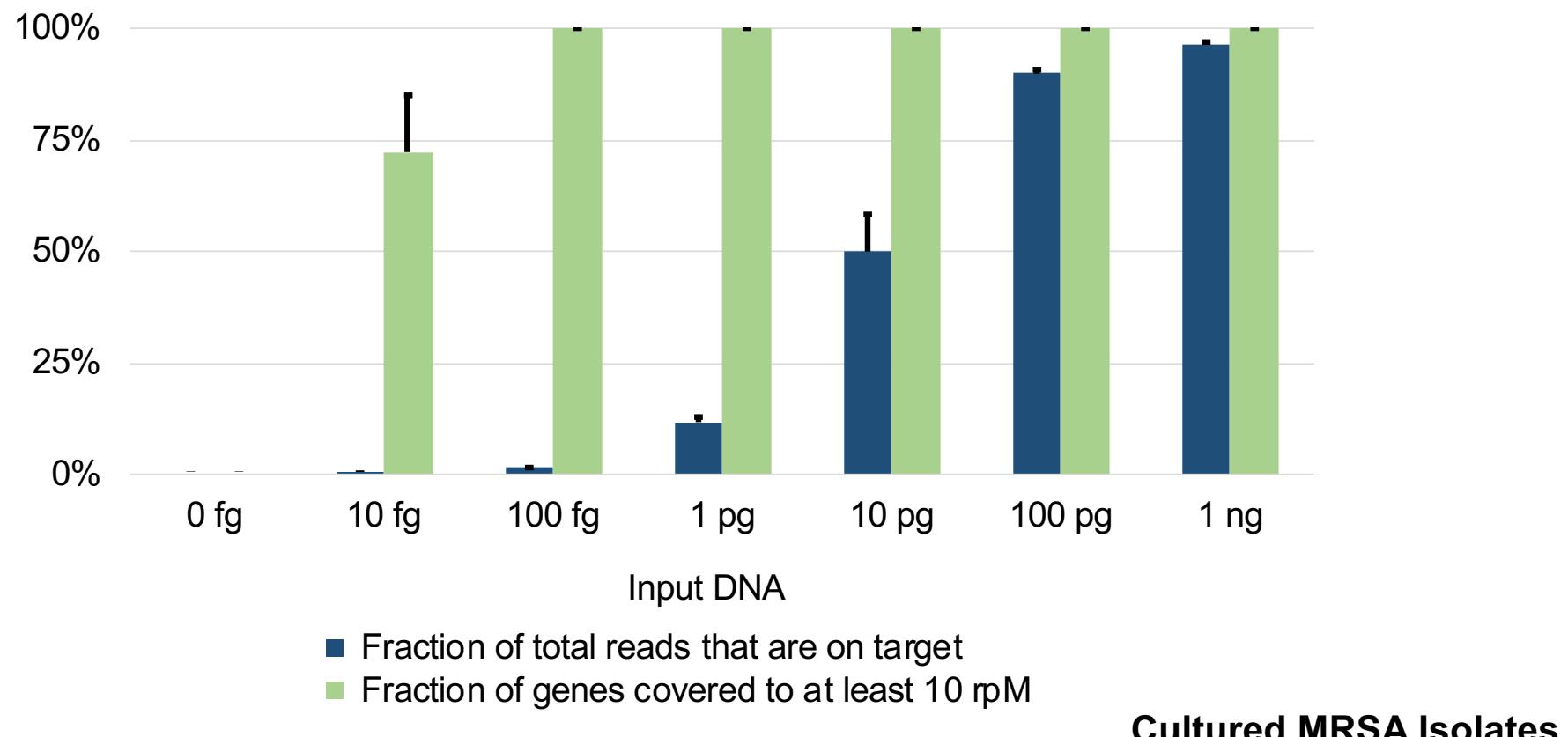
FLASH: how many reads are needed?
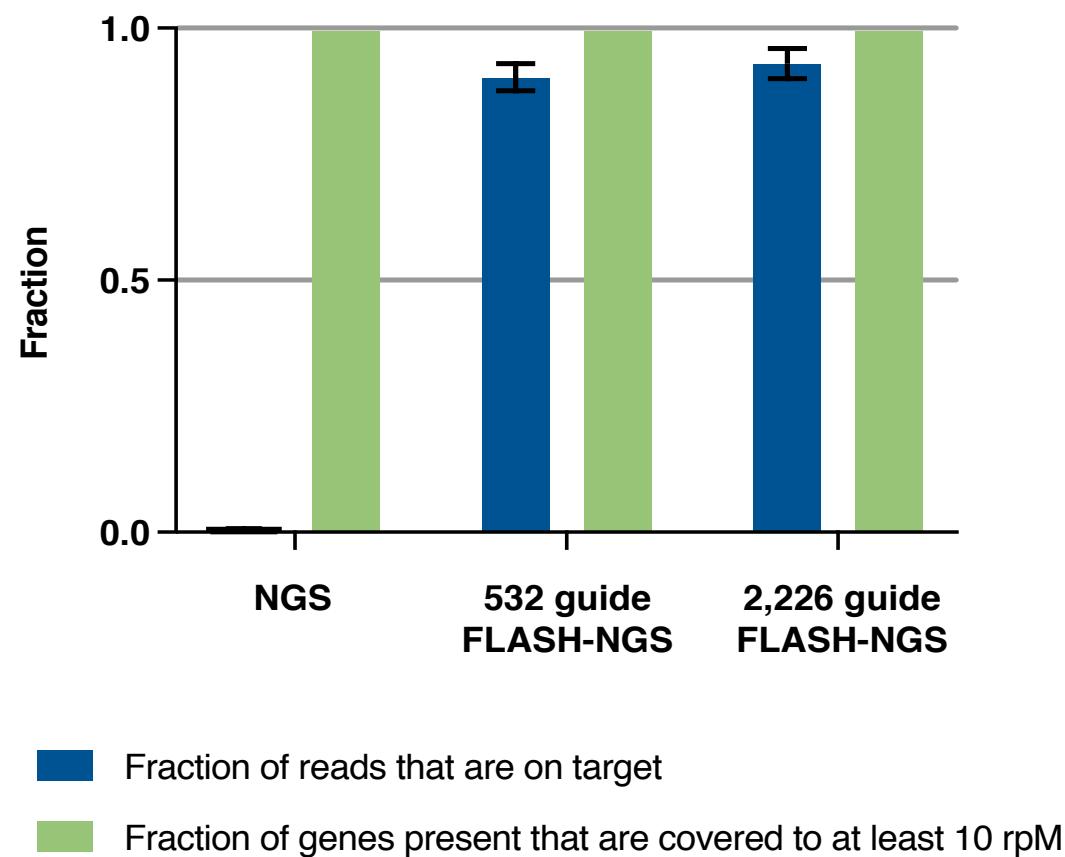
Quan, Langelier, Crawford et al. 2019. Nucleic Acids Research.

FLASH limit of detection

Input DNA

- ■ Fraction of total reads that are on target
- ■ Fraction of genes covered to at least 10 rpM

Cultured MRSA Isolates
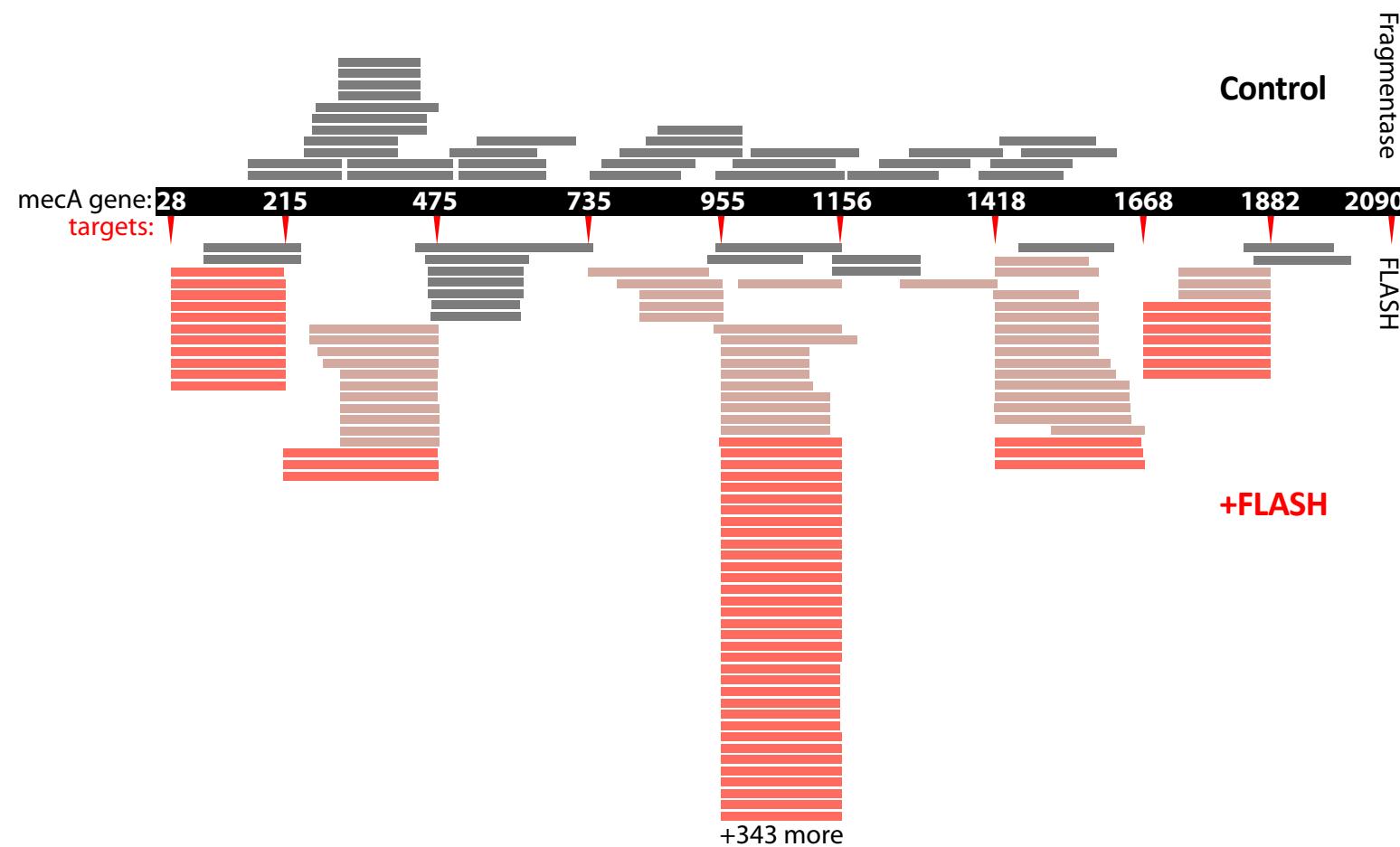
Quan, Langelier, Crawford et al. 2019. Nucleic Acids Research.

FLASH: Is there an upper limit to guide RNA multiplexing?

Fraction of reads that are on target

Fraction of genes present that are covered to at least 10 rpM
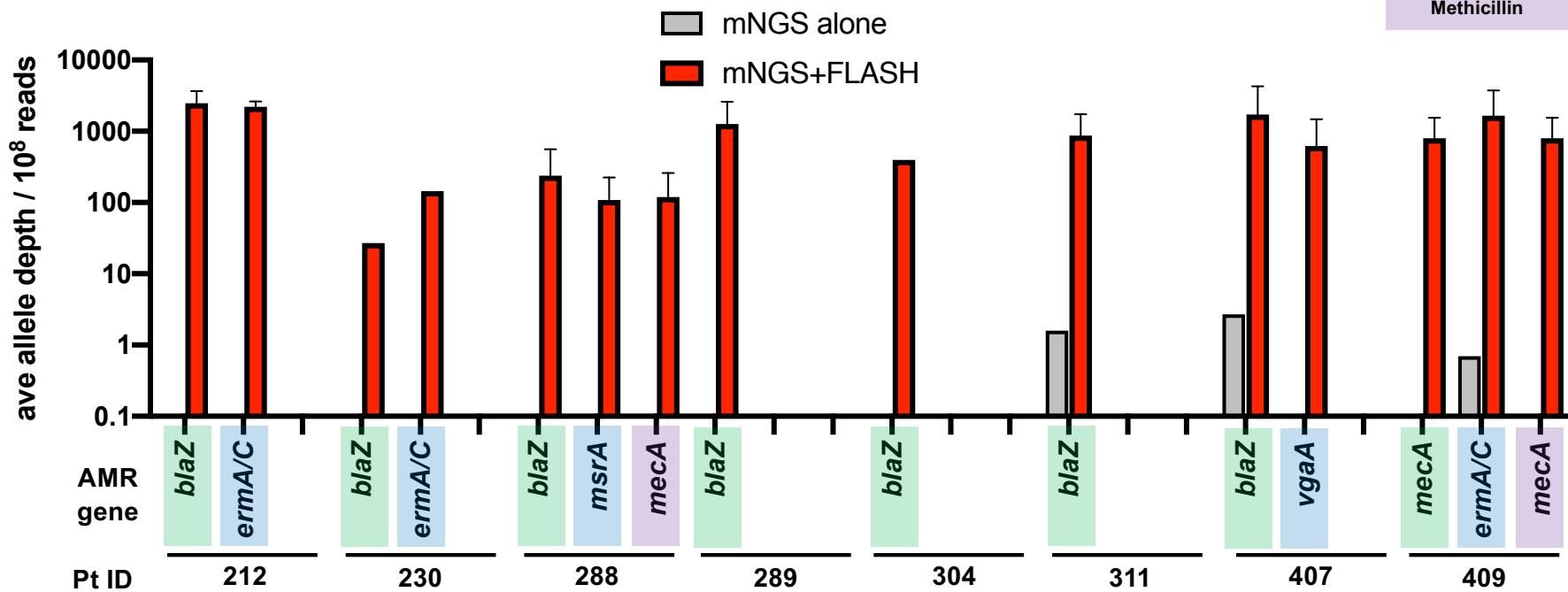
Quan, Langelier, Crawford et al. 2019. Nucleic Acids Research.

FLASH Cas9 Targeted mNGS - Example

FLASH-mNGS on direct clinical samples
Staph aureus LRTI

Phenotypic Resistance

| Penicillin |
| Clindamycin |
| Methicillin |

☐ mNGS alone
☐ mNGS+FLASH

ave allele depth / 10^8 reads

AMR gene																
blaZ	*ermA/C*	*blaZ*	*ermA/C*	*blaZ*	*msrA*	*mecA*	*blaZ*	*blaZ*	*blaZ*	*blaZ*	*vgaA*	*mecA*	*ermA/C*	*mecA*

| Pt ID | 212 | 230 | 288 | 289 | 304 | 311 | 407 | 409 |

Tracheal aspirate

mNGS + FLASH vs culture for AMR prediction

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combined	83%	95%	91%
+ FLASH	95%	93%	93%
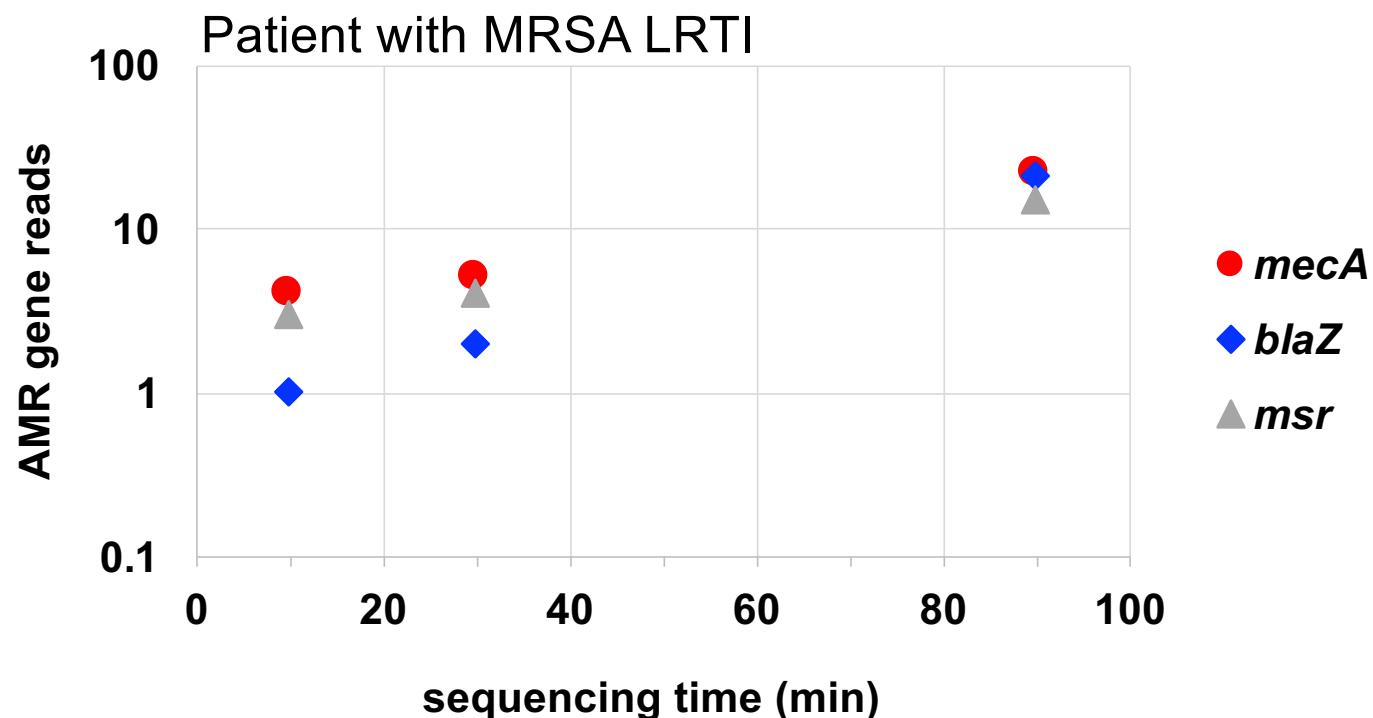
All (n = 31)

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DNA-Seq	52%	92%	80%
RNA-Seq	69%	96%	88%
combined	88%	92%	91%
+ FLASH	97%	89%	92%

Rapid mNGS-FLASH via Nanopore?

Rapid mNGS-FLASH via Nanopore

Patient with MRSA LRTI

AMR gene reads

sequencing time (min)

- ● *mecA*
- ◆ *blaZ*
- ▲ *msr*
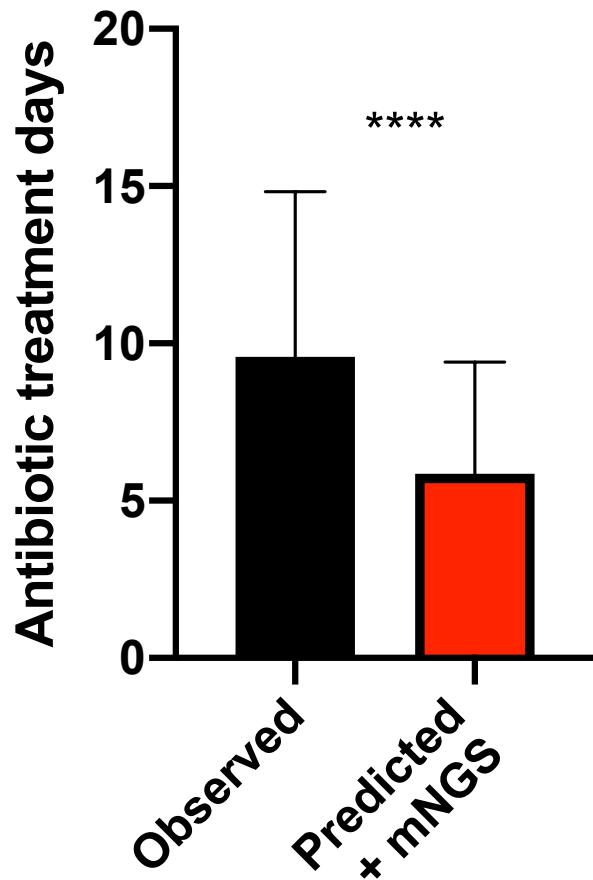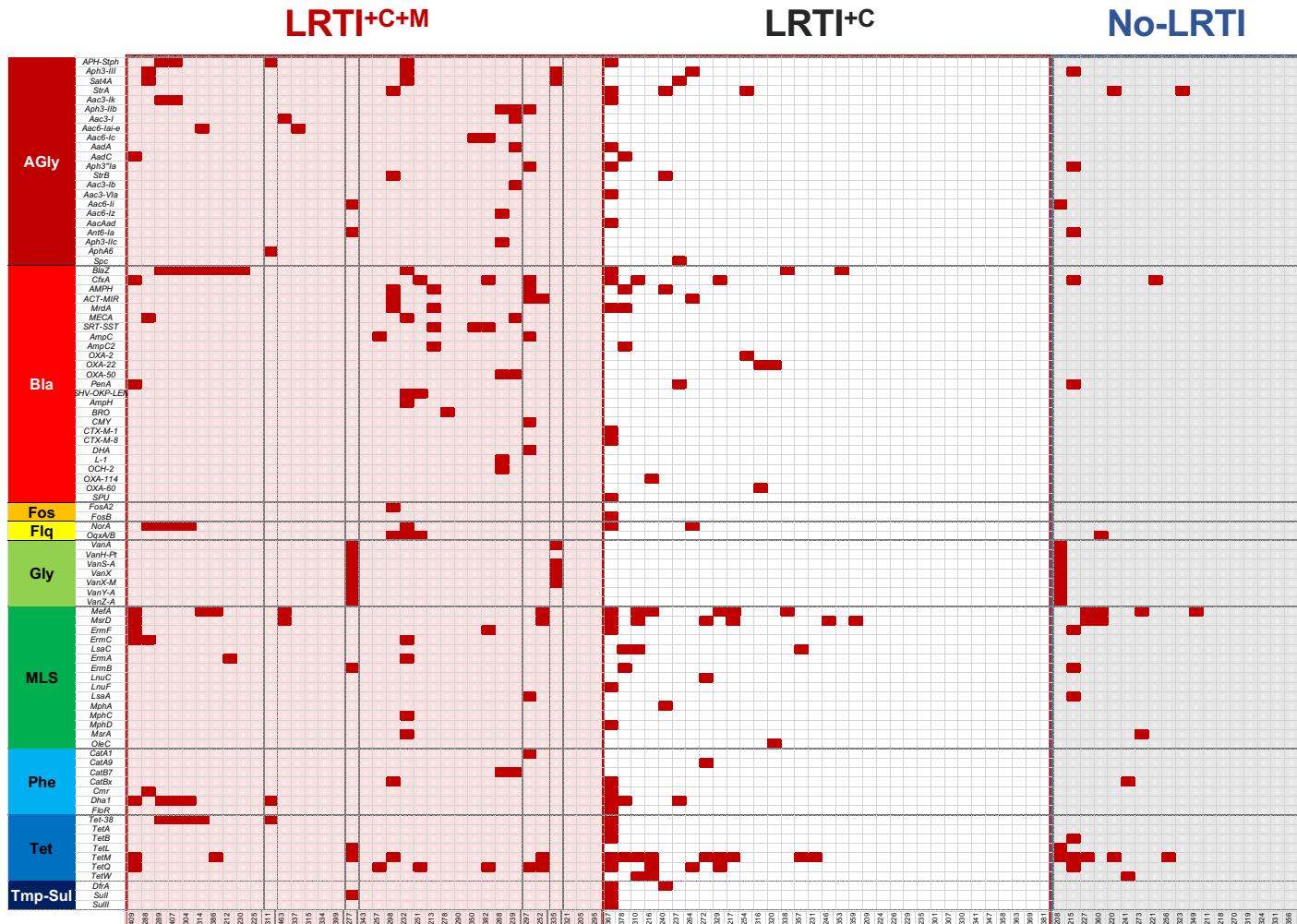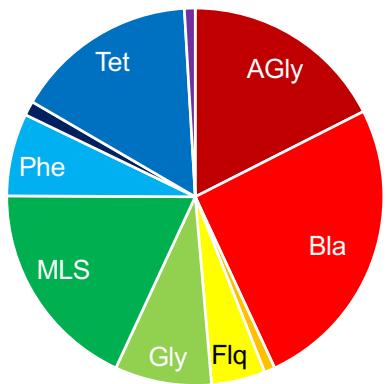
Wayne Deng PhD; Charles Chiu, MD PhD

Precision Antibiotic Stewardship
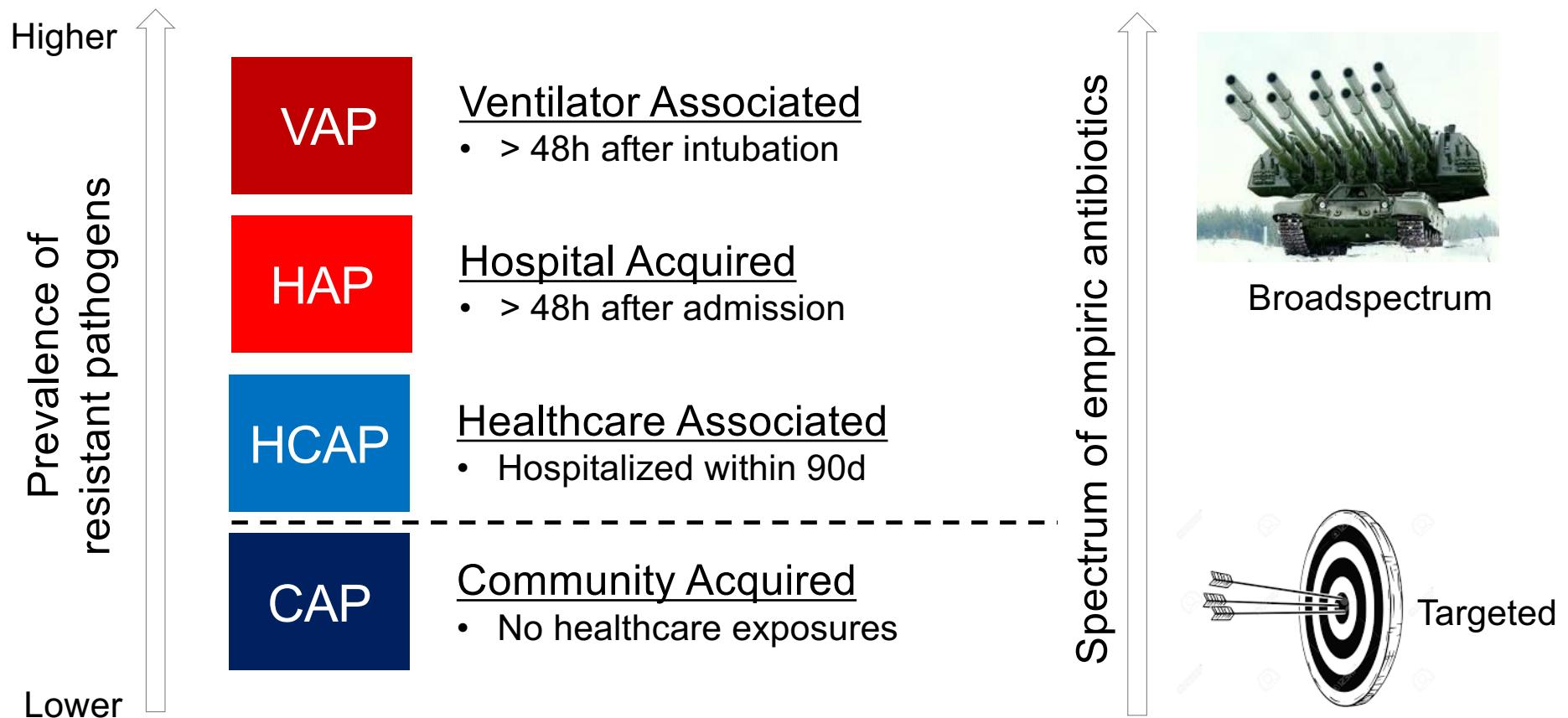
- **Median time to AST: 93 hours**
- **Empiric antibiotics: 91% of patients**
- **Theoretical impact of mNGS:**
  - Adjudicated optimal Δ antibiotics based on 48h mNGS turnaround
  - Compared against actual antibiotics received during hospitalization
  - **39% reduction** in unnecessary days of antibiotic therapy
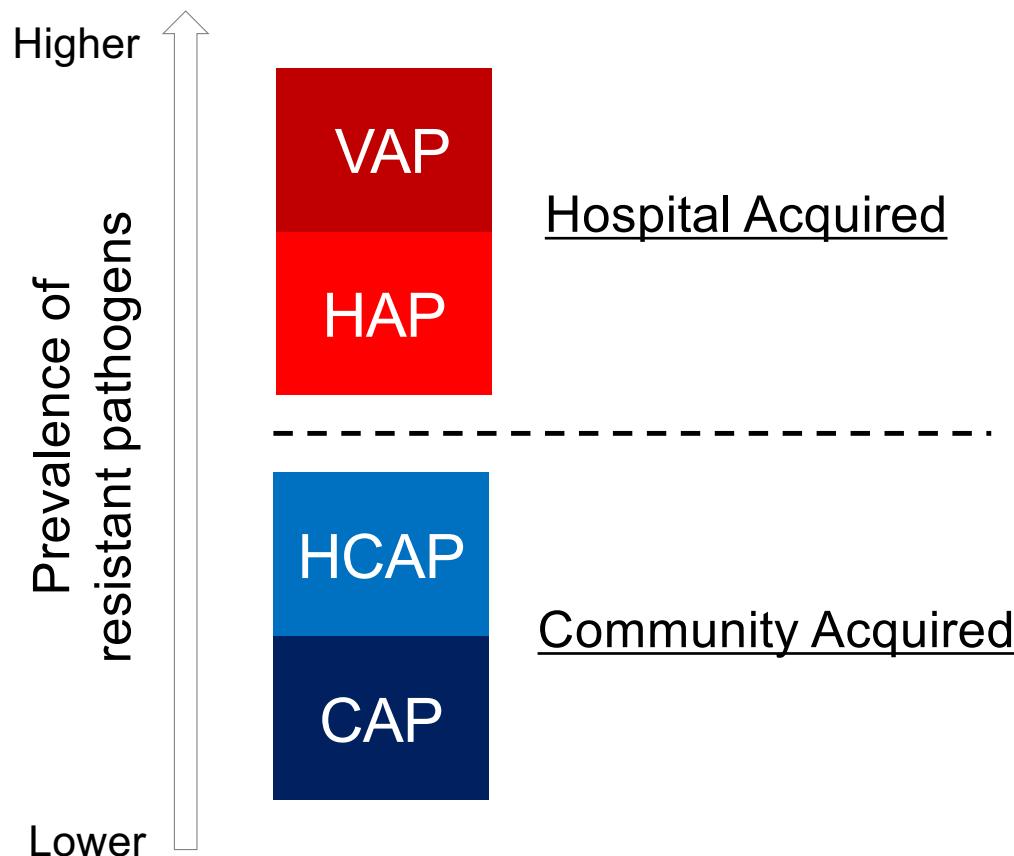
ICU Respiratory Resistome

# Epidemiolocal classification of pneumonia

Epidemiolocal classification of pneumonia

Kalil et al. *Clinical Infectious Diseases.* 2016.

AMR gene burden differs based on epidemiological classification of pneumonia

Airway β-lactamase genes in acute trauma patients at time of ICU admission vs 48 hours later

n = 15

Jen Mulliken, MD

Resistome of a hospital room

ESBL and carbapenemase genes

β-lactamase genes

OXA-23_Bla

AIM-1_Bla ⟵

OXA-211_Bla

OXA-2_Bla

OXA-237_Bla

TEM-1D_Bla

IND-1_Bla ⟵

OXA-50_Bla ⟵

CTX-M-1_Bla ⟵

BlaZ_Bla

CfxA_Bla

CME-1_Bla ⟵

OCH-2_Bla ⟵

GOB-16_Bla ⟵

CEPH-AQU_Bla

CMY-MOX_Bla ⟵
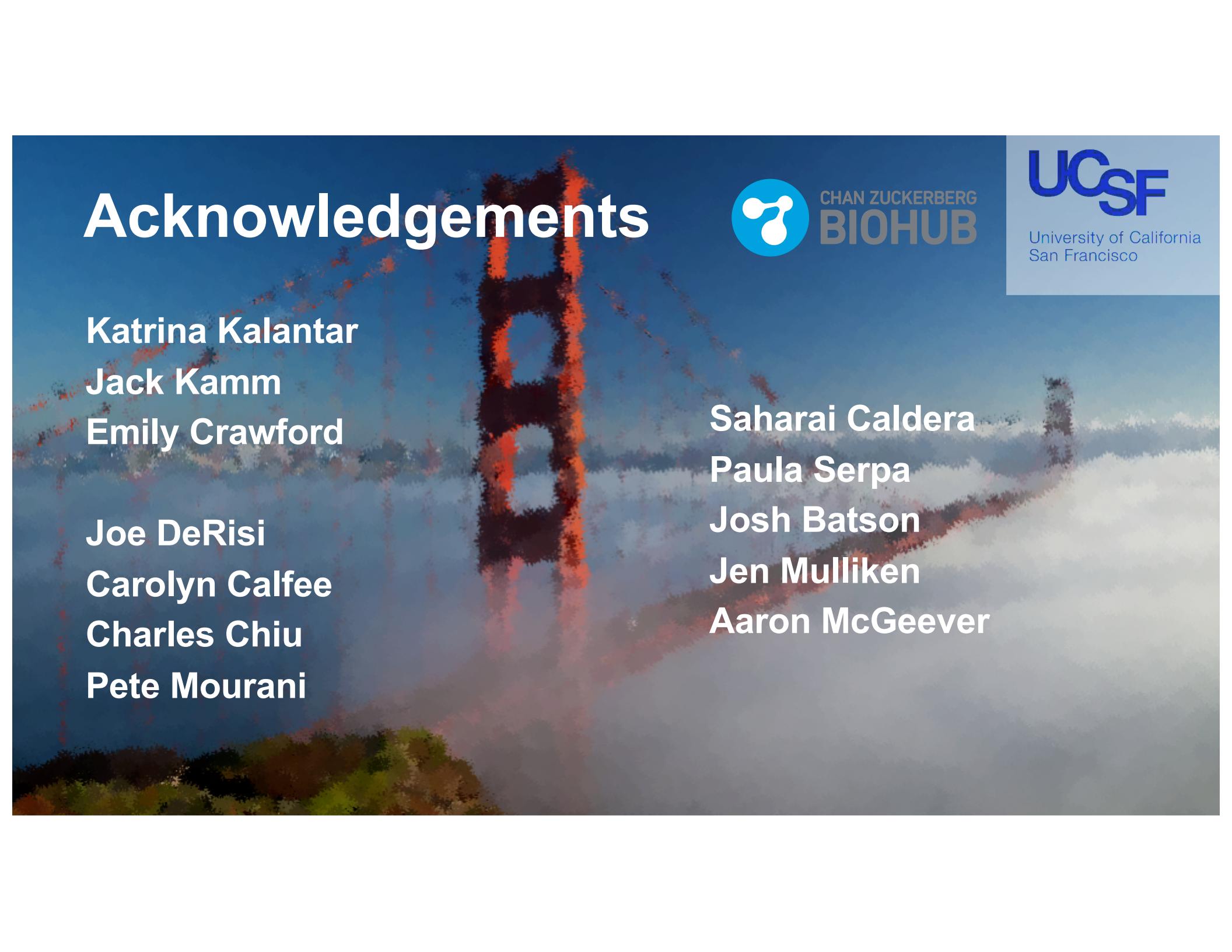
OXA-60_Bla ⟵

Future mNGS AMR Directions

- **Hospital resistome surveillance**

- **Dynamics of microbiome and AMR genes based on daily antibiotic exposure**

  - Children with acute respiratory illnesses
  - Lung transplant recipients

- **mNGS-FLASH for sepsis pathogen diagnosis**

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