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EMJ Microbiology & Infectious Diseases

Review of ECCMID 2023

Editor's Pick

Antibiotic Stewardship Attitudes and Beliefs Among Frontline Staff Nurses: Impact of Virtual Education

Interviews

Anne Wyllie, Louise Dyson, and Radhika Polisetty share insights from their careers and research

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Contents

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Aims and Scope

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Welcome letter

Evgenia Koutsouki
Editor

We are delighted to bring you this issue of *EMJ Microbiology & Infectious Diseases,* covering the European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), which this year took place in Copenhagen, Denmark. ECCMID was a hub of activity and ideas, and our team enjoyed having fruitful conversations with experts in the field. We are delighted to bring you some of the highlights from the congress in this issue.

Antimicrobial resistance was a recurring theme in this year's congress and we have chosen to cover this with a feature discussing antimicrobial resistance in special populations. We also had the opportunity to speak with Anne Wyllie, the winner for the 2023 Young Investigator Award in Clinical Microbiology and Infectious Diseases, at ECCMID and we are proud to feature an interview with her, highlighting her work into using saliva as a sample for COVID-19 detection, alongside interviews with other experts in the field.

Our Editor's Pick focuses on antibiotic stewardship attitudes and beliefs among frontline staff nurses, highlighting the barriers to nursing staff involvement and potential solutions to overcome these barriers.

I would like to extend my gratitude to the EMJ team and to our Editorial Board, interviewees, authors, and peer reviewers who have worked hard to bring together this collection of engaging content. I hope you enjoy reading through our articles. Look out later this year for our coverage of IDWeek, which will take place in Boston, USA.

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Foreword

Dear Colleagues,

I am delighted to welcome you to the latest issue of *EMJ Microbiology and Infectious Diseases*. You will find a variety of peer-reviewed articles covering pertinent topics within the field, plus content featured at the 33rd European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), which took place both online and in-person in Copenhagen, Denmark.

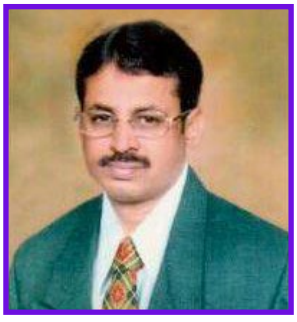
My Editor's Pick is 'Antibiotic Stewardship Attitudes and Beliefs Among Frontline Staff Nurses: Impact of Virtual Education'. Antimicrobial stewardship is vital in an era of multi-drug resistant bacteria, as highlighted by several ECCMID 2023 sessions. This timely research article explores the attitudes of nursing staff towards antimicrobial stewardship and evaluates the impact of virtual education methods in enhancing understanding and participation in antimicrobial stewardship. Such research is necessary to understand antimicrobial stewardship knowledge gaps, raise awareness, and aid development of strategies to help address this challenge.

There is ongoing need for infectious disease research. The residual impact of the COVID-19 pandemic still leaves its mark, and there are continued threats from other infectious diseases. An insightful research article into the epidemiological features of severe acute respiratory syndrome coronavirus 2 variants in Pieria, Greece, is also included in this issue, alongside an interesting article discussing the first *Francisella novicida* case in Argentina and the challenges associated with diagnosis.

An enlightening interview with Louise Dyson, The Zeeman Institute for Systems Biology and Infectious Disease Epidemiology Research, School of Life Sciences and Mathematics Institute, University of Warwick, Coventry, UK, is also included. It covers systematic non-adherence to mass drug administration, neglected tropical diseases, and the UK's response to the COVID-19 pandemic.

For those who were unable to attend, you can also find a review of highlights from ECCMID 2023. This congress review covers late-breaking news, research abstracts, and topical features of key sessions.

I would like to thank all those who have contributed to the successful creation of this edition of *EMJ Microbiology and Infectious Diseases*. I hope you enjoy reading the journal and take away valuable insights for your daily practice.



Rajeshwar Reddy Kasarla

Professor and Head, Microbiology Department, Universal College of Medical Sciences, Bhairahawa, Nepal

ECCMID 2023

Review of the 33rd European Congress of Clinical Microbiology and Infectious Diseases (ECCMID) 2023

Location:	Copenhagen, Denmark
Date:	15th–18th April 2023
Citation:	EMJ Microbiol Infect Dis. 2023; DOI/10.33590/emjmicrobiolinfectdis/10305560. https://doi.org/10.33590/emjmicrobiolinfectdis/10305560.

This year, the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) celebrated their 40th birthday by bringing together over 15,000 experts in Copenhagen, Denmark, for their 33rd European Congress of Clinical Microbiology and Infectious Diseases (ECCMID).

At the opening ceremony, Annelies Zinkernagel, ESCMID president, looked back on how the field of microbiology and infectious diseases has changed since the start of the society. While 40 years ago, mortality from infectious diseases was decreasing thanks to sanitation, water, antibiotics, vaccinations, and the public health system, mortality started to increase again in the 1980s. Since then, the HIV epidemic has taken its toll, the antimicrobial resistance pandemic has continued to grow, and diseases such as polio, Ebola, zika, and most recently mpox have emerged and re-emerged. Additionally, the COVID-19 pandemic has changed the world, bringing a shift in public awareness and perception of infectious threats. This has given ESCMID a great opportunity and responsibility, which is why a plan was created to tackle this. First, by leading the way in guiding the practice, education, and training of specialists in clinical microbiology and infectious diseases globally; second, by preparing for and rapidly responding to emerging infections; and, finally, by driving forward the response to antimicrobial resistance.

"To lead the fight against infections, we must think and act globally," stated Zinkernagel. Due to globalisaton, antimicrobial resistance and emerging infections can now spread around the world, and a global approach is crucial to fight them. While ESCMID is a European organisation, their mission is to be a diverse and inclusive society, to fight infections irrespective of continental borders, and to empower their experts through state-of-the-art knowledge, education, guidelines, and training.

ECCMID is based around three core strategic pillars: scientific content, education and professional development, and experience and engagement. Going forward, the society pledges to achieve these by maintaining a hybrid approach to the congress and continuing to improve it; releasing year-round content; enhancing scientific topics of interest; highlighting ESCMID strategic priorities; engaging under-represented groups and developing hands-on and immersive experiences; and personalising the ECCMID experience.

"To lead the fight against infections, we must think and act globally."

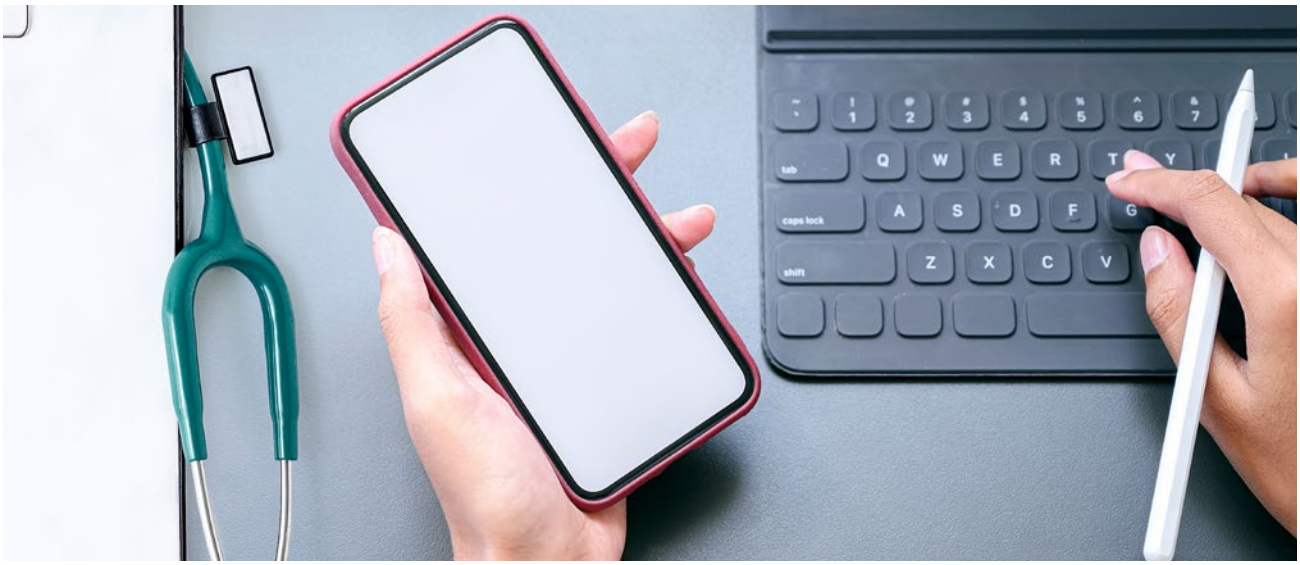
This year's programme was dominated by topics such as viral/bacterial infection and disease, as well as new antibacterial agents, with COVID-19 taking a step back. In total, over 6,000 abstracts were submitted, with the top categories covering a range of subjects, including severe sepsis, bacteraemia, and endocarditis; hospital epidemiology, transmission, surveillance, and screening; and molecular diagnostics. With 148 sessions and 54 integrated symposia, the congress brought together 725 speakers and chairs from 67 different countries.

The opening ceremony concluded with the presentation of multiple awards. First, The ESCMID Young Investigator Award in Clinical Microbiology was awarded to Anne Wyllie, Yale School of Medicine, New Haven, Connecticut, USA, for their work on saliva as a reliable sample type for sustainable surveillance and outbreak response efforts; and Oliver Van Hecke, University of Oxford, UK, for their work entitled 'Smooth seas do not make skilful sailors: the challenges and opportunities of antimicrobial stewardship in South African primary healthcare'.

Two ESCMID Young Investigator Awards in Infectious Diseases were also awarded to Belén Gutiérrez-Gutiérrez, Universidad de Sevilla, Spain, for their research on personalised medicine in infections caused by multidrug-resistant Gram-negative bacteria; and Jacob Bodilsen, Aalborg University, Denmark, for their research titled 'Head over heels: how I fell in love with CNS infections'. Finally, the ESCMID Excellence Award in Science was presented to Gunnar Kahlmeter, European Committee on Antimicrobial Susceptibility Testing (EUCAST) Development Laboratory Växjö, Sweden.

The team was delighted to be a part of this congress and are looking forward to the next congress, which will be held 27th–30th April 2024 in Barcelona, Spain. This *EMJ Microbiology & Infectious Diseases* issue includes summaries of the most pertinent ECCMID press releases and abstracts presented at the congress, as well as an interview with ESCMID Young Investigator Award recipient Anne Wyllie. Read on for more insights from this year's congress.

"This year's programme was dominated by topics such as viral/bacterial infection and disease, as well as new antibacterial agents."



Virtual Ward Safe to Treat Patients with Mpox

VIRTUAL wards can be used to safely treat patients with mpox, formerly known as monkeypox, eliminating the need for admission to a hospital, according to data presented at ECCMID 2023. The viral infection that had previously circulated in animals in West and Central Africa has led to a global outbreak in 2022. While mpox was classed as a high consequence infectious disease, involving a need for admission in a specialised unit, this classification was based on case fatality data from Africa, transmissibility, and the absence of vaccines or effective treatment. As the number of cases has grown in London, UK, it has become clear that mortality rates are lower than previously reported. Furthermore, the rapidly increasing number of cases has overwhelmed specialist units.

The Hospital for Tropical Diseases and Central and North West London NHS Foundation Trust, UK, has created a virtual ward, allowing patients with mpox to be treated at home. Care involved regular assessments by phone, including a review of symptoms, mental wellbeing, and isolation circumstances, as well as monitoring of changes in rash through photographs. The patients could contact their caregivers via a dedicated advice line and prescription medication was delivered to their home.

Emily Shaw, Hospital for Tropical Diseases, University College London Hospitals NHS Foundation Trust, UK, evaluated case notes of 221 patients diagnosed with mpox between May and August 2022. In total, 191 were managed as outpatients in a virtual ward, of whom 60 received treatments for their symptoms and painkillers, and 35 received antibiotics following infections occurring as a complication of mpox. Admission was needed for 30 patients, most commonly for soft tissue infections requiring intravenous antibiotic therapy. Admissions were generally short, and most patients completed the rest of their treatment on a virtual ward. The median time spent on a virtual ward was 10 days, and telephone assessment and photographs were used to determine when patients could be discharged from the virtual ward.

It is estimated that the virtual ward saved 2,100 hospital bed days, equating to a cost saving of approximately 1.05 million GBP. Shaw concluded: "We demonstrate that a virtual ward can be rapidly established to respond to emerging health threats and the majority of individuals with mpox can be safely managed virtually."

"Care involved regular assessments by phone, including a review of symptoms, mental wellbeing, and isolation circumstances."

Effectiveness of Mask-Wearing Questionable Against COVID-19 Transmission

SURGICAL masks have been integral to the infection control measures implemented globally to combat coronavirus transmission. A study conducted in a London, UK, hospital during the first 10 months of Omicron activity has brought forward interesting evidence that questions how effective this was; this research was presented at ECCMID 2023 in Copenhagen, Denmark, between 15th–18th April.

Investigating the risk-benefit of mask-wearing, as the severity of infection with COVID-19 decreased with time, researchers of St George's Hospital NHS Foundation Trust, UK, collected data over a 40-week period from 4th December 2021 to 10th September 2022. This period analysed the phase where the Omicron variant was dominant and presents data from before and after the UK National Health Service (NHS) lifted the mask mandate for all staff and visitors. During the first phase, from 4th December 2021 to 1st June 2022, all staff and visitors were required to wear masks in all areas of the hospital. Then in Phase II, from 2nd June 2022 to 10th September 2022, this policy was removed (except for some high-risk intensive care wards). Hospital severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection rate was adjusted by underlying community infection rate identified by routine admission screening.

The analysis discovered that the community surge in infections in June 2022, after the removal of the mask policy, was not associated with a statistically significant change in rate of hospital-acquired SARS-CoV-2 infection in the study group. The infection rate was no higher than the rate when masks were obligatory. There was also no delayed effect observed in Weeks 26–40 of the study period. The same was found in a control group, who continued to wear masks, experiencing no immediate or delayed change in infection rates.

The lead author, Ben Patterson, St George's University Hospitals NHS Foundation Trust, stated: "Our study found no evidence that mandatory masking of staff impacts the rate of hospital SARS-CoV-2 infection with the omicron variant." Patterson went on to add the disclaimer: "That does not mean masks are worthless against Omicron, but their real-world benefit in isolation appears to be, at best, modest in a healthcare setting." Limitations were acknowledged: the observational design of this study prevented the researchers from proving causation. Additionally, staff adherence to the mask-wearing policy was not assessed and staff infection rates were not determined.

Senior author, Aodhan Breathnach, St George's University Hospitals NHS Foundation Trust, highlighted the usefulness of these findings moving forwards: "We hope this empirical evidence can help inform a rational and proportionate mask policy in health services." These results will certainly influence hospitals and national governing bodies moving forwards, as they put together and enforce their policies for protective equipment in this new phase of the COVID-19 pandemic.

"We hope this empirical evidence can help inform a rational and proportionate mask policy in health services."

Metagenomic Sequencing Betters Conventional Tests to Detect Antimicrobial Resistance

ANTIMICROBIAL resistance is a significant challenge when treating bloodstream infections. Bloodstream infections can rapidly lead to sepsis, multiple organ failure, and even death. Hence, the early and appropriate antibiotic therapy is essential for managing the infection. In clinical settings, the current method used to identify the pathogen causing the infection is time-consuming and laborious. Contrastingly, clinical metagenomics sequences all the genetic material, including infectious pathogens in a sample all at once; therefore, this would reduce time spent running tests, waiting for results, and running more tests.

At this year's ECCMID annual meeting, study lead, Kumeren Govender, John Radcliffe Hospital, University of Oxford, UK, revealed that metagenomic sequencing can generate fast and actionable antimicrobial resistance predictions to treat bloodstream infections much faster than conventional laboratory tests, highlighting the potential to save lives and better manage antibiotic usage. The researchers randomly selected 210 positive and 61 negative blood culture specimens for metagenomic sequencing from the Oxford University Hospital's (OUH) microbiology laboratory between December 2020 and October 2022, the Oxford Nanopore GridION platform was used to sequence the DNA. They used sequences to identify the species of pathogen causing infections, and to spot common species that can contaminate blood cultures.

Sequencing identified 99% of infecting pathogens, including polymicrobial infections and contaminants, and gave negative results in 100% of culture negative samples. In some cases, sequencing identified probable causes of infection missed by routine cultures, and in some other instances detected uncultivable species where a result could not be ascertained. Sequencing could be utilised to detect antibiotic resistance in ten of the most common causes of infections. A total of 741 resistant and 4,047 sensitive combinations of antibiotics and pathogens were studied. The results of traditional culture-based testing and sequencing agreed 92% of the time, and similar performance could be obtained from raw reads after only two hours of sequencing; overall agreement was 90%.

The authors stated this is an exciting breakthrough as it can diagnose the cause of patients' infections faster and more completely than has been possible before. The researchers are attempting to overcome the remaining barriers to metagenomic sequencing being used more widely, which includes the high cost, improving accuracy, and creating improved laboratory expertise in these new technologies and simpler workflows for interpreting results.

"Metagenomic sequencing can generate fast and actionable antimicrobial resistance predictions."

Omicron May Be More Deadly than Seasonal Influenza

BREAKING research presented at ECCMID 2023 in Copenhagen, Denmark, suggests the Omicron variant of severe acute respiratory syndrome coronavirus 2 is associated with a higher death rate than those hospitalised with seasonal influenza. Despite previous research suggesting that Omicron is less virulent than the Delta and alpha strains, Alaa Atamna and colleagues, Rabin Medical Center, Belinison Hospital, Israel, found that adults hospitalised with influenza were 55% less likely to die within 30 days than those hospitalised with Omicron during the 2021–2022 influenza season.

In December 2021, influenza re-emerged in Israel after being undetected since March 2020. Concurrently, Omicron had been established as the predominant COVID-19 variant, substituting Delta. Therefore, the research team sought to investigate clinical outcomes in patients hospitalised with the Omicron variant and those hospitalised with influenza. Patients hospitalised with laboratory confirmed COVID-19 (167 patients; average age: 71 years; 58% male) and influenza infection (221 patients; average age: 65 years; 41% male) between December 2021 and January 2022 were included in the study.

Within 30 days, 63 patients died. Of these, 19 (9%) had been admitted with influenza while 44 (26%) had been admitted with Omicron. Furthermore, patients with Omicron tended to have higher overall comorbidity scores, were more likely to have high blood pressure and diabetes, and were more likely to require mechanical ventilation and more assistance with activities and daily tasks such as washing and dressing. However, asthma was more common in those hospitalised with influenza.

Atamna, Rabin Medical Center, Belinison Hospital, commented: "A possible reason for the higher Omicron death rate is that patients admitted with Omicron were older with additional major underlying illnesses such as diabetes and chronic kidney disease." They added that "the difference might also be due to an exaggerated immune response in COVID-19, and that vaccination against COVID-19 was far lower among patients with Omicron." Atamna summarised that there is one basic step people can take to alter the trajectory of both the influenza and COVID-19 pandemics: getting vaccinated, especially if you are older and have underlying illnesses. ●

"Adults hospitalised with influenza were 55% less likely to die within 30 days than those hospitalised with Omicron."

Dutch Study Suggests that Influenza Can Trigger Heart Attacks

INFLUENZA and heart attacks have previously been linked in a 2018 Canadian study investigating individuals hospitalised for heart attacks. However, the Canadian study did not incorporate information from death records; hence, out-of-hospitals deaths from heart attacks were not included.

At ECCMID 2023 in Copenhagen, Denmark, Athenarijn de Boer, University Medical Center (UMC) Utrecht, the Netherlands, presented a study revealing that individuals who are diagnosed with influenza are six times more likely to have a heart attack in the week after they test positive for the influenza virus than they are in the year before or afterwards. De Boer and colleagues used test results from 16 laboratories across the Netherlands, covering approximately 40% of the population, along with death and hospital records, to provide a more comprehensive understanding.

The researchers revealed that, between 2008 and 2019, 26,221 cases of influenza were confirmed by the laboratories, where 401 individuals had at least one heart attack within 1 year of their influenza diagnosis. Out of the total 419 heart attacks, 25 were within the first 7 days of flu diagnosis, 217 were in the year before diagnosis, and 177 were in the year after influenza diagnosis but did not have a heart attack in the first 7 days. Within a year of being diagnosed with influenza, 139 out of the 401 individuals died of any cause.

The study population were 6.16 times more likely to have a heart attack in the 7 days following an influenza diagnosis than in the year before or after. The Canadian study had a figure of 6.05. However, when excluding data from death records, as in the Canadian study, the increase in heart attack in the first week reduced to 2.42 times, thereby demonstrating the impact that incomplete data can have on results.

> **"Out of the total 419 heart attacks, 25 were within the first 7 days of flu diagnosis."**

The differences in testing practices between the two countries may explain the weaker association found in the Canadian study, as testing for influenza in out-of-hospital settings is less common in the Netherlands than in Canada. Nonetheless, the association is still prominent, and by utilising similar methodology to the Canadian authors, the researchers have been able to corroborate that the increased risk applies across different populations. Additionally, the findings highlight the significance of vaccination, as well as awareness of heart attack symptoms among flu patients and those treating them. ●

Finding New Approaches to Treat Amyotrophic Lateral Sclerosis

FAECAL microbiota transplantation could be used as to alter gut microbiota in patients with amyotrophic lateral sclerosis (ALS), according to research presented at the ECCMID 2023 annual meeting.

ALS is the most common motor neurone disease, where motor neurones in the spinal cord and brain degenerate, leading to paralysis, physical disability, and death. It is difficult to treat because it is inherited in 5–10% of cases but 'sporadic' in 90%, where the cause is unknown.

Gut microbiota composition could be linked to many neurological disorders through the gut–brain axis, with specific microbiota activating pro-inflammatory pathways after losing T cell numbers and suppressing function. This could have therapeutic benefits to patients with ALS.

Researchers allocated patients with ALS who had symptoms for more than 18 months into faecal microbiota transplantation (n=28) or placebo (n=14) groups in a randomised trial. Patients will be infused with gut microbes at the start of the study and Month 6. Stool, saliva, and blood samples will be collected on procedure days to investigate how the transplant affects gut microbiota, immune cells, and inflammatory status.

Researchers will also take three intestinal biopsies from both groups: at the start of the study, at 6 months, and at 12 months. The primary outcome is a significant change in T cell numbers between the groups at 6 months.

> **"Anyone can develop ALS, regardless of race or socioeconomic background."**

The profile of gut microbiome in six patients at the start of the study showed a much higher relative abundance of *Proteobacteria*. This can activate the immune system, alerting the body to illness and triggering the release of molecules that cause inflammation.

Anyone can develop ALS, regardless of race or socioeconomic background. Author Luca Masucci, Catholic University of the Sacred Heart, Rome, Italy, stated: "With this information, we could potentially provide new approaches for treatments by altering or interfering with these inflammatory pathways. We hope to have all our data from this trial to analyse in 2024." ●

COVID-19 Vaccines Saved Over 1 Million Lives Since the End of 2020

NOVEL research presented at this year's ECCMID annual meeting shared evidence that COVID-19 vaccination directly saved at least 1,004,927 lives from December 2020 to March 2023. Of the lives saved, 95% were in adults aged 60 and older. The new estimates come from the World Health Organization (WHO) and Margaux Meslé, Epidemiologist, WHO, who underlined the huge impact of these vaccines and the need for countries with lower vaccination rates to focus on vaccinating older populations.

Following the emergence of severe acute respiratory syndrome coronavirus 2 in early 2020, countries in the WHO European Region introduced COVID-19 vaccine programmes to protect populations from the disease, especially focusing on the severe impact on vulnerable groups. Researchers analysed the weekly reported deaths alongside the reported number of doses in 26 European countries between December 2020 and March 2023.

The results of this analysis showed that most lives saved were people aged 60 and older (up to 96%); these groups were identified during the pandemic as being most vulnerable to severe disease. The results also demonstrated that the largest number of lives were saved during the Omicron wave when at least 568,064 deaths were prevented. This equates to over half of all the deaths prevented by COVID-19 vaccination. "We see from our research, the large numbers of lives saved by COVID-19 vaccines across Europe during the pandemic," stated Richard Pebody, Head of High Threat Pathogen Team, WHO. "However, too many people in vulnerable groups across the WHO European Region remain unvaccinated or partially vaccinated. We urge people who are eligible and who have not yet taken the vaccine to do so." ●

"The largest number of lives were saved during the Omicron wave when at least 568,064 deaths were prevented."

Study Reveals How Gut Microbiota Changes in Infants

AN ITALIAN study has revealed how gut microbiota alters in the first few months of an infant's life. Researchers at the Universities of Genoa and Florence, Italy, and the San Jacopo Hospital, Pistoia, Italy, tracked changes in the gut microbiota in the first 3 years of life. They released the primary data covering the first 0–3 months of life at the 2023 ECCMID annual meeting in Copenhagen, Denmark.

Primary research from the CI.EMME study demonstrates that from birth, the intestinal tract becomes colonised by many species of bacteria, protozoa, fungi, and viruses. Collectively, these are known as the gut microbiota. In an infant, this microbiota grows and alters in the first few months of life. If the gut microbiota is disrupted from normal growth, it is more likely that the individual will develop health conditions in future, such as Type 1 diabetes, inflammatory bowel disease, and asthma.

Researchers examined stool samples collected from 165 infants at delivery (T0); following hospital discharge (T1, within 2–3 days of birth), or at later stages in those who required intensive care (T2); and at 3 months of age (T3). These samples were stored until processing. In total, 495 samples were collected; of these, 370 were processed and analysed (T0=71; T1=136; T2=13; T3=150). Using genetic profiling, researchers were able to detect a greater number of bacterial species in the T0 cohort than in the T1/T2 or T3 cohort. This suggests that the gut microbiome evolves rapidly in the first 3 months of life.

Marked changes over time were detected in some species of bacteria, including *Lactobacillaceae* (T0), *Staphylococcaceae* (T1), and *Bifidobacteriaceae* (T3). A higher proportion of infants born via caesarean section than vaginal delivery had *Bifidobacteriaceae* in their stool samples at Stage T0 and T3. Other factors were examined, such as breastfeeding and weight at birth, but the time of stool sample collection had the biggest link to bacterial diversity (T0, T1, and T3).

Lead study author Vincenzo Di Pilato, University of Genoa, stated the importance of this research: "Given that the development of the gut microbiota is fundamental to health later in life, it is vital to learn all we can about how this collection of microbes matures." Di Pilato went on to conclude: "A better knowledge of how the gut microbiota develops from being nearly sterile at birth towards a diverse healthy ecosystem later in life would us to identify unhealthy, or dysbiotic, microbiota. We might then be able to 'correct' the bacterial imbalance, and so increase the odds of good health later in life." ●

"From birth, the intestinal tract becomes colonised by many species of bacteria, protozoa, fungi, and viruses."

Present and Future Considerations for Sepsis Management

Authors: Jivitesh Newoor, Editorial Assistant

Citation: EMJ Microbiol Infect Dis. 2023; DOI/10.33590/emjmicrobiolinfectdis/10300722. https://doi.org/10.33590/emjmicrobiolinfectdis/10300722.

IN A HIGHLY interesting session on sepsis management during the 33rd annual European Congress of Clinical Microbiology and Infectious Diseases (ECCMID) congress, held in Copenhagen, Denmark, between 15th–18th April, speakers discussed the definition of sepsis, as well as the present and future considerations for sepsis management. The session was co-chaired by Thierry Calandra, Lausanne University Hospital, Switzerland, and Willem Joost Wiersinga, Division of Infectious Diseases of the Amsterdam University Medical Centers (UMC), University of Amsterdam, the Netherlands.

Calandra introduced the International Sepsis Forum (ISF), a not-for-profit organisation, with a mission to reduce the global burden of sepsis and improve the care of patients with sepsis. Calandra presented the joint ISF-ECCMID Sepsis Award to Claire Dahyot-Fizelier, University of Poitiers, France, who went on to present their session on the use of ceftriaxone to prevent early ventilator-acquired pneumonia (VAP) in patients with brain injuries and are comatose.

PREVENTING EARLY VENTILATOR-ACQUIRED PNEUMONIA

Patients with brain injuries in intensive care units (ICU) are particularly vulnerable to VAP, yet very little research has been completed. Prior to 2013, only one trial reported the beneficial effect of antibiotic-prophylaxis after tracheal intubation.

A randomised double blind, placebo-controlled clinical study conducted by Dahyot-Fizelier and colleagues aimed to assess the efficacy of a single dose of ceftriaxone (2 g) in preventing early VAP. The trial included patients from eight centres in the 'AtlanRea' research network. The secondary goals were to measure the incidence of all-VAP and type of bacteria, antibiotic exposition, mechanical ventilation exposition, ICU and hospital stay, neurological prognosis, and mortality.

A total of 345 patients were randomised into two groups to receive ceftriaxone or placebo 12 hours after tracheal intubation, where the primary outcome was the proportion of patients developing an early-VAP, with a cut-off period of 7 days.

> **"The complexity of sepsis cannot be fully understood by single-timepoint and reductionist studies."**

Patients who received ceftriaxone displayed a decreased risk of developing early-VAP, from 33% to 14%. The same trend was observed for the secondary outcomes. At Day 15, the median ICU free-days and median hospital-free days were higher in the ceftriaxone group, and no safety complications or difference in resistance acquisition were observed within these groups. Dahyot-Fizelier concluded by stating that a single dose of ceftriaxone protects patients with brain injuries from early-VAP, antibiotic and mechanical ventilation exposition, and mortality (at Day 28), as well as ICU and hospital exposition (at Day 60).

PROGNOSTIC AND PREDICTIVE ENRICHMENT IN SEPSIS

Tom van der Poll, Department of Medicine, Amsterdam UMC, University of Amsterdam, the Netherlands, emphasised that precision medicine for sepsis is only in its infancy. They discussed the introduction of prognostic and predictive enrichment in sepsis, where prognostic enrichment refers to the selection and classification of patients with high or low risk of mortality. Van der Poll recommended that patients with a good prognosis (i.e., a low mortality risk) should be treated with standard of care, whereas predictive enrichment should be applied to predict which patients may benefit from certain interventions.

Van der Poll discussed the SCARLET trial, which evaluated the effect of soluble thrombomodulin, an anticoagulant protein, in patients with sepsis. The researchers attempted to enrich the population in a prognostic and a predictive manner. For prognostic enrichment, they selected patients based on cardiovascular and/or respiratory failure; however, for predictive enrichment, they solely selected patients with coagulopathy. Van der Poll discussed various clinical trials that have utilised proteomic analysis to aid informed treatment decisions. The speaker reviewed the need to identify patients who may benefit from soluble thrombomodulin treatment and emphasised the need for the integration of real-time spatial-dynamic information on the host response linked to clinical decision-making tools.

Van der Poll concluded by acknowledging that sepsis is highly complex, non-linear, and spatially dynamic system. The complexity of sepsis cannot be fully understood by single-timepoint and reductionist studies, hence the focus should be on longitudinal and continuous biological data collection. They stated that the complexity of sepsis will require a huge multidisciplinary effort, wherein computational approaches derived from complex systems science must be integrated with biological data.

IMPROVING SEPSIS DIAGNOSIS

Brigitte Lamy, Nice University Hospital, France, highlighted that patients with sepsis are a highly heterogenous population, thereby increasing the demand for personalised medicine. A higher rate of mortality was observed when the time to receive the appropriate antimicrobial treatment was more than 12 hours; hence, fast diagnosis is necessary to avoid these complications.

Lamy listed the various characteristics of an ideal diagnostic test, such as an accurate, rapid, inexpensive test that can be performed directly on (blood) samples and available at a point of care (<30 minutes). The ideal test would also remain unaffected by antimicrobial therapy, be able to differentiate contaminants from pathogens, and would permit informed decision making regarding antibiotic choices. Lamy offered recommendations to tackle the issue of high heterogeneity in patients by using biomarkers, artificial intelligence, and microbiological findings, as well as the prospect of using a single biomarker to accurately identify patients with sepsis. They acknowledged that new biomarkers are currently being investigated, such as circulating microRNA, as well as endothelial-related biomarkers aimed at indicating severity and predicting sepsis incidence.

Lamy concluded by stating that the cost-effectiveness of these rapid methods is still unclear; emerging and promising technologies are rapidly becoming available, but it is too early to have firm evidence on whether they can help with sepsis diagnosis. Hence, there is a need for better diagnostics permitting for the rapid identification of pathogens and characterisation of the host response, well-designed clinical trials with enrichment strategies to better manage patient heterogeneity, and biomarker tests for sepsis.

CONCLUDING REMARKS

The session went on to discuss sepsis management in low- and middle-income countries, as well as implementing biomarker-driven immunotherapy. Flavia Machado, Federal University of São Paulo, Brazil, highlighted the importance of understanding challenges, increasing awareness, prevention, and survivorship, as well as improving recognition, treatment, and research capacity.

Evangelos Giamarellos-Bourboulis, National and Kapodistrian University of Athens, Greece, underscored the main challenge of patient heterogeneity and emphasised the need for biomarker-guided therapeutics. Giamarellos-Bourboulis discussed the need for biomarkers that are informative to a degree, whereby certain pathways that, for example, impact mortality can be directly targeted using therapies, thereby advocating for the treatment based on biomarkers, irrespective of physical and clinical signs.

A common theme throughout this session revolved around personalised medicine in sepsis management being a rapidly emerging and promising field, with substantial potential to improve patient outcomes. However, further randomised controlled trials are requisite to investigate the feasibility of utilising biomarkers for sepsis management. ●

"There is a need for better diagnostics permitting for the rapid identification of pathogens."

Antimicrobial Stewardship: Insights from Paediatrics, Intensive Care, Emergency Medicine, and Dental Practice

Authors: Darcy Richards, Editorial Assistant

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ANTIMICROBIAL stewardship (AMS) was a key focus at the 33rd European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), which took place both virtually and in-person in Copenhagen, Denmark, between the 15th–18th April 2023. This high priority topic was explored during the symposium entitled 'Antimicrobial stewardship in special populations'. In this thought-provoking session, experts shared perspectives on AMS in different patient groups and settings.

ANTIMICROBIAL STEWARDSHIP IN SPECIAL PATIENT POPULATIONS

With the ongoing threat of antimicrobial resistance, stewardship is of high importance, and the need for AMS strategies to be implemented at local, national, and international levels across all factions of healthcare is becoming increasingly evident. Resistance, combined with a paucity in novel antimicrobials, has seen a shift in focus to optimising antibiotic spectrum, dose, duration, and indication. The special populations discussed in the session were paediatric and dental patients, as well as patients in the intensive care unit (ICU) and emergency department (ED).

Antimicrobial use is often high in special patient populations, and strategies to reduce this is a key facet for AMS. Terhi Tapiainen, Head of Pediatric Infectious Disease, Oulu University Hospital, Finland, and University of Oulu, Finland, discussed paediatric AMS, noting that antimicrobial consumption is high amongst this population. This is largely secondary to respiratory infections, of which acute otitis media is the commonest indication. Child day-care centres are a source for microbial exposure in young children, and attendance increases acute otitis media risk two- to three-fold.

> **"Approximately 50% of ED antibiotic prescriptions are unnecessary or inappropriate."**

Jan De Waele, Department of Intensive Care Medicine, Ghent University Hospital, Belgium, discussed how antibiotic usage in critical care settings is also high. On an average day, 70% of patients in intensive care receive antibiotics. In 30–60% of these cases, antibiotics are inappropriate, unnecessary, or suboptimal, De Waele stated. In addition to this, Teske Schoffelen, Department of Internal Medicine, Division of Infectious Diseases and Radboud Center for Infectious Diseases, Radboud University Medical Center, Nijmegen, the Netherlands, stated that 10–20% of ED attendances are infection-related, and approximately 50% of ED antibiotic prescriptions are unnecessary or inappropriate. These statements highlight the need for AMS strategies and implementation in these settings.

Furthermore, Leanne Teoh, National Health and Medical Council Early Career Fellow, University of Melbourne, Australia, discussed AMS in dentistry. Oral diseases are the most common chronic health condition worldwide, and dental prescribing accounts for 10% of all global antimicrobial prescriptions. Teoh discussed how rates of prophylactic and therapeutic overprescribing can be as high as 80% in some countries, highlighting how dentistry can play a huge role in contributing to global AMS.

Antimicrobial course duration was identified as a target for improving AMS. De Waele discussed findings from the DIANA study,[1] which looked at AMS practices across the world. The results showed that local antimicrobial guidelines were only available in 65% of the 152 hospitals involved, and empirical use of broad-spectrum and combination antibiotic therapy was frequent, but de-escalation was only performed in 16% of cases. The study further found that average antibiotic course duration was approximately 10 days, which is longer than guideline recommendations.

Tapiainen discussed the impact of community-acquired infections on AMS in the paediatric population by presenting data from four randomised controlled trials that evaluated antibiotic treatment duration for community-acquired pneumonia. These showed that shorter treatment courses were as effective as longer courses. Tapiainen further added that following these results, Finland now recommends a 5-day antibiotic treatment course for community-acquired pneumonia in paediatric outpatient settings. However, trial data regarding antibiotic duration for acute otitis media is less clear.

Tailoring AMS strategies to different settings and patient cohorts was another theme of the session. De Waele discussed how the ICU is a "hotspot for multi-drug resistant pathogens," which will impact AMS strategies; and explored antimicrobial pharmacokinetics in critical illness, explaining how drug plasma concentrations are altered depending on patient factors, such as abnormal haemodynamics, organ dysfunction, or use of organ support devices. In light of this, De Waele concluded that ICU AMS programmes need to be tailored to the specific requirements of these patients, in whom there are additional factors that need to be considered.

Schoffelen discussed additional considerations for the ED setting, including the rationale for antibiotic prescription, appropriate cultures, and microbiological testing.

These should be performed to aid pathogen identification and antimicrobial sensitivity for de-escalation and directed therapy, given that empirical antibiotics started in ED are often carried on in the community, or by other hospital physicians. Schoffelen further added that these microbiological tests should be followed-up post-ED discharge to ensure that appropriate de-escalation takes place.

Schoffelen further explored whether blood cultures should be performed in the ED setting through data from a systematic literature review. Whilst reiterating that all patients presenting to ED with sepsis should have blood cultures taken, Schoffelen stated that blood cultures may not need to be routinely performed in patients who are not septic and present to the ED with community-acquired pneumonia, urinary tract infection with systemic symptoms, or skin and soft tissue infections, with the exception of special populations, such as those with immune compromise; a diagnosis of diabetes; extensive comorbidities; risk of infections caused by non-standard pathogens; and endovascular devices, pacemakers, or valvular prostheses. Schoffelen highlighted that this is a good practice statement rather than a recommendation, due to the low level of evidence in the literature review, and concluded that there is a need for future clinical trials to help strengthen the evidence on blood culture omission in selected patients.

Improved and rapid diagnostics were identified as an important consideration in the approach towards improving AMS in the future.

Schoffelen stated that rapid diagnostics and biomarkers will aid clinicians in making decisions on whether to commence or withhold antibiotics. Earlier identification of causative pathogens could lead to earlier de-escalation of empirical antibiotics and earlier commencement of targeted antimicrobial therapy, De Waele commented. This would reduce exposure to broad-spectrum antimicrobials, which is key to AMS strategies. Further to this, De Waele discussed that improved diagnostics with the ability to differentiate between infectious disease and infectious disease mimics could help reduce antibiotic consumption, leading to a reduced overall antibiotic exposure. This is not only beneficial from an AMS stance, but could also limit any potential patient harm from unnecessary antibiotic treatment.

CONCLUSION

Antimicrobial resistance is a major concern for not only those working in the field of microbiology and infectious diseases, but for healthcare professionals working across all specialties, and in the community. The speakers highlighted areas that need to be addressed in order to improve AMS across different clinical settings and patient cohorts. The session emphasised the need for these strategies to be adopted in clinical practice globally, and highlighted the challenges with AMS, as well as the considerations for tailoring approaches when developing AMS strategies for different populations. ●

"Improved and rapid diagnostics were identified as an important consideration in the approach towards improving AMS."

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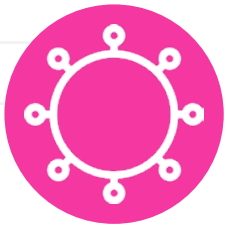
Clostridioides difficile Infection: Targeting an Unwelcome and Persistent Threat

This symposium review is based on an integrated session that took place at the 33rd European Congress of Clinical Microbiology and Infectious Diseases (ECCMID) 2023 on 17th April, held in Copenhagen, Denmark



Chairpeople:	Mark Wilcox,[1,2] Anne Gonzales-Luna[3]
Speakers:	Sarah Tschudin-Sutter,[4] John Coia,[5] Esther Calbo,[6] Benoît Guery[7]

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Meeting Summary

An integrated symposium, with an esteemed panel of experts, discussed the burden and treatment approaches for *Clostridioides difficile* infection (CDI). The session took place on 17th April 2023 as part of the 33rd European Congress of Clinical Microbiology and Infectious Diseases (ECCMID) 2023 in Copenhagen, Denmark.

The session was co-chaired by Mark Wilcox, Head of Microbiology Research & Development and Infection Lead at Leeds Teaching Hospitals NHS Trust, UK, and Professor of Medical Microbiology at the University of Leeds, UK; and Anne Gonzales-Luna, Assistant Professor at the University of Houston College of Pharmacy, Texas, USA.

Sarah Tschudin-Sutter, Professor and Head Division of Hospital Epidemiology at the University Hospital Basel, Switzerland, explored the changing landscape for CDI, including the epidemiology and surveillance of infection, and approaches to diagnosis.

John Coia, Professor Emeritus at the Institute of Regional Health Research, University of South Denmark, and Honorary Research Fellow at Glasgow University, UK; and Esther Calbo, Professor and Head of Infectious Disease at the Hospital Universitari Mútua de Terrassa, Spain, gave an overview of the burden of recurrent CDI, dysbiosis in CDI, and the role in recurrent CDI, mortality risk, and the importance of targeting recurrence.

Calbo also presented an interactive case study that allowed audience members to engage and review the patient case presented. The faculty panel explored with the audience how to approach assessment and treatment to achieve the best outcome for the patient case.

Benoît Guery, Associate Professor Infectious Disease at the Centre Hospitalier Universitaire Vaudois, Switzerland, presented data from the EXTEND study, which explored extended-pulse dosing regimens of antibiotics (fidaxomicin) in CDI.

Introduction

Mark Wilcox

Clostridioides difficile (formerly known as *Clostridium difficile* and commonly referred to as *C. difficile*) is a Gram-positive anaerobic bacterium that causes CDI.[1] It is a leading cause of healthcare-associated infections and is considered a global public health threat.[1]

Wilcox opened the symposium highlighting the epidemiological burden of CDI. In the USA, CDI is considered an urgent threat, with approximately 223,900 cases per year compared with approximately 124,000 CDI cases per year in Europe.[2,3] Wilcox believes that these figures

are underestimated due to attainment issues, and noted that *C. difficile* is associated with high mortality rates (3,700 deaths in Europe and 12,800 deaths in the USA each year).[2,3] Wilcox emphasised the burden of CDI, stating mortality within 28 days post-diagnosis rates are higher than the recognised burden and mortality associated with meningitis. Approximately 25% of patients treated for CDI may experience recurrence, which can be due to relapse with the same strain or reinfection with another *C. difficile* strain, and up to 65% of these patients may experience further recurrences.[4-6] Wilcox emphasised that *C. difficile* is "extremely good at finding weaknesses in a system." Notably, also, selecting an optimal treatment is critical to ensuring patients do not experience CDI recurrence.

The Changing Landscape of *Clostridioides difficile* Infection

Sarah Tschudin-Sutter

Tschudin-Sutter commenced with a comprehensive overview of the global CDI epidemiology.[7] In the USA, crude rates of healthcare-associated CDI have declined from 92.8 cases per 100,000 persons (2011) to 50.1 cases per 100,000 persons (2020), indicating the success of healthcare setting interventions.[8,9] However, community-associated infections remain stable at 51.2 cases per 100,000 persons (2020) compared to 48.2 cases per 100,000 persons in 2011.[8,9]

Recent data shows that *C. difficile* was identified as the most common pathogen in patients with acute infectious gastroenteritis (32.2%) in the USA outpatient setting.[10] In Europe, CDI ribotype (RT) distribution has changed. Toxinotype IIIb (027, 181, and 176) has declined in many European countries, but high prevalence rates were seen in Eastern European countries, the region with the lowest testing rate.[11] Tschudin-Sutter highlighted that community CDI cases are often "undetected due to the absence of clinical suspicion," accounting for three times more undiagnosed adults in the community compared with the hospital setting (approximately 111,000 compared with 37,000 cases per year in Europe, respectively).[11] Tschudin-Sutter then compared the global epidemiology associated with CDI, where data is "lacking" or underestimated in many regions, however, remains an important cause of diarrhoea, with differing distribution of RT and sequence types.[12-15]

Tschudin-Sutter noted the COVID-19 pandemic had a varied impact on CDI rates, with reports of both an increase and decrease in incidence. In the southeastern USA, CDI incidence increased during the pandemic period (March 2020–March 2021) by 4.2% per month (95% confidence interval [CI]: 1.7–6.8; p=0.001; pandemic trend change rate ratio: 1.04 [95% CI: 1.01–1.07]), particularly in smaller community hospitals, "possibly due to staffing and resource constraints," stated Tschudin-Sutter.[16] In contrast, the Netherlands reported a lower annual incidence of CDI during the pandemic period (2020), which may be due to lower testing rates, but a higher percentage of severe cases, especially in the second wave of the pandemic (September 2020–January 2021).[17] In severe cases, this increase was related to delayed community-onset CDI diagnosis (time to detection ≥8 days from start of symptoms).[17] In the UK, overall CDI rates have generally declined (2007–2022) due to the success of interventions.[18] However, a large increase in hospital- and community-onset CDI cases occurred during the COVID-19 pandemic (April 2021–March 2022), representing a 9-year high, and a 3-year consecutive increase.[18] Tschudin-Sutter emphasised the importance of returning to conventional infection prevention and control practices, and building resiliency in such programmes in light of this data.[19]

Tschudin-Sutter highlighted the importance of the 'One Health' concept in managing CDI. The identification of possible sources is important for the understanding of CDI epidemiology. A multinational European study found that 22.4% of retail potatoes tested positive and may serve as a vector for introducing *C. difficile* spores into households where, if ingested, they could multiply in sensitive hosts.[20] In terms of potential emerging *C. difficile* resistance, a study in Czechia found diverse *C. difficile* strains in waste- and surface-water samples, including a newly identified plasmid-mediated resistance to metronidazole, a drug used for the treatment of CDI.[21] There are also reports of potential reduced susceptibility to vancomycin reported in Africa, the Middle East, and the USA.[22–24] Tschudin-Sutter noted that

"ongoing clarification" is needed to determine whether this is an emerging problem.

The Patient With Recurrent *Clostridioides difficile* Infection: Understanding Risk Factors and the Role of the Microbiome

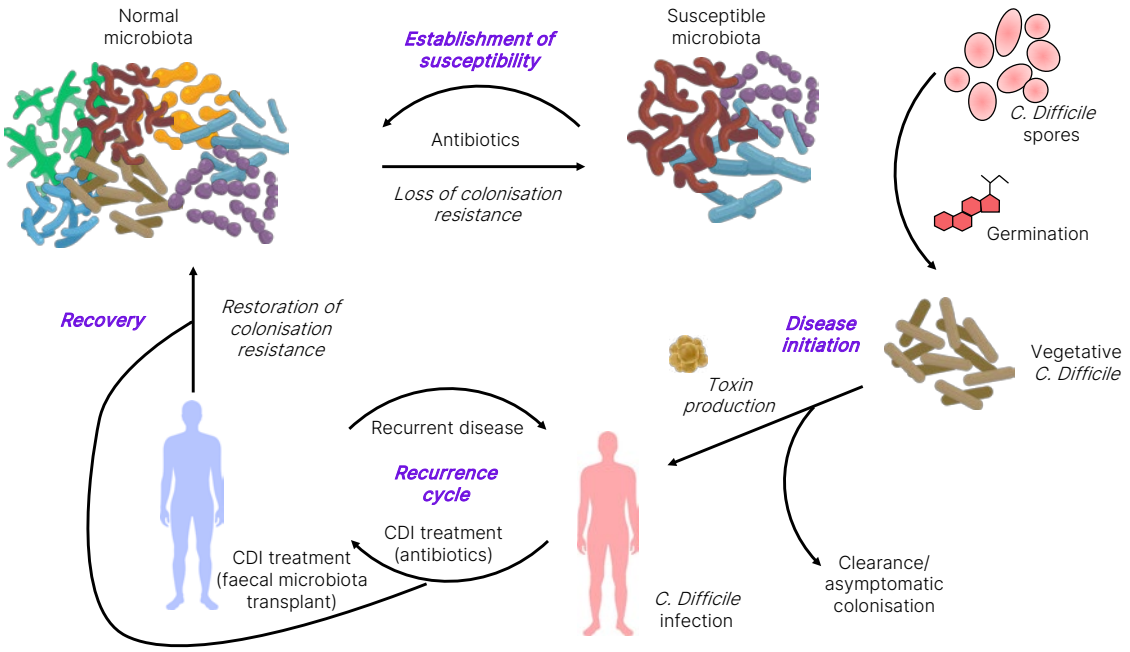
John Coia and Esther Calbo

Coia gave an overview of the burden of recurrent CDI, with a focus on the gut microbiome, which normally protects against CDI through 'colonisation resistance'. However, disruption of the gut microbiota increases susceptibility to recurrent CDI by allowing ingested *C. difficile* spores to germinate, multiply, and produce exotoxins.[25] These elicit a profound inflammatory response leading to epithelial cell death and underlying connective tissue disturbance, resulting in characteristic features of CDI, such as colitis, colonic inflammation, and profuse diarrhoea (Figure 1).[25]

The most common cause of gut microbiota disturbance is antibiotic therapy.[25] Although appropriate antibiotics targeting *C. difficile* resolve symptoms and restore the microbiota over many months, a significant minority of patients develop subsequent cycles of recurrent CDI with associated morbidity and mortality.[25]

The European Society of Clinical Microbiology and Infectious Diseases (ESCMID) guidelines define CDI recurrence as a renewed presentation of CDI within 8 weeks of the resolution of symptoms from the previous episode.[26] Coia noted that "discriminating between relapse from reinfection is not routinely available in clinical practice;" however, multi-locus variable-number tandem-repeat analysis has identified 75% of first recurrences are due to relapse with the same strain.[4] Whole genome sequencing studies have confirmed that the majority of recurrences, particularly early recurrences, are due to relapse, and some later cases also due to relapse.[27] Widespread use of whole genome sequencing in clinical practice is expected to help differentiate relapse and reinfection.[5]

Figure 1: The cycle of recurrent *Clostridioides difficile* infection.[25]



Adapted from Britton and Young.[25]

C. difficile: Clostridioides difficile; CDI: *Clostridioides difficile* infection.

A systematic literature review of large-sized studies with more than 1,000 patients (n=27; of which 16 were from the USA) found overall CDI recurrence rates of 17% (range: 2–57%).[1] The highest rates were seen in Canada (18%), the USA (17%), and Europe (UK [22%], Poland [22%], Germany [18%], and Spain [57%]).[1] Coia indicated that the cut-off for a recurrent episode in most studies was ≤8 weeks after the first episode, and most only report overall recurrence rates without considering number of recurrences. The median recurrence rate from all studies was 17% (range: 0–64%).[1] Recurrence rates were lower in community-associated than healthcare-associated CDI, possibly reflecting the younger age and lower exposure to healthcare facilities in this group.[5]

The European Centre for Disease Prevention and Control (ECDC) report for CDI from a multinational hospital surveillance (23 countries) between 2016–2017 with more than 18.3 million patient admissions and over 109 million patient days, identified a 6.4% recurrence rate (n=2,439 out of 37,857), with a crude incidence density of 0.22 recurrent CDI cases per 10,000 patient-days.[28] Recurrence was most common in tertiary hospitals, and twice as likely to have a complicated course of infection than non-recurrent cases (25% versus 14%; p<0.0001), and higher mortality related to recurrent CDI cases (31% versus 21%; p=0.003).[28] Up to 35% of CDI cases recur, with 20% recurring after a single episode, 40% after two episodes, and 65% after three episodes.[4,5] Recurrent CDI is associated with increased morbidity, mortality, and inpatient hospital costs compared with non-recurrent CDI.[29-31]

The ESCMID guidelines highlight several risk factors associated with recurrent CDI,[26] including age >65 years (relative risk: 1.63 [95% CI: 1.24–2.14]; p=0.00050);[32] prior CDI episode (particularly previous severe CDI); healthcare-associated CDI (admission within the last 3 months); concomitant non-CDI antibiotic use after diagnosis (relative risk: 1.76 [95% CI: 1.52–2.05]; p<0.00001);[32] and gastric acid suppression, such as proton-pump inhibitor (PPI) use, during or after CDI diagnosis (22.1% versus 17.3% without; odds ratio: 1.52 [95% CI: 1.20–1.94]; p<0.00100).[33] Other risk factors include severe underlying disease, such as inflammatory bowel disease, renal insufficiency, inadequate immune response to *C. difficile* toxins A and B, and virulence of the infecting strain.[26,34] Narrow-spectrum antibiotics such as fidaxomicin or vancomycin are associated with a lower rate of CDI recurrence, with fidaxomicin having a lower recurrence rate compared to vancomycin.[35,36]

Whilst these risk factors are helpful, Coia noted that "there are no specific tests or markers that accurately predict patients' likelihood of developing recurrent CDI," although age >65 years is considered the most important risk factor.[26] This is an important unmet need not only for the prognosis of these patients, but also for helping better targeting of therapeutic options.

The Role of Dysbiosis in Recurrent *Clostridioides difficile* Infection

Recurrence of CDI is likely caused by a combination of microbiome disruption factors, including failure to re-establish the colonic microflora, the presence of *C. difficile* spores in the intestines, and a suboptimal host immune response to the infecting organism and its toxins.[37]

Dysbiosis, as generated by broad-spectrum antibiotics, is an imbalance in gut microbiota, characterised by reduced microbiota diversity, an increased proportion of other species (e.g., *Escherichia coli*, *Proteus mirabilis*, and *Enterococcus faecalis*), a loss of resistance to colonisation, and an increase in pro-inflammatory cytokine synthesis, and has been proposed as a key factor in CDI recurrence.[35,38] The mechanisms through which gut microbial dysbiosis drives CDI are complex and not fully understood; however, the gut microbiota is mainly composed of *Firmicutes* (64%) and *Bacteroidetes* (23%). Gene sequencing studies have shown alterations in microbial composition of *Bacteroidetes* and *Firmicutes,* and marked decreased species diversity in patients with recurrent CDI, as well as in patients with non-*C. difficile* diarrhoea compared with healthy controls.[38-40] Calbo discussed the molecular mechanisms underlying dysbiosis in CDI, including the role of intestinal bile acid composition and spore germination, gut microbiota competition for nutrient niches inhibiting *C. difficile* growth, and zinc and other elements facilitating metabolic adaptation of *C. difficile*.

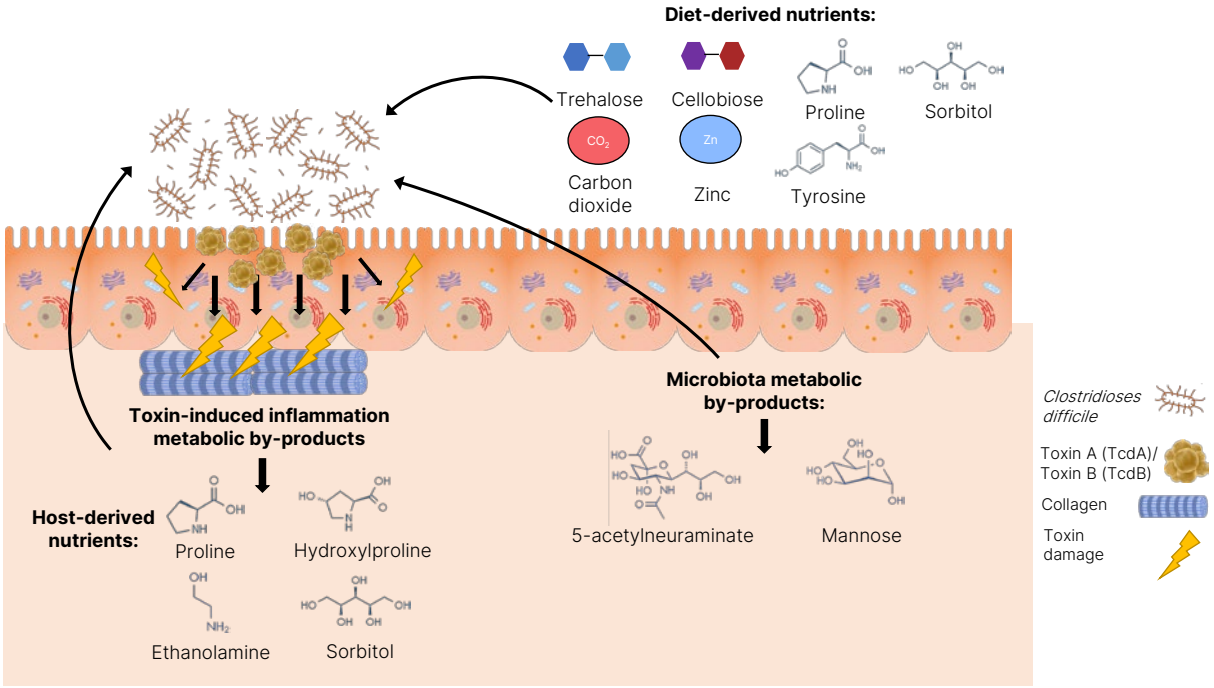
Alterations in the gut metabolome and expansion of antibiotic-resistant enterococci alter the gut metabolic environment and reprogramme *C. difficile* metabolism by a parallel process of

nutrient restriction and cross-feeding, and may also play a role in CDI pathogenesis.[41,42]

Calbo stated that there are more than 2,000 different bile acids, so a simplification of the process is that primary bile acids (cholate derivates) promote *C. difficile* spore germination (with co-germinants such as amino acids, calcium, and glycine) while 7α-dehydroxylation by gut microbiota to generate secondary bile acids inhibits spore germination and vegetative *C. difficile* cell growth, depleting the pool of primary bile acids.[43,44] Intestinal bacteria that mediate 7α-dehydroxylation have been shown to be protective against CDI in a mouse model.[45] Furthermore, a Phase II, double-blinded, placebo-controlled study investigating stool samples (n=113) from patients with recurrent CDI (N=27) who were administered a microbiota-based live biotherapeutic, showed a reduction in dominant primary bile acids and concurrently increased secondary bile acids, which correlated with "clinical cure."[46]

Regarding nutrient competition, there are many important molecules, including gut microbial-derived short chain fatty acids, such as propionate, acetate, and butyrate, which play a role in maintaining intestinal barrier integrity, inhibit pro-inflammatory cytokines, and serve as an energy source for colonic epithelium cells, which are associated with CDI resistance.[43] The gut microbiota competes for nutrients with *C. difficile* by depleting carbohydrates, amino acids, glycine (a co-germinant for spores), and cholesterol (by producing coprostanol).[43,47] *C. difficile* has adapted its metabolism to transport and uptake metal ions, such as zinc sequestration by calprotectin (Figure 2), and uses mannitol as a primary nutrient, which is abundant in post-antibiotic environments.[47] Furthermore, *C. difficile* also produces bacteriostatic compounds such as p-cresol (a tyrosine metabolite) and sorbitol.[43]

Figure 2: An overview of the nutrients *Clostridioides difficile* utilises and their origin during infection of the gut.[46]



Adapted from Marshall et al.[46]

C. difficile: Clostridioides difficile.

Understanding the Risk Factors and Outcomes of Recurrent *Clostridioides difficile* Infection

Calbo emphasised the importance of targeting recurrence to improve CDI outcomes, a composite of recurrence of CDI, refractory CDI, severity, and mortality risk.[48] However, Calbo stated studies on CDI mortality are "scarce and vary widely in methodology." Some studies describe rates of poor mortality outcomes related to specific RTs, such as BI/NAP1/027 and 078.[48]

Calbo noted that CDI mortality risk factors are similar to those for CDI recurrence, including age, number of comorbidities, cancer, and BMI.[49] Poor outcomes in CDI result in 14.0–25.0% recurrence, 4.0–20.0% refractory, and approximately 6.0% mortality, with in-hospital mortality ranging from 8.0–37.2%.[48] Moreover, outcome drivers include *C. difficile* virulence factors, host factors such as age and malignancy, and treatment.[49,50] A retrospective single centre cohort study on almost 4,000 patients with CDI found that recurrent CDI is associated with an increased risk of death at 3 and 6 months; and a UK study with 6,682 patients with CDI, including 1,140 patients with recurrent CDI, found an increased risk of mortality and complication at 12 months.[29,30] Additional studies show that CDI is characterised by a high delayed and unrelated mortality rate (18% at 75 days), associated with age (>65 years), comorbidity, and faecal incontinence.[51] Calbo investigated CDI in patients with cancer, and found a higher risk of CDI recurrence (13%) and mortality (27%), particularly late mortality (3 months after initial episode: 13%).[52] Calbo concluded that in select populations (the elderly, patients with cancer, and patients with recurrent CDI), delayed mortality rates may be higher than early mortality rates.

Clostridioides difficile Infection in Practice: Interactive Case Study

Expert Panel

During an interactive session involving the audience by use of a mobile application, Calbo outlined the case of an 86-year-old female living in a skilled nursing facility, with a history of chronic oedema and kidney disease, hospitalised 2 months prior with heart failure, where they were diagnosed with gastroesophageal reflux disease, and started on a PPI. The patient had developed cellulitis 20 days prior, without systemic signs of infection, and was treated with antibiotics (oral clindamycin 300 mg four times daily [QID]). The patient subsequently developed abdominal pains and diarrhoea approximately 1 week after completing the course of antibiotics, and tested positive for CDI.

The panel and audience, consisting of participants from a wide range of countries (such as Germany, Italy, Spain, the UK, and the USA) and specialities (including infectious disease specialists and clinical microbiologists), agreed (93.2%) that the patient was at risk of recurrence due to age (>65 years), prior antimicrobial exposure and PPI use, and environment (living in a skilled nursing facility and prior hospitalisation within the past 3 months).[26,32-34]

For the initial CDI, 63.5% of the audience selected fidaxomicin (200 mg twice daily [BID] for 10 days) as the best therapy for this patient, while 30.4% selected vancomycin (125 mg QID for 10 days), and 6.1% chose metronidazole (500 mg three times daily for 10 days). Wilcox emphasised that guidelines "no longer recommend" metronidazole as a first-line therapy for primary CDI, whether there is a risk of recurrence or not.[26] They further emphasised the importance of adherence to the guidelines to improve outcomes for patients, with fidaxomicin and vancomycin being standard of care. Fidaxomicin should be used as first-line, followed by vancomycin if fidaxomicin is not available. Metronidazole is no longer recommended.[26]

Despite receiving fidaxomicin for 10 days for the initial CDI, the patient experienced two subsequent recurrent episodes, approximately 4 weeks after completing the first course, and approximately 3 weeks after completing the second course (fidaxomicin [200 mg BID for 10 days] plus bezlotoxumab infusion [10 mg/kg administered on Day 6 of fidaxomicin]). Calbo confirmed the patient was toxin positive in the last recurrence, with symptoms of pain and fever.

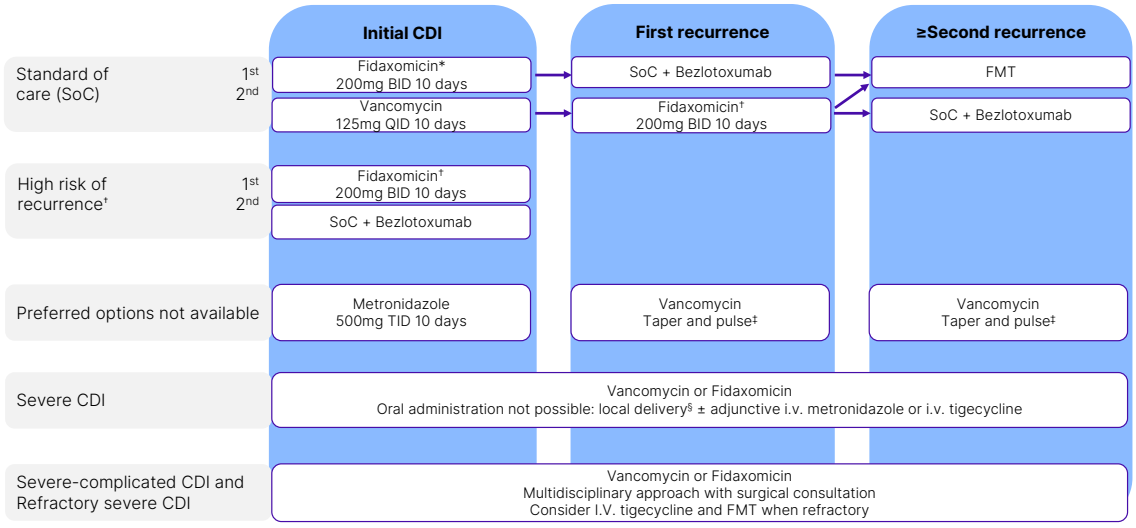
The panel agreed on faecal microbiota transplantation (FMT) as the next step despite acknowledging the risks, with Wilcox advising caution for use of FMT prior to recurrent CDI, and before exploring optimal and alternative treatment pathways (Figure 3).[26] Gonzales-Luna questioned that perhaps the first episode of CDI could have been treated with fidaxomicin plus bezlotoxumab. Guery confirmed that this would have been an option, but there was no strong data supporting it. This lack of data may also raise some cost concerns. Coia emphasised the importance of diagnostics and identifying risk factors for recurrent CDI to select the appropriate treatment, and Wilcox highlighted the need to determine where current and emerging treatments fit in the therapeutic pathway.

Extended Dosing in *Clostridioides difficile* Infection: EXTEND Study

Benoît Guery

Guery discussed the use of fidaxomicin, a narrow-spectrum macrocyclic antimicrobial, for treating CDI, with a focus on the extended dosing approach.[35] Fidaxomicin selectively targets *C. difficile* by inhibiting RNA polymerase, while having minimal effects on gut commensals.[35] Guery suggested that fidaxomicin preserves the gut microbiota compared with broad-spectrum antibiotics, and "reduces the recurrence of CDI."[36,53] This may be partly due to gut microbiota such as *Proteobacteria* or *Bacteroidetes* lacking the fidaxomicin binding site.[34]

Figure 3: European Society of Clinical Microbiology and Infectious Diseases (ESCMID)-suggested treatment recommendations.[26]



*Risk stratification for risk of recurrence may be applied for selective use of fidaxomicin in case of limited access or resources.

†Consider extended fidaxomicin: 200 mg BID on Day 1-5, 200 mg q48h on Day 7–25. Most important risk factor for recurrence is age >65–70 years. Additional risk factors to consider are healthcare-associated CDI, prior hospitalisation ≤3 months, prior CDI episode, continued non-CDI antibiotic use, and PPI therapy. The risk of recurrence is assumed higher with more risk factors present.

‡Vancomycin taper and pulse: 2 weeks 125 mg QID, followed by 1 week 125 mg BID, then 1 week 125 mg qd, then 1 week 125 mg q48h, and finally 125 mg q72h for 1 week.

§Rectal or nasoduodenal delivery.

Adapted from Van Prehn et al.[26]

BID: twice daily; CDI: *Clostridioides difficile* infection; FMT: faecal microbiota transplantation; IV: intravenous; qd: once daily; QID: four times daily; q48h: administered at 48-hour intervals; q72h: administered at 72-hour intervals; SoC: standard of care; TID: three times daily.

In an *in vitro* study using a human chemostat gut model, fidaxomicin first-line was effective in reducing the total viable count of *C. difficile*, spore counts, and cytotoxin titre compared with vancomycin and metronidazole.[54] Alternative dosing regimens, including extended (20 days with 200 mg/L BID) and tapered-pulsed dosing (5 days 200 mg/L BID, followed by 20 days 200 mg/L once every other day) were effective in reducing *C. difficile* and toxin detection with no recurrence, while sparing microbiota.[54] Pulsed or tapered regimens enabled greater recovery of *Bifidobacteria* compared with the extended regimen.[54] Guery believes that this could be interesting in the gut healing process.

The EXTEND Phase IIIB/IV study is an open-label, randomised, multinational controlled trial conducted in 86 centres across 21 countries.[55] Patients >60 years old (N=364) were administered either an extended-pulsed fidaxomicin regimen (n=177; 200 mg BID on Days 1–5, followed by 200 mg once daily on alternate days over Days 7–25) or vancomycin (n=179; 125mg QID on Days 1–10).[55] The primary endpoint was sustained clinical cure 30 days after the end of the treatment (Day 55 for fidaxomicin and Day 40 for vancomycin), with follow-up at Day 90.[56] Approximately 58.1% of participants were female, most had non-severe CDI (63.5%), 78.9% of participants had not experienced a previous CDI occurrence in the 3 months prior, and approximately 72.0% received antibiotics for conditions other than CDI, indicating they were high-risk for CDI recurrence due to age (>60 years) and systemic antibiotic use.[26,55]

Although there was no difference in clinical cure, a significant decline in recurrence was observed between Day 40 and 55 (-15% and -14%, respectively; p<0.0001).[55] Extended-pulsed fidaxomicin was found to be superior to standard-dose vancomycin for sustained clinical cure of CDI, demonstrating that efficacy was preserved in patients with a high risk of recurrence.[55] The extended-pulsed fidaxomicin regimen is approved for use in Europe.[56,57] Subgroup analysis identified similar efficacy in the extended-pulsed fidaxomicin regimen with a preserved rate of sustained clinical cure regardless of risk factors, such as age (≥60 years), cancer diagnosis, CDI severity, prior CDI episodes, or infection with RT027.[58] Extended-pulsed fidaxomicin showed sustained clinical response rates of 74% at 30 days (n=34 out of 46) and 61% at 90 days (n=28 out of 46) in 46 high-risk patients with multiple CDI recurrences (57% ≥65 years old; 39% using PPI; and a mean of 3.5 previous CDI episodes) who failed tapered vancomycin treatment (75%).[59]

Guery proposed future research to address limitations of the EXTEND trial to consider the use of randomised control trials versus conventional administration, including patients under 60 years old. They suggested comparing extended fidaxomicin with vancomycin and bezlotoxumab as standard of care, and versus fidaxomicin pulsed approach. Additionally, Guery suggested the need for data on multiple recurrence, especially in cases where FMT is not available.

Question and Answer Session

Gonzales-Luna asked Calbo if fidaxomicin resistance was tested in the patient case, and Calbo responded that they did not, as there is not currently "a problem." Tschudin-Sutter identified the importance of routine surveillance for vancomycin resistance, and Coia emphasised the importance of conducting susceptibility testing properly and recommended "reference laboratories do monitor isolates for the potential of resistance for fidaxomicin," while Wilcox supported the need for ongoing surveillance of minimal inhibitory concentrations.

The panel were asked about retesting protocols post-CDI infection. Coia said that "waiting 28 days from positive CDI cases is too long," while Guery and Calbo indicated in their clinical practice testing only occurs in symptomatic patients (i.e., those with diarrhoea).

Regarding the importance of dysbiosis and the potential role of diet and foodstuffs, Coia and Wilcox called for further understanding of this, as well as the role of One Health in *C. difficile* transmission.

Regarding treatment approaches, Coia recommended following ESCMID guidelines for first-line therapy and dosage approach, with fidaxomicin (200 mg BID for 10 days) as standard of care, or vancomycin (125 mg QID for 10 days) when not available (Figure 3).[26]

Calbo recommended that the more risk factors a patient has for CDI recurrence, fidaxomicin plus bezlotoxumab should be used. Gonzales-Luna asked whether tapered or pulsed vancomycin or fidaxomicin could be used in cases where other options are unavailable or unsuitable, and Guery agreed that data supports the use of these approaches, but highlighted gaps for multiple recurrence.

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Providing Expert Consultation in a World Living with COVID-19

This 'Meet the Experts' symposium took place on 16th April 2023 as part of the 33rd European Congress of Clinical Microbiology and Infectious Diseases (ECCMID) in Copenhagen, Denmark

Chairpeople: Paolo Antonio Grossi,[1] Ann-Brit Eg Hansen[2,3]

Speakers: Tobias Welte,[4] Stephen Thomas,[5] Marta Boffito,[6] Roger Paredes[7,8]

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Meeting Summary

After a welcome by Paolo Antonio Grossi, the appointed chair by the European Society of Clinical Microbiology and Infectious Diseases (ESCMID), Department of Medicine & Surgery, University of Insubria, Varese, Italy; Ann-Brit Eg Hansen, Department of Infectious Diseases, Copenhagen University Hospital – Amager and Hvidovre, Denmark and Department of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark, summarised the objectives of the meeting and introduced the speakers. In their opening presentation, Tobias Welte, Department of Pulmonary and Infectious Diseases, Hannover University School of Medicine, Germany, described how the COVID-19 pandemic has evolved, in terms of variants, mortality rates, vaccinations, immunity, and antivirals. Welte then presented a hypothetical case study to illustrate how older patients with comorbidities can initially have mild symptoms, but may then deteriorate and require hospitalisation. Stephen Thomas, State University of New York Upstate Medical University, Syracuse, USA, then described the efficacy and potential side effects of the BNT162b2 (Pfizer–BioNTech, New York City, USA, and Mainz, Germany, respectively) vaccine, and the need for, and benefits of, booster doses. Thomas also described the added benefits of the newer bivalent vaccines in a world where COVID-19 is constantly mutating. Marta Boffito, HIV, Sexual and Gender Health, Dermatology, Chelsea and Westminster Hospital NHS Foundation Trust, Imperial College London, UK, then outlined various factors that increase the risk of progression to severe COVID-19 disease, including older age, immunocompromised status, and underlying health conditions (e.g., obesity, hypertension, heart disease, and chronic kidney disease [CKD]). Such patients can benefit from antiviral medications such as nirmatrelvir/ritonavir, although potential drug–drug interactions must be considered. Roger Paredes, Department of Infectious Diseases and IrsiCaixa AIDS Research Institute, Hospital Universitari Germans Trias i Pujol, Badalona, Catalonia, Spain, and Center for Global Health and Diseases, Case Western Reserve University School of Medicine, Cleveland, Ohio, USA, revisited the case study to highlight the importance of early COVID-19 diagnosis among high-risk patients to enable the use of nirmatrelvir/ritonavir, which is only approved within 5 days of symptom onset for non-hospitalised adults at increased risk of progression to severe COVID-19. Paredes went on to discuss antiviral treatments in more detail, describing a randomised controlled trial (EPIC-HR) and two large real-world studies that showed that nirmatrelvir/ritonavir could significantly reduce the risk of hospitalisation and death due to COVID-19 among high-risk patients. To conclude, Hansen highlighted the importance of regular updates to COVID-19 management guidelines, given the ongoing and evolving nature of COVID-19, as well as the importance of identifying high-risk patients early in their disease course to enable the use of nirmatrelvir/ritonavir.

A COVID-19 Perfect Storm: Reviewing a Clinical Challenge

Tobias Welte

Since COVID-19 was first identified in late 2019 in Wuhan, China, key variants have included the Alpha and Delta variants.[1] However, by the end of 2022, most cases were Omicron,[2] which is more transmissible but less deadly. There are multiple variants of Omicron, of which some are termed "variants of concern." Most recently, XBB.1.5 was dominant in Western Europe, and this has increased transmissibility.[3]

Despite the reduced mortality risk of COVID-19, the high incidence means that there are still patients with COVID-19 who need hospital treatment and some who die, especially high-risk patients. Fortunately, low-risk patient groups (i.e., younger people without comorbidities) have good immunogenicity due to high vaccination rates[4] and exposure to infection. However, vaccinations for low-risk groups have reduced dramatically, and due to waning immunity and lower infection rates over the summer, immunogenicity is falling, the impact of which is not yet known.

The World Health Organization (WHO) recently acknowledged COVID-19 as an ongoing public health emergency.[5] Recommendations include: 100% vaccination rates for high-priority groups; to increase uptake and ensure availability of medical countermeasures; improved surveillance; and continued engagement with communities.[5]

Welte presented a hypothetical case study of a 70-year-old female who had a positive home COVID-19 test 6 days after symptom onset (Figure 1). They were receiving amlodipine for hypertension and had mild renal impairment. They had received two primary vaccine doses, and a booster 8 months prior to contracting the virus. The patient's COVID-19 symptoms up to Day 6 were mild.

Typical primary symptoms of COVID-19 include headache, fatigue, shortness of breath, cough, loss of sense of taste or smell, muscle aches, nausea, and diarrhoea.[6] These symptoms typically last for approximately 1 week, followed by a decrease in viral load.[7] For many patients, their initial host immune response controls the infection and they then recover. However, in some patients, symptoms can become more severe during a second immune response phase, when infection can result in COVID-19 pneumonia.[8] Some of these patients go on to have systemic hyperinflammation, which can damage the lung and other organs.[8]

Approximately 25–30% of people with COVID-19 are asymptomatic.[9,10] At the other end of the spectrum, approximately 15% are admitted to hospital, and 5% require care in an intensive care unit (ICU).[11] Mechanical ventilation is required by approximately 4% of patients who are hospitalised, increasing to 11% of those with severe acute respiratory infections.[12] In-hospital mortality is approximately 6%, but increases to approximately 16% in patients with severe acute respiratory infection.[12]

If antiviral medications are to be given, this needs to be done early in the disease course, while the viral load is still high. However, the case patient only tested on Day 6 (Figure 1). By Day 8, they had deteriorated, with signs of COVID-19 pneumonia, O_2 saturation <94%, and breathing difficulties. The patient was admitted to hospital, where they were given O_2 support and corticosteroids. A CT scan revealed bilateral parenchymal opacities in >50% of the total chest area. The patient deteriorated, went into acute renal failure, which is a strong predictor of mortality, and ultimately required invasive mechanical ventilation in the ICU.

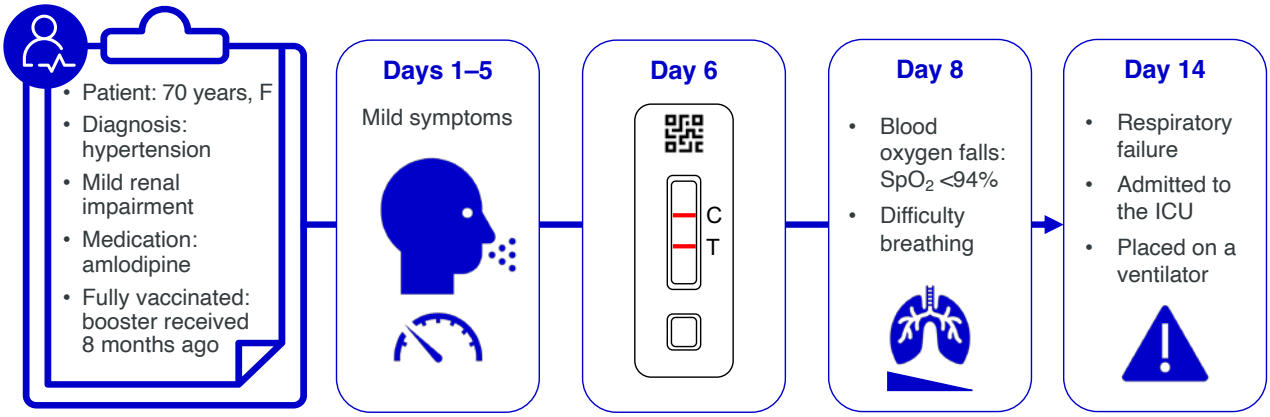
Currently, immunocompromised patients (i.e., transplant recipients and patients with cancer or autoimmune disease) are considered to be at the highest risk from COVID-19. However, older adults with comorbidities are also at increased risk of severe COVID-19 disease, and ways to manage such patients are discussed below.

Expert COVID-19 Consultation: Vaccination for the Prevention of COVID-19

Stephen Thomas

The recommended primary vaccination course with the BNT162b2 vaccine is: three doses ×3 µg/dose for children aged 6 months–4 years; two doses ×10/dose µg for children aged 5–11 years

Figure 1: Hypothetical COVID-19 case timeline.



C: control; F: female; ICU: intensive care unit; SpO_2: oxygen saturation; T: test.

(three doses if severely immunocompromised); and two doses ×30 µg/doses for children aged ≥12 years and adults (three doses if severely immunocompromised).

As with all vaccines, there is the potential for adverse reactions, including hypersensitivity, anaphylaxis, myocarditis, and pericarditis.[13] The risks of hypersensitivity and anaphylaxis are similar to other routinely used vaccines, but appropriate treatments should be available at vaccination centres. Myocarditis and pericarditis are very rare reactions, occurring at a rate of less than one case per 10,000 vaccinations; they have been observed more often in young males than other demographic groups, and after a second dose.[13,14] Myocarditis and pericarditis generally occur within 14 days after vaccination.[13,14] Healthcare providers should, therefore, be alert to the signs and symptoms of myocarditis and pericarditis, namely acute and persisting chest pain, shortness of breath, or palpitations.

Among individuals aged ≥16 years, BNT162b2 vaccine efficacy in preventing first COVID-19 occurrence was >90% at the time of the study, when Wuhan/Wild type and Alpha variants were the predominant circulating strains.[13] Among individuals aged 5–11 years and 12–15 years, a similar safety profile and vaccine efficacy was observed for BNT162b2. Immunobridging criteria were also met in children aged 5–11 years and adolescents aged 12–15 years at 1 month after dose 2, when compared with young adults aged 16–25 years. Among younger children (6 months–4 years), BNT162b2 resulted in >70% vaccine efficacy in preventing first COVID-19 occurrence when the Omicron variant of severe acute respiratory syndrome coronavirus 2 (BA.2) was the predominant circulating strain. Immunobridging criteria were also met in children aged 6 months–4 years at 1 month after dose 3, when compared with young adults aged 16–25 years.[13]

Later, a small Phase I study showed that immunogenicity waned over time after the second dose of BNT162b2, and also that protection against the Beta variant was lower than against the Wild type variant.[15] However, a booster dose among adults aged 18–55 and 65–85 years resulted in increased immunity against both strains.[15] A larger, Phase II study further supported the need for, and potential benefit of, a booster dose.[13]

In a large study, individuals aged ≥16 years who had received two doses of BNT162b2 were randomised 1:1 to a booster dose of BNT162b2 or placebo.[16] The boosters were administered a median of 11 months after the second dose, and the main strain circulating at the time of the study was Delta. After a median follow-up of 2.5 months, only five individuals who had received a booster dose of BNT162b2 developed COVID-19, compared with 109 in the placebo group (giving a

vaccine efficacy of 96%), and there were no new safety signals.[16]

After these studies, the Omicron BA.1 variant became dominant in early 2022.[17] It became apparent that the components of the vaccine needed to be better matched to the circulating strains, hence a bivalent vaccine that contained the original Wuhan component plus BA.1 was developed.[13] This had a similar safety profile to the original vaccine, and induced neutralising antibodies to both strains. In the summer of 2022, Omicron BA.5 and BA.4 appeared,[17] and a bivalent vaccine containing the original strain and BA.4/5 was developed.[13]

Among individuals who had received three doses of the original BNT162b2 vaccine, a booster dose of the bivalent BNT162b2 original/Omicron BA.4/5 vaccine elicited similar neutralising antibody titres against the original strain as a booster dose of the original vaccine, but higher neutralising antibody titres against various other Omicron sublineages, such as BA.4, BA.5, BA.4.6, BQ.1.1, XBB.1, and BA.2.75.2.[18]

A large, retrospective study of individuals aged ≥12 years with a positive COVID-19 test result was undertaken at a time when Omicron BA.4.6, BA.5, BQ.1, and BQ.1.1 sublineages were predominant.[19] The bivalent vaccines were shown to offer better protection against severe disease than the original vaccine (efficacy against hospitalisation: 59% versus 25%; efficacy against hospitalisation or death: 62% versus 25%), with similar results among adults (≥18 years), older adults (≥65 years), and those without previous COVID-19 infection.[19]

In summary, Thomas highlighted the need to match vaccine components to circulating COVID-19 variants.

Identifying Patients at Risk of Severe Disease Progression

Marta Boffito

Boffito recapped the case study presented earlier of the 70-year-old female with hypertension and mild renal impairment, who only tested for COVID-19 after 6 days of mild symptoms, and then progressed to severe disease (Figure 1). Boffito highlighted the importance of identifying patients at risk of severe disease progression so that testing is done in a timely manner, and treatments can be administered earlier.

Risk factors for severe COVID-19 include: older age; immunocompromised status, due to disease or immunosuppressants; underlying health conditions; and current or recent pregnancy.[20-22] According to data from the Centers for Disease Control and Prevention (CDC), the risk of COVID-19 infection is similar across age groups, but the risk of hospitalisation increases five-fold among those aged 65–74 years and 15-fold among those aged ≥85 years, compared with individuals aged 18–29 years.[23] The risk of death increases even more substantially, being 65-fold and 360-fold higher among people aged 65–74 and ≥85 years, respectively, versus 18–29 years.[23]

Patients who are immunocompromised are also at increased risk of severe COVID-19, including:[24] haematopoietic stem cell transplant recipients; solid organ transplant recipients taking immunosuppressive therapy; patients undergoing treatment for solid or haematological cancers; patients with moderate or severe primary immunodeficiency; patients with advanced or untreated HIV infection; and patients taking immunosuppressive or immunomodulatory treatments.

Underlying health conditions that can increase the risk of severe COVID-19 include:[25] diabetes, obesity, and hypertension; ischaemic heart disease and history of heart failure; solid organ tumours; chronic respiratory disease and chronic kidney disease; and neurological conditions.

Boffito described several studies that have looked at the risk associated with various underlying heath conditions in more depth. A systematic review has shown that patients with chronic obstructive pulmonary disease (COPD) who develop COVID-19 are at a 1.4-fold increased risk of hospitalisation and death compared with people without COPD.[26] Among patients with CKD, the risk of death from COVID-19 was approximately doubled after adjustment for confounding variables.[27] In a small study of 41 patients who had received a kidney

or kidney/pancreas transplant and developed COVID-19, 56% required hospitalisation, even though all but one had received two or more doses of a COVID-19 vaccine.[28]

Indeed, several studies have demonstrated a residual vulnerability in patients with underlying health conditions, even after vaccination. A retrospective cohort study of adults who developed COVID-19 during the Omicron era, despite having received three or more COVID-19 vaccine doses, showed that significant risk factors for hospitalisation were: hypertension (2.3-fold increased risk); CKD (2.2-fold); myocardial infarction/heart failure (2.2-fold); age (1.2-fold per 10-year increase); and time since last vaccination dose,[29] consistent with the observed waning of immunity described by Thomas. A prospective cohort study of adults in the UK who had received one or two vaccine doses produced similar results, and identified significant risk factors for severe COVID-19, including: CKD (increasing from 1.3-fold increased risk of hospitalisation for patients with Stage 3 CKD, up to 12.8-fold for kidney transplant recipients); bone marrow or solid organ transplantation (6.8-fold); blood cancer (1.9-fold); diabetes (1.3–1.8-fold, depending on glycated haemoglobin levels); atrial fibrillation (1.4-fold); congestive cardiac failure (1.4-fold); COPD (1.3-fold); coronary heart disease (1.3-fold); and stroke (1.2-fold).[30] This study also showed that the risk of COVID-19-related hospitalisation was reduced by 79% after two versus one dose of vaccine,[30] highlighting the importance of multiple vaccine doses. However, this study did not look at the impact of booster doses on residual risk of severe disease in patients with underlying conditions.

Similarly, in patients who are immunocompromised, a healthcare database study found that the BNT162b2 vaccine was effective in individuals who were taking immunosuppressants (e.g., disease-modifying antirheumatic drugs and glucocorticoids), but that such patients were at higher risk of COVID-19 infection and hospitalisation than immunocompetent individuals.[31]

Together, these studies highlight a range of comorbidities that physicians and patients need to be aware of to facilitate timely intervention. Boffito revisited the case study, in which a patient with hypertension and mild renal failure, in whom COVID-19 was not confirmed until Day 6, was hospitalised and admitted to ICU, demonstrating the potential serious consequences of failing to act promptly in patients with risk factors.

The evidence for intervention in the form of antiviral treatment was discussed by Parades and is outlined in the next section, but Boffito summarised the latest WHO guidelines for antiviral treatments for COVID-19 in people with the highest risk of hospitalisation, including:[32] a strong recommendation for nirmatrelvir/ritonavir; weak or conditional recommendations for molnupiravir and remdesivir; and a strong recommendation against monoclonal antibody treatment with sotrovimab.

When prescribing nirmatrelvir/ritonavir, potential drug–drug interactions must be considered.[33,34] Although nirmatrelvir/ritonavir can be given with various other medications, there are some contraindications, so these should be checked.[33,34]

Overall, it should be recognised that the speed of the development of COVID-19 vaccines and treatments has been unprecedented.[35] However, it is important to identify and treat high-risk patients early in their disease course, which can also help to reduce transmission.[36] Treatment with oral antivirals is manageable for various high-risk patient groups,[34] and although knowledge of drug–drug interactions is key, this potential problem should not stop most patients from receiving oral antivirals, as the majority of drug–drug interactions are manageable.[33,34]

What is the Evidence for Antiviral Therapy?

Roger Paredes

Going back to the case study (Figure 1), the 70-year-old female patient with hypertension could potentially have benefitted from nirmatrelvir/ritonavir, had their COVID-19 been diagnosed earlier. Given the patient's age and comorbidities, particularly hypertension, they would have been considered to be at increased risk of severe COVID-19.[25,37] However,

nirmatrelvir/ritonavir should be given as soon as possible after the onset of COVID-19 symptoms, and within 5 days.[33]

COVID-19 symptoms can appear 2–14 days after exposure;[6] and viral loads are highest around the time of symptom onset and decline thereafter.[38-40] As cases are unlikely to be detected before symptom onset, detection as soon as possible after symptom onset is therefore important in high-risk patients, and this can be achieved using various diagnostic tests.[38] High-risk patients can progress quickly to severe disease, highlighting the need for rapid therapeutic intervention.[8,36] Modelling studies using viral shedding duration as a surrogate for COVID-19 severity suggest that early testing and treating are a promising approach for reducing the risk of severe disease.[41]

Antivirals can target different parts of the viral replication mechanism, including:[42-44] binding to the angiotensin-converting enzyme 2 receptor (e.g., neutralising monoclonal antibodies, convalescent plasma, and vaccines); viral entry or exit (e.g., human protease inhibitors); proteolysis (e.g., protease inhibitors); and RNA replication (e.g., RNA polymerase inhibitors).

The approved indication for nirmatrelvir/ritonavir in the European Union (EU) is for the treatment of COVID-19 in "adults who do not require supplemental O_2 and who are at increased risk for progressing to severe COVID-19".[33] It is, therefore, for patients who have not yet been hospitalised, although the 5-day course should be finished if a patient becomes hospitalised.[33] The recommended dose is 300 mg nirmatrelvir plus 100 mg ritonavir every 12 hours for 5 days.[33] This dose is also suitable for patients with mild-to-moderate hepatic impairment or mild renal impairment, but for those with moderate renal impairment, the dose of nirmatrelvir should be reduced to 150 mg.[33] Nirmatrelvir/ritonavir is not suitable for patients with severe hepatic or renal impairment.[33] Other contraindications include hypersensitivity to the ingredients, and co-administration with certain drugs that are potent *CYP3A* inducers, or that are highly dependent on *CYP3A* for clearance (for full details, please refer to the summary of product characteristics).[33]

Going back to the case patient (Figure 1), nirmatrelvir/ritonavir would have been appropriate for this patient if they had tested positive for COVID-19 within 5 days of symptom onset. However, as the patient was taking amlodipine, they would have required careful monitoring of therapeutic and adverse effects.[33]

Key evidence to support the use of nirmatrelvir/ritonavir comes from a large, Phase II/III, randomised, double-blind, placebo-controlled trial of non-hospitalised, unvaccinated, symptomatic adults with a first COVID-19 infection who were at high risk for progression to severe disease.[45] The EPIC-HR study was performed at a time when the Delta variant was dominant.[46] In total, 2,246 patients were randomised to nirmatrelvir/ritonavir 300/100 mg or placebo every 12 hours for 5 days.[45] The primary endpoint was the proportion of patients with COVID-19-related hospitalisation or death from any cause through Day 28, among patients who started treatment within 3 days of symptom onset. A key secondary endpoint was the same outcome among patients who started treatment within 5 days of symptom onset.[45] The most common risk factors for severe COVID-19 among the patients in the EPIC-HR study population were: BMI ≥25 kg/m^2 (80.5%), smoking (39.0%), and hypertension (32.9%); and 61.0% of patients had two or more risk factors.[45]

Among 697 patients who received nirmatrelvir/ritonavir within 3 days of symptom onset, 0.7% had COVID-19-related hospitalisation or death by Day 28 compared with 6.5% of 682 patients who received placebo, giving a relative risk reduction of 88.9% ($p<0.001$).[33,45] Among 2,085 patients who received treatment within 5 days of symptom onset, results were similar (0.9% versus 6.3%; relative risk reduction: 86.3%; $p<0.0001$).[33] There were 12 deaths, all of which occurred in the placebo arm.[33,45] The most common adverse events in the nirmatrelvir/ritonavir arm were dysgeusia (5.6%) and diarrhoea (3.1%).[33,45]

It should be noted, however, that EPIC-HR included unvaccinated patients with a first COVID-19 infection, whereas nowadays, most people have been vaccinated and/or infected. Although randomised controlled trials provide high-quality evidence and are the basis for regulatory approval, real-world evidence has various strengths, including: larger and more diverse patient populations, being more reflective

of clinical practice, and the potential for longer follow-up.[47,48] However, limitations of real-world evidence include selection bias, confounding, and variable data quality.[49]

A high-quality real-world study of nirmatrelvir/ritonavir has recently been published.[50] This Pfizer-sponsored study was conducted in California, USA, and included non-hospitalised patients aged ≥12 years with COVID-19 during April–October 2022 (i.e., the Omicron era).[50] The study was conducted within the Kaiser Permanente Southern California healthcare system.[50] Patients who had been prescribed nirmatrelvir/ritonavir were considered to be exposed from the dispensing date.[50] A total of 7,274 patients who had been prescribed nirmatrelvir/ritonavir were matched on date, age, sex, BMI, clinical status, vaccination history, comorbidities, and healthcare seeking during the previous year to 126,152 controls who were not prescribed nirmatrelvir/ritonavir.[50] Most patients had received two or more COVID-19 vaccine doses (93.9% of those prescribed nirmatrelvir/ritonavir and 85.1% of controls).[50]

After adjustment for differences in risk status, receipt of nirmatrelvir/ritonavir within 5 days of symptom onset was associated with an estimated effectiveness for preventing all-cause hospitalisation and death within 30 days of a positive COVID-19 test of 79.6% (p=0.008), while receipt at any time after symptom onset was associated with an estimated effectiveness of 53.6% (p=0.031).[50] For the prevention of all-cause ICU admission, mechanical ventilation, or death within 60 days, the estimated effectiveness rates were 89.2% (p=0.075) if given within 5 days, and 84.1% (p=0.027) if given at any time.[50] Limitations included: incomplete data, potential misclassification of immunity, unmeasured confounding, low event rates, and the use of all-cause endpoints.[50] Also, some patients may not have taken the drug as prescribed.[50] However, nirmatrelvir/ritonavir was found to help prevent hospitalisation and death in a highly vaccinated population, and early treatment was more beneficial than later treatment.

Another high-quality real-world study of nirmatrelvir/ritonavir was conducted independently in the USA.[51] This study included non-hospitalised, vaccinated adults who developed COVID-19 ≥1 month after vaccination.[51] A total of 1,130 patients who were prescribed nirmatrelvir/ritonavir were propensity score matched to 1,130 individuals who were not. Common comorbidities included hyperlipidaemia (57.5% versus 58.5%) and hypertension (52.2% versus 51.2%). Nirmatrelvir/ritonavir reduced the risk of all-cause emergency room visit, hospitalisation, or death at 30 days by 45% (p<0.005). Limitations include potential unmeasured confounding, retrospective data, and the use of all-cause endpoints. Nonetheless, a significant benefit of nirmatrelvir/ritonavir was confirmed.

Parades emphasised that antiviral treatment should not be considered a substitute for vaccination, as vaccination remains the primary strategy for protecting patients against severe COVID-19. However, antivirals are a valuable additional tool for reducing the residual risk of severe disease in vulnerable groups, such as the immunocompromised or patients with high comorbidities, particularly when started early.

Summary and Close

Ann-Brit Eg Hansen

Hansen concluded the meeting by saying that COVID-19 management guidelines need to be updated regularly by infectious disease specialists because the COVID-19 situation is ongoing and constantly evolving. The engagement of primary healthcare providers is also vital, to help identify patients who are at high risk of progression to severe COVID-19. This has to be done early in their COVID-19 disease course so that nirmatrelvir/ritonavir can be prescribed.

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Abstract Reviews

Sharing key insights into the latest research in microbiology and infectious diseases, from novel abstracts presented at the 33rd European Congress of Clinical Microbiology and Infectious Diseases (ECCMID) 2023.

SARS-CoV-2 Pandemic and *Neisseria meningitidis* Serogroup B Invasive Infections: Insights From Italian Surveillance Data and Vaccination Rates

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BACKGROUND AND AIMS

Vaccination against *Neisseria meningitidis* serogroup B (MenB) has significantly aided the global efforts against invasive meningococcal disease (IMD).[1,2] Some reports have suggested that, despite the lack of clear effects on carrier status, MenB is highly effective in reducing the occurrence of incident cases in highly vaccinated populations.[2] Mitigation measures that have been implemented during 2020 against the severe acute respiratory syndrome coronavirus 2 pandemic have allegedly impaired the access to vaccination services, potentially impairing the delivery of childhood vaccinations, including MenB. Therefore, a recent increase in incident cases could be identified by retrospective analysis of corresponding data.

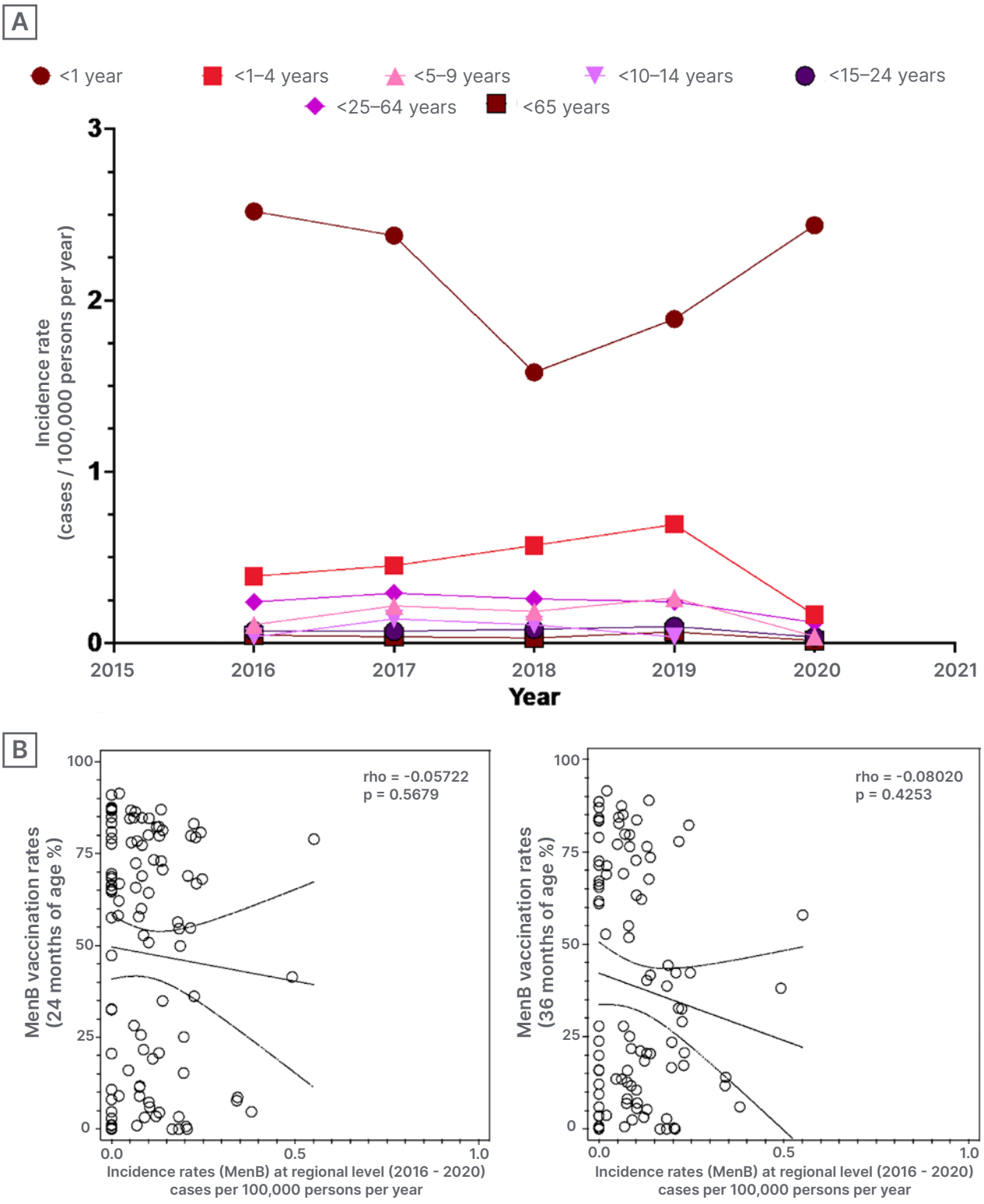
MATERIALS AND METHODS

The authors retrieved official Italian notification and surveillance data on IMD and vaccination rates for MenB between 2016–2020.[3,4] All data are provided as aggregate at regional level, and by age group at national level. Excess incidence rates were calculated with their 95% confidence intervals (CI) as per cent values for 2020 compared with the average for 2016–2019. Correlation between incidence rates and vaccination rates was estimated through calculation of the Spearman's rank correlation coefficient (rho). Data included in this analysis were retrieved as anonymous and collective from National Health Service (NHS) repositories; therefore, no preventive ethical approval was required for their analysis.

RESULTS

A total of 34 cases of MenB IMD were reported in 2020, compared with 84 in 2019, 71 in 2018, 74 in 2017, and 67 in 2016. The standardised incidence rate for IMD associated with MenB was 2,811 per 100,000 persons in 2020. Corresponding excess incidence rates were estimated in -56.51%

Figure 1: (A) Incidence rates (2016–2020) for invasive meningococcal diseases associated with *Neisseria meningitidis* serogroup B; and (B) correlation between vaccination rates for *Neisseria meningitidis* serogroup B (24 months of age and 36 months of age) and invasive meningococcal disease at regional level, Italy, 2016–2020.



MenB: meningitidis serogroup B; rho: Spearman's rank correlation coefficient.

(95% CI: 27.3–-85.7%), with heterogeneities by age groups, and among the Italian regions. Incidence rates decreased in all age groups but newborns (+14.9%; 95% CI: -23.1–+53.1% [Figure 1A]). Corresponding vaccination rates increased from 14.7% at 24 months in 2016 to 66.3% in 2020 (70.0% in 2019; Wilcoxon signed rank test; p=0.226), and from 7.7% at 36 months in 2016 to 72.9% in 2020 (48.5% in 2019; p <0.001). No correlation was identified between incidence rates and vaccination rates, neither for vaccination rates at age 24 months (rho=-0.057; p=0.568), nor for 36 months (rho=-0.080; p=0.425 [Figure 1B]).

CONCLUSION

During 2020, vaccination rates for MenB were only moderately affected by mitigation measures, while incidence rates dramatically decreased compared to the previous years, in both raw and crude figures. Vaccination rates unexpectedly increased from 2019 to the end of 2020. Still, as no correlation was identified between vaccination rates and incidence rates, the most likely explanation resides in a positive effect of non-pharmaceutic measures elicited by the severe acute respiratory syndrome coronavirus 2 pandemic, as otherwise suggested by epidemiological studied on other respiratory pathogens (e.g., respiratory syncytial virus, influenza, etc.).[5-7]

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Out-of-Season Epidemic of Respiratory Syncytial Virus in Denmark in the Summer/Autumn of 2021, with More Cases and Admissions than Seen in Previous Winter Seasons and a Shift in Affected Age Groups Towards Older Children Aged 2–5 Years

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Keywords: COVID-19 interventions, epidemiology, national surveillance, out-of-season epidemic, public health, respiratory syncytial virus (RSV).

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BACKGROUND AND AIMS

During the COVID-19 pandemic, non-pharmaceutical interventions suppressed the circulation of many respiratory viruses, including respiratory syncytial virus (RSV).[1,2] Thus, the expected winter season of 2020/21 did not appear in Denmark. However, as restrictions were lifted during spring 2021, an unusually large RSV epidemic occurred in the summer/autumn of 2021. The aim of this study was to compare the RSV summer/autumn epidemic with previous winter seasons using national Danish registries.

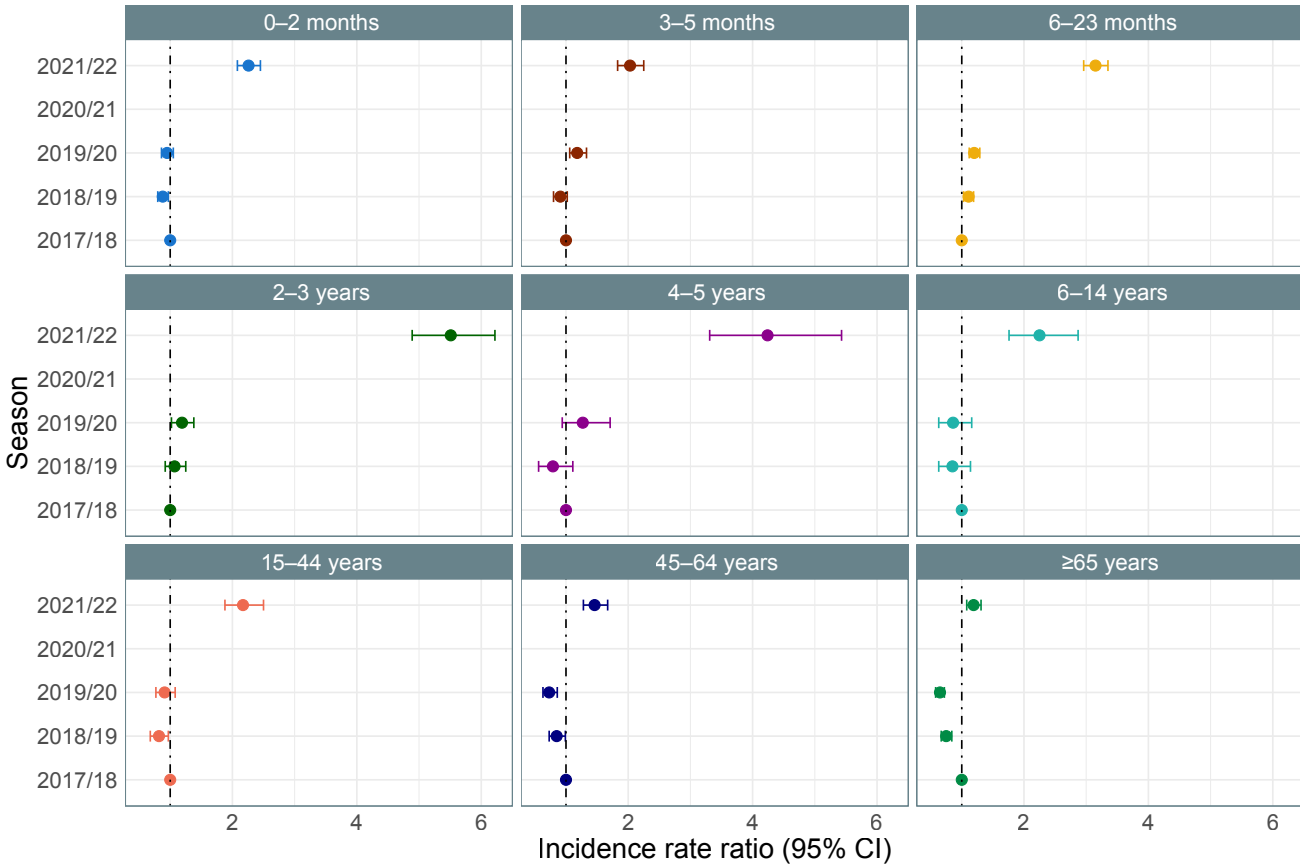
MATERIALS AND METHODS

A retrospective, register-based study was conducted to analyse the occurrence of RSV in the Danish population in three typical pre-COVID-19 RSV seasons (2017/18, 2018/19, and 2019/20) in comparison to the unusual RSV epidemic during the summer/autumn of 2021. Laboratory-confirmed RSV cases were identified using the Danish Microbiology Database (MiBa).[3] Cases were linked to the National Patient Register[4] to identify RSV-related admissions and patients receiving intensive care treatment (ICT) during an RSV-related admission. Seasonal incidence rates (IR) per 1,000 person-years of RSV cases, RSV-related admissions, and ICT were calculated with 95% confidence interval (CI) for each season, stratified by age group. The seasonal IRs were compared by calculating IR ratios (IRR) with 95% CI using the 2017/18 season as reference. Finally, to explore if a more severe type of RSV was circulating in the summer/autumn, the relative risk (RR) of a case being admitted to hospital and an admitted case receiving ICT was calculated with 95% CI using the 2017/18 season as reference.

RESULTS

For the summer/autumn epidemic, IRs of RSV cases exceeded previous winter seasons for all age groups. Compared with the winter season of 2017/18, the highest IRRs of cases in the summer/autumn epidemic were detected among children aged 2–3 years and 4–5 years, with IRRs of 5.5 (95% CI: 4.9–6.2) and 4.2 (95% CI: 3.3–5.4), respectively (Figure 1).

Figure 1: Incidence rate ratio of respiratory syncytial virus cases by season and age group using the 2017/18 season as the reference season.



CI: confidence interval.

For hospital admissions, IRs were significantly higher in the summer/autumn epidemic than in the 2017/18 season, except for the age groups of 6–14 years and ≥65 years. The highest IRRs were detected in children aged 2–3 years and 4–5 years, with 3.7 (95% CI: 3.0–4.6) and 4.3 (95% CI: 2.6–7.1), respectively.

The risk of a case being admitted in the summer/autumn epidemic compared with the winter season of 2017/18 was significantly lower in most age groups. This was especially the case for children ≤5 years, where RRs ranged between 0.6 (95% CI: 0.4–0.6) and 0.9 (95% CI: 0.8–0.9), suggesting that the cases detected in the summer/autumn epidemic were milder than in previous seasons.

The risk of an admitted case receiving ICT was only significantly higher in the summer/autumn epidemic than in the 2017/18 season for children aged 3–5 months and children aged 2–3 years with RRs of 1.5 (95% CI: 1.1–2.1) and 2.3 (95% CI: 1.1–4.8), respectively.

CONCLUSION

The summer/autumn RSV epidemic of 2021 was considerably larger than previous RSV winter seasons in terms of both confirmed cases and hospital admissions. A shift in age groups affected by RSV was observed, most prominently in children who are 2–5 years old. The authors speculate that this age shift may be explained by an immunity debt due to suppression of RSV in the winter of 2021. Further, there were no indications that the specific RSV type circulating in the summer/autumn epidemic of 2021 per se caused

more severe disease as the risk of a case being admitted was lower and the risk of an admitted case receiving ICT was similar when compared to previous winter seasons.

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Abstract Highlights

The following selected highlights spotlight several interesting and timely abstracts presented at the 2023 European Congress of Clinical Microbiology and Infectious Diseases (ECCMID) congress, covering topics such as subacute and chronic meningitis diagnosis, multidisciplinary approaches for *Clostridioides difficile* treatment, and bloodstream infection risk factors.

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Risk Factors for Gram-negative Bloodstream Infection Revealed

FOLLOW-UP blood cultures (FUBC) are controversial; while positive FUBCs are an effective prognostic marker associated with increased survival, they are also associated with longer hospital stays and increased treatment duration.

Researchers at the Infectious Diseases Unit and the Clinical Pharmacology Unit, IRCCS Azienda Ospedaliero Universitaria di Bologna, Italy, conducted a systematic review and meta-analysis to assess the impact of FUBCs on the outcome and management of patients with Gram-negative bloodstream infection (GN-BSI) along with the risk factors for persistent GN-BSI. PubMed-MEDLINE, Scopus, and Cochrane Library Database were independently searched until 24th June 2022 to retrieve randomised control trials and observational studies. Studies were excluded if quantitative target outcome results for intervention or comparator group were missing, or if data adjusted for cofounders was unavailable. Data was extracted and the quality of each included paper was assessed according to the risk of bias 2 tool for randomised control trials, and ROBINS-1 tool for observational studies. The meta-analysis was then performed by pooling adjusted odds ratios (OR) using a random-effect model with inverse variance method.

The database search identified 3,747 articles, which were screened, resulting in the identification of 11 studies. Overall, the execution of FUBCs was associated with a significantly lower risk of mortality (OR: 0.58; 95% confidence interval [CI]: 0.49–0.70) without heterogeneity ($p=0.68$; $I^2=0.0\%$) and publication bias. However, FUBCs were also associated with increased treatment duration (standardised mean difference: 0.65; 95% CI: 0.45–0.84) and longer hospitalisation (standardised mean difference: 0.75; 95% CI: 0.19–1.31). Regarding independent risk factors for a positive FUBCs, end-stage renal disease (N=3; OR: 2.99; 95% CI: 1.77–5.05), central venous catheters (N=4; OR: 3.30; 95% CI: 1.82–5.95); infections due to extended spectrum β-lactamase or carbapenemase-producing *Enterobacteriaceae* (N=4; OR: 3.24; 95% CI: 2.01–5.23); resistance to empirical treatment (N=3; OR: 2.70; 95% CI: 1.65–4.41); and an unfavourable response within 48 hours (N=2; OR: 2.99; 95% CI: 1.44–6.24) emerged. No substantial heterogeneity or publication bias were found.

Overall, the analysis could aid with the stratification of patients at low or high risk for persistent bacteraemia, thus optimising the use of FUBCs in patients with GN-BSI.

"The database search identified 3,747 articles, which were screened, resulting in the identification of 11 studies."

Multidisciplinary Review and Diagnostic Approaches Align for the Treatment of *Clostridioides Difficile*

A RECENT prospective, multidisciplinary review has recognised the value that comes with the alignment of multidisciplinary review and diagnostic approaches in the treatment of *Clostridioides difficile* infection (CDI).

Researchers from Beaumont Hospital, Dublin, Republic of Ireland, and the Royal College of Surgeons in Ireland (RCSI) ran a retrospective study to assess the current two-step testing guideline approach for clinicians to diagnose CDI. At Beaumont Hospital, the testing protocol was amended in 2015, first using a PCR test to check for *C. difficile*, and following this with an enzyme immunoassay (EIA) to check for *C. difficile* toxin if the PCR test indicated a positive result.

The impacts of this approach, as well as surveillance categories and treatment for CDI infection, were reviewed using data from between 2016–2021 held in a centralised database. Data included laboratory results for *C. difficile*, treatment details, case category, and origin of cases. Every positive PCR result was given a weekly prospective, multidisciplinary review.

Researchers created three categories for cases of CDI: case definition criteria fulfilled; case definition unmet, but clinical treatment for CDI indicated; and case not meeting definitions, with no treatment necessity. Sensitivity and specificity analyses determined CDI from positive EIA toxin results. Researchers also utilised χ^2 analyses in order to investigate possible associations existing between the case definition and EIA toxin results.

Data included 1,305 PCR results positive for *C. difficile*. Of these, 43.1% were positive for EIA toxin and 56.9% negative. Around one-third of results failed to meet case definition, and no treatment was therefore needed; 59.2% of results were positive for CDI; and 6.7% of cases did not meet definition, but treatment was clinically indicated. Of those requiring treatment, 43.0% were toxin-negative. Toxin-positive patients were more likely to meet the case definition for CDI (odds ratio: 4.6; $p<0.01$; 95% confidence interval: 3.6–6.0).

Researchers concluded that when clinicians are diagnosing CDI, it is important not to depend on the results of a single laboratory test. Using a multidisciplinary approach is optimal for patient management, as well as diagnosing definite cases of CDI.

"Data included laboratory results for *C. difficile*, treatment details, case category, and origin of cases."

What Increases 30-Day Mortality Risk in Bloodstream Infections?

WORLDWIDE incidence of vancomycin-resistant enterococci is increasing. Therefore, researchers from the Trieste University Hospital, Italy, investigated enterococcal blood stream infections risk factors and 30-day mortality, focusing on *Enterococcus* species, vancomycin resistance, and appropriate treatment. The team also investigated if the timing of receiving appropriate treatment had an impact on 30-day mortality.

Most cases of nosocomial bloodstream infections are caused by *Enterococcus faecalis* and *Enterococcus faecium*. All patients aged >18 years who had a positive blood culture of either were retrospectively included in this study. Appropriate antibiotic therapy was defined as active therapy against isolated *Enterococcus* commencing within 24 hours of diagnosis, and lasting for a minimum of 5 days.

Of the 584 patients included in this study, 93 had vancomycin-resistant *E. faecium*. The 30-day mortality was analysed with a multivariable Cox model. The 30-day mortality rate for vancomycin-resistant *E. faecium* bacteraemia was higher when compared to vancomycin-sensitive *E. faecium* and vancomycin-sensitive *E. faecalis* (hazard ratio [HR]: 1.701; 95% confidence interval [CI]: 1.214–2.383; p=0.002). However, male gender and an infectious disease consultation were independently associated with lower mortality (HR: 0.666; 95% CI: 0.481–0.921; p=0.014; and HR: 0.504; 95% CI: 0.352–0.719; p<0.001, respectively).

"Of the 584 patients included in this study, 93 had vancomycin-resistant *E. faecium*."

Further, the mortality rate was 11.4% when antimicrobial treatment commenced within 24 hours; however, this rose when active therapy started later. Appropriate antimicrobial treatment was also associated with lower mortality (HR: 0.682; 95% CI: 0.488–0.955; p=0.026). However, Pitt bacteremia score (PBS) and complicated bacteremia are independently associated with higher mortality (HR: 1.269; 95% CI: 1.192–1.350; p<0.001; and HR: 1.818; 95% CI: 1.304–2.535; p<0.001, respectively).

Delayed antimicrobial treatment is associated with a higher 30-day mortality rate, and there is a higher risk of 30-day mortality with vancomycin-resistant *E. faecium* bacteraemia.

Aetiologic Diagnosis of Subacute and Chronic Meningitis Remains Challenging

NEW research confirms the challenges in diagnosing the aetiology of subacute or chronic meningitis (SOCM). While diagnostic tests to identify causes of SOCM have expanded, with metagenomic next-generation sequencing to aid the identification of novel or unexpected pathogens, these techniques are expensive and not always accessible, especially in low- or middle-income countries.

A team reviewed medical records and discharge letters of patients 16 years and older (median age: 37 years) with SOCM who were admitted between March 2015–September 2019 in Mashhad, Iran, as well as online patient registration forms of those admitted between October 2019–October 2022. They scored outcomes using the Glasgow Outcome Scale (GOS).

In total, 183 episodes of SOCM were diagnosed. The most common infectious cause of SOCM was tuberculous meningitis, with 86 (47%) cases, followed by Brucella meningitis with 45 (24.6%) cases. In 72 (39.3%) cases, aetiology was confirmed; however, it remained unknown in 45 (24.6%) and presumptive in 66 (36.1%). Mortality rate before hospital discharge was 14.3%, but 44 (29.3%) patients who survived experienced unfavourable outcomes. Of note, patients with an unknown aetiology were at higher risk of in-hospital death compared with those with proven or presumptive diagnosis (31.1% versus 8.7%; p<0.001; odds ratio: 4.74; 95% confidence interval: 1.996–11.267).

The team concluded that determining the cause of SOCM remains a challenge, as less than 40% of episodes led to a cause-specific diagnosis. Approximately half of the patients experienced unfavourable outcomes, and those with an unknown cause had a five-times higher risk of in-hospital death. Therefore, more efforts to find the causes of SOCM are necessary to improve patient outcomes, and more rapid, accurate, and low-cost tests are necessary for this.

"In 72 (39.3%) cases, aetiology was confirmed; however, it remained unknown in 45 (24.6%)."

Interviews

Anne L. Wyllie, Louise Dyson, and Radhika Polisetty spoke with EMJ, sharing insights into their careers and inspiring research. The experts also covered several other key topics in the field, including antimicrobial resistance, the COVID-19 pandemic, and the One Health approach.

Featuring: Anne L. Wyllie, Louise Dyson, and Radhika Polisetty

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Q1 After completing your BSc in Immunology and Microbiology and MSc in Cancer Immunology in Auckland, New Zealand, you moved to Utrecht, the Netherlands, to undertake a PhD on the surveillance of pneumococcal carriage in all ages. What or who influenced you to pursue this line of work?

While I selected the immunology and microbiology major during my biomedical sciences undergraduate degree, this was purely due to my interest in immunology, as I did not actually like microbiology at all. Through this, I became fascinated with cancer immunology and completed my masters in this, before moving overseas, first to London, UK, and then to Amsterdam, the Netherlands. Without career experience in the laboratory, I struggled to secure a research position. Fortunately, a former colleague connected me to the research group of Debby Bogaert and Krzysztof Trzciński at the University Medical Centre (UMC) Utrecht, and they thankfully gave me a chance. I very gratefully accepted the role of laboratory technician, despite it being microbiology-focused. Their supervision and research group was incredible though and I quickly found myself absolutely gripped by the work we were doing. My colleague and I would actually run down the corridors to eagerly share results with them as they came through. Our work broadly focused on the respiratory microbiome together with a particular focus on *Streptococcus pneumoniae*. This is where my work in salivary diagnostics started. Trzciński had been combing through literature from the early 1900s and noticed all the pneumococcal research involved use of saliva samples. We decided to revisit this sample type to investigate whether it could help to improve detection of pneumococcal carriage, particularly in older adults, and this formed the basis of my PhD thesis.

Q2 You have won several awards over the last 3 years, including the 2021 COVID-19 Research Award from Yale School of Public Health, New Haven, Connecticut, USA. Can you explain the research you performed that resulted in you receiving this award?

Having developed sensitive sampling and detection methods for using saliva to improve the detection of pneumococcus, when witnessing the numerous challenges arising from complete reliance on nasopharyngeal swabs at the start of the COVD-19 pandemic, I wondered whether saliva could prove useful in this setting. My colleagues at Yale were supportive of this idea and within a few weeks we had obtained a robust dataset showing saliva to perform at least as well as, and in many cases better than, the 'gold standard' nasopharyngeal swab. Recognising early on that there would be a need for frequent, repeat testing as communities re-opened and that nasopharyngeal swabs would not be ideal for that, we worked to further optimise saliva for severe respiratory syndrome coronavirus 2 (SARS-CoV-2) detection. A major driver in this was to increase access to testing, removing barriers from sample collection through to cost. This motivation led to the development of SalivaDirect™ (Yale School of Public Health, New Haven, Connecticut, USA), an RNA extraction-free PCR test we validated for use on numerous PCR instruments and with many different reagents. This allows labs to more quickly and easily implement it into their own settings, utilising their existing infrastructure and supply chains, and thereby supporting a lower cost testing option for patients.

Q3 You were also awarded the 2023 Young Investigator Award in Clinical Microbiology and Infectious Diseases at the European Congress of Clinical Microbiology and Infectious Diseases (ECCMID) for your work into testing saliva for COVID-19 detection. Can you summarise your research in this area and the outcomes.

I am incredibly honoured to have received the 2023 Young Investigator Award in Clinical Microbiology and Infectious Diseases, and the 33rd ECCMID Congress in Denmark will always be a memorable point in my career. This award was in recognition of the validation and optimisation of saliva as a reliable sample type for the detection of SARS-CoV-2, which led to the development of a low-cost, simplified, open-source PCR test to help increase access to testing during the COVID-19 pandemic. Importantly, we have continued to build upon this and have worked to expand this testing approach for the detection of other respiratory pathogens such as influenza, respiratory syncytial virus (RSV), human metapneumovirus, and pneumococcus. We also demonstrated its flexibility for responding to local outbreaks, quickly adapting and validating it for the detection of mpox virus as cases spread around the USA.

Q4 Following on from this research, you developed SalivaDirect™ to advance the use of saliva-based testing. Could you explain the protocol and give insight into your future vision for this project?

The protocol is incredibly straightforward and has even been likened to a high school biology project. We ask that a passive drool sample be collected in a simple, laboratory plastic tube (such as a 2, 5, or 25 mL Eppendorf tube [Eppendorf, Hamburg, Germany]). No buffers or preservatives are required, as we have shown that the detection of SARS-CoV-2 remains stable for at least 7 days. On arrival at the lab, a very small aliquot of this sample is taken, of just 50 µl. Due to the flexibility in our protocol, to work with the different safety requirements in each lab, the sample is then subjected to a heat pre-treatment step and/or the addition of proteinase K, before testing 5 µl in PCR with the USA Centers for Disease Control and Prevention (CDC)'s N1 primer/probe together with human ribonuclease P for sample quality control.

This streamlined and importantly low-cost approach facilitated large testing programmes, keeping schools and workplaces open. Going forward, I hope that it can support sustainable surveillance programmes as we continue to monitor SARS-CoV-2 around the world. In addition to this, we have expanded the assay for the multiplexed detection of other respiratory viruses. With this, I hope that we extend surveillance efforts more broadly.

Due to the rapid advances in diagnostics and the large-scale testing programmes over the past 3 years, SARS-CoV-2 has now been studied more closely than any other respiratory pathogen. This leap forward presents a unique opportunity. With the cutting-edge tools available, we now have the opportunity to expand the depth and intensity of this scrutiny to other respiratory viruses, examining the interplay between early infection and downstream disease. Going forward, I hope that SalivaDirect can play a role in this. With ease of collection and low testing costs, it is well-suited to support sustainable surveillance programmes. This can be applied more broadly in applications such as monitoring the RSV strains circulating as new therapeutics and vaccines move through the pipeline. Being flexible, the assay can be rapidly updated to reply to outbreaks of other respiratory pathogens of concern. As an academic researcher, I am excited that its low cost can help make our research funding go further, so that more samples can be collected and tested compared to when more expensive methods are applied, meaning we can obtain more data.

Q5 What are the main challenges associated with using saliva for infectious disease detection over the use of nasopharyngeal swabs?

The main barriers to the greater utilisation of saliva-based tests for the detection of infectious disease are awareness and acceptance at the laboratory level, as saliva is not a traditional diagnostic sample type. As such, when the COVID-19 pandemic started in 2020, most laboratories simply were not familiar with working with saliva, and they neither had the experience nor the skillset, while my lab was set up through nearly a decade of my work with saliva. This meant clinical laboratories were not equipped with robust go-to methods or protocols to process saliva effectively and reliably. Saliva is a more complex sample type than the transport media swabs are typically placed into and, therefore, requires methods specifically suited to this. Just as a laboratory protocol for swabs would not be expected to reliably process a blood sample, we should not simply expect that a method that works for swabs will perform comparably for saliva. Unfortunately, many labs initially applied their methods for swabs to saliva, had them fail, and concluded saliva did not work for SARS-CoV-2 detection. In reality, their methods failed. Fast forward 3 years and nearly 10 million SalivaDirect COVID-19 PCR tests later, labs have been achieving equally great results with saliva samples. There have also been many more robust saliva-based methods successfully developed and applied all around the world.[1]

"A major driver in this was to increase access to testing, removing barriers from sample collection through to cost."

Importantly, this is not about mastering difficult or complicated techniques. It is because they are utilising methods specifically developed or evaluated to produce sensitive and reliable saliva-based testing outcomes.

However, a major challenge to more widespread testing, which is perhaps not surprising for a new testing approach, is that saliva-based PCR test validation has been held to greater levels of regulatory scrutiny. For example, anterior nasal swabs had previously been viewed as being less sensitive than the gold standard nasopharyngeal swab. However, anterior nasal swabs were more rapidly accepted as a sample type than saliva for SARS-CoV-2. Nonetheless, accumulating evidence has proven certain saliva-based PCR tests are at least as sensitive as nasopharyngeal swabs when used properly. Research from us and others also showed saliva-based PCR tests accurately detected the Omicron variant of SARS-CoV-2 as soon as 4 days earlier during infection compared to nasal swabs.

"This award was in recognition of the validation and optimisation of saliva as a reliable sample type."

It has been a battle to correct the amount of misinformation about saliva-based tests that has circulated in the literature and the media, leading to this hesitation from labs and regulatory agencies. It is unfortunate that in the race to contain COVID-19, flaws in study design (such as inadequately controlled populations, improper sample collection, or inefficient testing methods) or the reporting of study results led to an array of discrepant findings and incomplete conclusions. On the other hand, I am optimistic and hopeful about the solid work that is continuing by many around the world. The more we share, publish, and implement properly validated methods of saliva-based testing, the sooner saliva will become another gold-standard sample type for sensitive and reliable pathogen detection. Compared to last year's ECCMID in Lisbon, Portugal, the dialogue describing saliva-based testing has advanced considerably. Even earlier critics have begun to re-evaluate their beliefs and approaches as new information and real-world evidence comes to light.

Q6 Do you see this type of testing having wider applicability to other infectious diseases and can this type of testing be used on a global scale?

The COVID-19 pandemic reinvigorated global interest in saliva-based diagnostics. We demonstrated how well this sample type compares with the gold-standard for SARS-CoV-2 detection, particularly for large screening programs and repeat testing that had been required in K–12 school systems, universities, businesses, and congregate living communities. So, it is a logical next step to apply saliva methods to detection of other respiratory pathogens. While the typical respiratory pathogens largely disappeared during the early pandemic period, anticipating their return, we worked to expand SalivaDirect for the multiplexed detection of influenza and RSV in addition to SARS-CoV-2. Currently, the biggest challenge involved with this expansion is sourcing adequate numbers of paired nasopharyngeal and saliva samples from positive individuals. We have also demonstrated its flexibility to serve outbreak response efforts. As you note, our lab and others quickly determined mpox could be detectable in saliva samples and this should be investigated further. Rather than testing samples from skin lesions, which develop at later stages in disease progression, it should be evaluated whether saliva-based tests could allow for earlier diagnosis and prevention of further transmission.

In low- and middle-income countries, where disparities in access to infectious disease testing are often greatest, saliva-based diagnostics could potentially help change the public health landscape. As SalivaDirect was developed in response to many access-related problems, it is particularly well-suited for diagnosing and monitoring infectious diseases in low-resource settings. First, it is incredibly easy to collect saliva from subjects, so highly skilled healthcare workers are not required to assist sample collection. Second, we designed the SalivaDirect assay to decrease resource utilisation and increase throughput, which is important for overburdened labs worldwide. Third, we have shown that expensive tubes, buffers, special equipment, and cold chain transport are not required. Furthermore and especially important in resource constrained countries, our assay can

be used with multiple different reagents and PCR equipment. Together, these attributes make the saliva-based testing process much cheaper than other approaches. And if you want to monitor disease in the most difficult environments, you need to have a sustainable, reliable, low-cost solution, with which the public will comply.

Before the pandemic, saliva-based tests had been available for detection of HIV, DNA analysis, and numerous other applications. There is even a decent body of evidence in the literature with studies conducted prior to the COVID-19 pandemic, demonstrating the comparable performance of saliva for the detection of a wide variety of respiratory pathogens. Other studies have explored saliva for the detection of human papillomavirus, norovirus, leishmania, malaria, Zika virus, Chikungunya, Karposi's sarcoma-associated herpesvirus, and Epstein–Barr virus, to name a few. As a result of COVID-19, we are seeing a surge in the development of saliva-based tests. Our colleagues at Yale have also explored the suitability of saliva compared to sputum for the detection of tuberculosis in Uganda. Saliva techniques are being validated for detection of diabetes, gastrointestinal infections, sexually transmitted diseases, viral infections like hepatitis B, and cancer, as well as several bacterial infections. Last year, the first saliva-based pregnancy test became available, and encouraging research is progressing for earlier and easier diagnosis of concussions and lead poisoning using saliva samples.

Q7 Alongside the COVID-19 pandemic, the world has recently seen other infectious disease outbreaks, such as mpox. Do you think there are any gaps in infectious disease surveillance programmes and how can we potentially bridge these to improve outbreak prevention?

More research has been published about SARS-CoV-2 and the disease it causes than for any other virus of interest. Instead of squirreling away in our labs independently, in our collective response to the unfolding pandemic we broke through institutional barriers crossing academia, industry, non-profits, private organisations, and governments. Exceptional worldwide allocation of resources turned the tide against COVID-19, but epic levels of collaborative research generated the underlying scientific, technological, and medical solutions of disease mitigation and treatment. During this time, we witnessed the importance of, and need for real-time surveillance systems to reliably link vast inputs from multiple geographies and health systems. COVID-19 outbreaks in neighbouring states and countries became highly predictive of future infectivity at home. From variant to variant, the challenges of tracking SARS-CoV-2 transmission revealed the gaps in our surveillance systems, with strains slipping through these gaps at times and going undetected until they had spread quite far.

> **"We must keep open the recently established lines of communication and patterns of information sharing."**

Transitioning from the COVID-19 emergency, we can improve surveillance efforts through ongoing development of laboratory infrastructure, as we still do not have enough capacity. We must keep open the recently established lines of communication and patterns of information sharing. My team and I have experienced first-hand how partnerships between public health departments, academia, and the private sector contributed to improved infectious disease surveillance and delivery of care. In a perfect world, all public health labs would have a protocol like SalivaDirect in place, allowing them to quickly scale up inexpensive testing efforts when needed, without needing to stock expensive proprietary reagents and collection kits.

In the community, trust building remains essential, as is education about the safety and value of frequent screening, especially for our most vulnerable patients and under-resourced communities. Our research shows people do not want to have repeated nasopharyngeal swabs, with even nasal swabs quickly becoming irritating and inconvenient. Both methods, as well as blood draws, can increase patient aversion to testing, which clearly limits sustainable surveillance testing of asymptomatic individuals, and can hinder retention of research study participants. Invasive sample collection methods also require trained healthcare personnel, leading to staffing

bottlenecks and higher costs, probably the highest expense of these testing approaches. Significantly decreasing costs helps fund more research studies or generate more data points.

On the other hand, saliva as a sample for SARS-CoV-2 testing reduces collection burdens on healthcare providers, improves patient compliance, speeds lab throughput, lowers overall costs, and, most importantly, can detect infections at earlier, pre-symptomatic stages. That means public and private healthcare systems could potentially better detect outbreaks, prevent transmission, and stretch budgets for SARS-CoV-2 testing by including saliva as a sample in their testing portfolio.

Saliva samples collected once or twice annually in settings such as dentists, schools, or employers, could permit affordable health screening, which is key to deployment of preventative health measures.

As an academic, I am excited about the research potential recently created. Being so easy to collect, we can use saliva in research to better understand what goes on in the community before individuals end up at the hospital, which typically only represents the more severe cases. Through community surveillance efforts we can explore the interplay of multiple respiratory pathogens in circulation at a given time and place. We can better assess the consequences of one infection preceding another and how certain co-infections might amplify or mute disease progression. The implementation of multiple low-cost approaches that complement each other can help increase the efficacy and timeliness of disease surveillance. For example, wastewater surveillance is an inexpensive, low resource approach that can detect the circulation of pathogens in the community before symptomatic patients present to health centres. For some communities during the pandemic, municipal wastewater testing projects served as advance warning systems, helping communities mobilise resources and activate testing programs. Building on unconventional partnerships like these requires little investment, and could pay-off day-to-day or for future public health emergencies.

For disease surveillance programs to be effective and sustainable, ongoing sampling across large, diverse populations must be easy to carry out, acceptable, and much less expensive. Saliva self-collection combined with saliva-specific PCR assays like SalivaDirect represent a safe, simple, scalable, and cost-effective solution.

Q8 What were your three main highlights from the ECCMID 2023 Congress?

The programme and the attendees are the major highlights for me each year. This year proved no different with such a vast range of content on offer. On most days I found I had double or even triple booked myself when bookmarking all the sessions I wanted to attend. Fortunately, this year I had the pleasure of being able to support the attendance of two of my lab members, so we were able to take a divide and conquer approach and fill each other in on the various sessions. It was an absolute joy to share this year's conference experience with them as not only have they been instrumental to the development of SalivaDirect, but this was also their first conference experience, so there was a lot of excitement through it all. I also really value the opportunity that ECCMID provides to reconnect with colleagues who I have either worked with, collaborated with, or met at prior ECCMID events. Of course, it was certainly a highlight to attend this year's congress as an ECCMID awardee. It was such a pleasure to meet the other awardees and learn not only about their work but their experiences through their research careers.

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Q1 What is yaws and how can your research support the eradication and elimination of this neglected tropical disease? How have you collaborated with Michael Marks, Associate Professor at London School of Hygiene and Tropical Medicine, UK, to achieve this goal?

Yaws is a bacterial infection primarily seen in children, causing lesions in the skin and sometimes in the bones. It is one of a handful of diseases currently targeted by the World Health Organization (WHO) for eradication by 2030. This is an ambitious goal, especially as there are only two diseases that have ever been officially eradicated: smallpox and rinderpest. My work with Michael Marks aims to support disease eradication efforts by using mathematical modelling to infer missing information from the data and predict the effect of different potential intervention policies.

Q2 Why does the WHO recommend mass drug administration (MDA) as a strategy to control or eliminate many neglected tropical diseases?

MDA is an intervention policy for treatable diseases like yaws. The idea is to repeatedly give out drugs to the total population, including those with no symptoms or sign of infection. While it is not possible to reach absolutely everyone in one round of MDA, if each round has reasonable coverage and we miss different people in different rounds, then most people should eventually be treated. When the prevalence of disease is reduced, there are fewer infectious individuals and so fewer new infections. In this way we hope to gradually reduce the prevalence of disease and eventually eliminate the disease in the population.

Q3 Please explain why it is important to measure and model the effects of systemic non-adherence to MDA campaigns. Going forward, what implications may this have for the design of subsequent MDA programmes?

The success of an MDA campaign rests on reaching the majority of the population. If we instead systematically miss a significant group, they might provide a reservoir of infection, allowing disease resurgence after we stop the intervention. When some people are more likely to miss successive rounds of treatment, we call this systematic non-adherence. Significant work has been done for specific treatment campaigns to identify the causes of systematic non-adherence, and ways of reaching groups that are not receiving treatment. Our work instead aims to find ways of measuring the extent of the problem and estimating the effects of it on disease elimination. In particular, if we don't take systematic non-adherence into account then we may overestimate the effect of an intervention and stop too early, leading to disease

"This is an ambitious goal, especially as there are only two diseases that have ever been officially eradicated."

resurgence. The modelling framework is quite broad and can be applied to various infections that are targeted with MDA. It can also be used to investigate other sorts of interventions that can have systematic effects over many rounds, such as repeated community testing or multiple vaccine doses.

Q4 Could you tell us about your involvement in the UK's response to the COVID-19 pandemic? Were any of your reports considered by the Scientific Advisory Group for Emergencies (SAGE) to support the government response to COVID-19?

During the COVID-19 pandemic, I was a member of Scientific Pandemic Influenza Group on Modelling, Operational sub-group (SPI-M-O), the modelling subgroup of SAGE, supporting the UK government response. We had regular meetings, weekly during the main part of the pandemic and reducing to fortnightly in 'quieter' times. For each meeting there was a set of commissions, i.e., questions that were asked of the modellers with research to be completed and presented at the meeting (often only a few days after the commission came in). I was co-author on more than 60 documents sent to SPI-M-O, of which more than 20 went on to be considered directly by SAGE. Other documents and contributions at meetings were incorporated into consensus statements written by the SPI-M-O secretariat to be sent to SAGE. The whole SPI-M-O process was a bit like an extremely rapid peer review, in which leading experts in the field presented their response to commissions asked and others critiqued, asked for clarifications, and suggested improvements. For me, it was like a masterclass in a huge range of techniques and skills, not least how to present scientific insights in a clear and accessible way while communicating the limitations and uncertainties involved.

Q5 Could you discuss the key projects you have undertaken to date as a member of the Joint UNIversities Pandemic and Epidemiological Research (JUNIPER) Consortium?

In Autumn 2020, I was part of a group of SPI-M-O members from eight universities who formed the JUNIPER modelling consortium,[1] originally focused on collaborating on the COVID-19 response. Initially, the majority of our work was concentrated on responding to the direct commissions coming from SPI-M-O, but one of the advantages of forming a larger consortium was the ability to start planning ahead a little. In 2021, after the rapid expansion of the Alpha variant, and in response to the circulation of other variants of concern around the world, I led some work considering the potential for future waves of COVID-19 caused by variants of concern. The initial work, which was sent to SPI-M-O, included scoping of the international situation at the time and modelling of the potential impact of a novel (to the UK) variant on the UK pandemic. This work was later extended significantly to consider different putative variant characteristics, a more complex model including hospitalisations, and a consideration of the potential effect of border controls.

Q6 The WHO recently launched its 2021–2030 neglected tropical disease (NTD) roadmap. How are you able to support the WHO in targeting control efforts at this set of diseases?

The WHO launched the new NTD goals for 2030 and the roadmap towards them following intensive consultation with the global community, including managers of national NTD programmes, stakeholders in NTDs, and input from disease experts and modellers. I attended two meetings between the WHO and NTD disease modellers, facilitated by the NTD modelling consortium. The first was in 2019, consulting on the proposed goals, and the second more recently in 2022, discussing the design of strategies to meet the goals, and ways to measure and certify elimination. These consultations varied for the different NTDs, reflecting their varying degrees of progress towards elimination.

Q7 Have you been involved in investigating the 2022 mpox (formerly monkeypox) outbreak in England? How can infectious disease modelling groups inform and facilitate the implementation of public health strategies to prevent the transmission of mpox virus in human populations?

The JUNIPER modelling consortium has been modelling mpox in the UK, and I have been involved in some of these discussions, both internally and with the UK Health Security Agency (UKHSA). UKHSA work with JUNIPER and other groups, which formed part of the UKHSA Investigation into Monkeypox Outbreak in England: Technical Briefing 8.[2] This modelling aimed to investigate questions regarding the expected future of the mpox outbreak in the UK, including the expected size of the overall outbreak, time until the outbreak is over, and effects of vaccination. These analyses support policy decisions on public health strategies like vaccinations, and whether further interventions are required.

Q8 How important will the One Health approach be in controlling and preventing future pandemics?

The One Health approach recognises that human health is intrinsically linked to animal health and the environment. We can use this approach to identify places where infections with pandemic potential may emerge, and work together to reduce risk and respond to global health threats like COVID-19. It is essential to integrate these approaches with a focus on global equity, but also recognising the factors that lead to higher risk activities taking place. Sadly, both COVID-19 and mpox vaccinations have been very unequally distributed between the different countries and continents, highlighting persistent and ongoing global inequalities.

Q9 What are the potential impacts of climate change on emerging vector-borne infectious diseases in the UK and Europe?

The changing climate alters the regions of the world that can support different species. For vector-borne diseases, this also brings the potential for new regions to support disease transmission. Perhaps the most well-known example is that rising temperatures in Europe may render parts of Europe habitable to malarial mosquitoes. However, climate change has already begun to have an impact on vector-borne diseases in Europe, with increases in the number and geographical extent of infections with West Nile virus. The potential for infections expanding into new geographical regions presents difficulties for populations and health systems that are unused to identifying and treating these conditions.

Q10 What has been your most significant achievement during the course of 2022?

As has typically been the case since the COVID-19 pandemic began, this year has been a whirlwind of very different types of work, from the intensive short deadlines of government scientific advice,

"The One Health approach recognises that human health is intrinsically linked to animal health and the environment."

to university teaching in various forms, to much more long-term academic research. Throughout, it has been essential to form strong and supportive collaborations and work effectively together, and it is these teams that I am most proud of. Working together with other modellers (who might in normal academic life be somewhat in competition) in the JUNIPER consortium and in SPI-M-O has been a pleasure and a privilege, and I am proud of what we achieved together.

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Radhika Polisetty

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Q1 Please discuss the research contributions that led to you being awarded Fellow status by the Infectious Disease Society of America (IDSA) in August 2022.

I have been an active member of the IDSA for the past several years since completing my speciality pharmacy residency in infectious diseases (postgraduate year 2). As an infectious diseases pharmacist and a faculty member, I have been involved in taking care of patients, teaching infectious disease pharmacotherapy, and being engaged in clinical research at my hospital. I have created and implemented several protocols and order sets related to antimicrobial stewardship and presented our results as posters at Infectious Disease Week (IDWeek) meetings. Some examples include the 'Impact of a Two-Step Diagnostic Bundle on Hospital-Onset *Clostridioides difficile* Infection Rates and Treatment Across a Large Health System', 'Impact of Targeted Restrictions for Fluoroquinolones, in Two Community Hospitals', and a 'Multicentre Study to Evaluate the Impact of Antibiotic Time Out in Four Community Hospitals'. In addition, I have published several review articles for continuing medical education credit on various infectious diseases topics, such as 'Managing Clinical Expectations in Infections due to Gram-Positive Bacteria' and 'The Challenges of Hospital-Acquired and Ventilator-Associated Pneumonia and Recent Advancement in Antibiotic Treatment'. My recent research contributions to the literature on infectious diseases include 'Multicentre Project Evaluating the Nephrotoxicity of Vancomycin in Combination with Beta-Lactam Agents: Ceftolozane-Tazobactam vs. Piperacillin-Tazobactam' as well as a 'Multicenter Point Prevalence Evaluation of the Utilization and Safety of Drug Therapies for COVID-19 at the Onset of the Pandemic Timeline in the United States'. In addition to research, I am closely involved in the day-to-day operations and education of our clinical staff, including pharmacists, nurses, and physicians, and provide regular updates regarding COVID-19 therapeutics, antimicrobial stewardship pearls, drug shortages, and guideline updates to our providers. The IDSA considers the body of contributions to the field of infectious diseases in terms of direct patient care, teaching, and research when awarding Fellow status to its members. I am highly honoured to receive this designation.

Q2 Could you share the principal conclusions from your 2022 *EMJ Microbiology and Infectious Diseases* paper, 'Antibiotic Stewardship Attitudes and Beliefs Among Frontline Staff Nurses: Impact of Virtual Education'?

Nurses are vital healthcare team members who can play an important role in establishing or expanding antimicrobial stewardship programmes. However, nurses are often underutilised in antimicrobial stewardship activities and barriers to nursing involvement, such as lack of knowledge, scope of practice concerns, and time constraints, persist. This is partly because of the paucity of data on nursing attitudes and barriers towards antimicrobial stewardship, and because of the limited number of educational training programmes regarding antimicrobial stewardship that are

"Virtual education may be an option to increase nursing awareness and participation in antimicrobial stewardship."

designed specifically for nurses. This study was conducted to assess frontline staff nurses' baseline attitudes and beliefs towards antimicrobial stewardship, and to see if a virtual education campaign consisting of newsletters and tip sheets would affect those attitudes and beliefs. In our study, over 90% of the nurses surveyed considered themselves to be antibiotic stewards and wanted to participate in antimicrobial stewardship activities, such as assessing adverse drug reactions and educating patients. We also found that virtual education was effective in increasing the familiarity of our frontline nurses with the hospital antimicrobial stewardship programme. Therefore, virtual education may be an option to increase nursing awareness and participation in antimicrobial stewardship programmes, especially in resource-limited settings.

Q3 Your ePoster, entitled 'Impact of Implementing a Multidisciplinary Sepsis Bundle in a Community, Non-Teaching Hospital', was presented at the 31st European Congress of Clinical Microbiology and Infectious Diseases (ECCMID). Please provide an overview of the key take-home messages.

Sepsis is a cause of significant morbidity and mortality in the USA and around the world. The Surviving Sepsis Campaign guidelines, published in 2012, and since revised in 2018, made recommendations for early and goal-directed therapy in order to improve sepsis outcomes. Treatment bundles for 3-hour and 6-hour timeframes were recommended to improve compliance and outcomes. Guidelines suggest that increases in sepsis bundle compliance contribute to decreased sepsis mortality; however, implementation of these bundles remained a challenge, especially in resource-limited community settings. The purpose of the project was to decrease severe sepsis mortality at our institution to <20%, improve 3-hour bundle compliance to >31%, and improve sepsis alerts called in appropriate patients to >75%. In 2013, the executive leadership at our hospital established a multidisciplinary sepsis steering committee to address an observed sepsis mortality rate of 43.5% and 3-hour bundle compliance of 16%. The implementation of the multidisciplinary sepsis bundle with collaboration between critical care, emergency room, infectious diseases, pharmacy, and nursing was highly successful, and resulted in a bundle compliance of >60% and an average mortality rate of <20 % (28% decline from baseline; p=0.04) at our institution. Appropriate sepsis alerts called in also improved to an average of >80%. The success of the programme has been sustained over the past several years, and this initiative has greatly increased awareness of sepsis guidelines, criteria, and application. This study shows that strong administrative-level support, interactive web-based learning, a designated response team, and daily data sharing can lead to a successful sepsis initiative, even in resource-limited community settings.[1]

Q4 Please summarise your current research into antibiotic time-out and strategies to reduce rates of *Clostridium difficile*-associated diarrhoea.

Distinguishing acute *C. difficile* Infection (CDI) from colonisation is a challenge due to high rates of colonisation. PCR testing alone is not able to distinguish colonisation from infection, leading to overdiagnosis and unnecessary treatment. The purpose of this study was to evaluate the impact of this two-step testing algorithm bundled with education, antimicrobial stewardship programme support, and order set changes on hospital-onset CDI rates and *C. difficile* treatment across our health system. A two-step testing algorithm (PCR with enzyme immunoassay) was implemented between May 2021 and August 2021 across seven hospitals within the Northwestern Medicine Health System. Multifaceted education was delivered to leadership and clinicians in person as well as electronically. Antimicrobial stewardship team performed daily diagnostic prospective audit, result interpretation, and management support. The results showed that the hospital-onset CDI standardised infection rates reduced significantly from 0.80 to 0.57 ($p<0.001$). Although treatment of colonised patients remained high, a large number of patients safely avoided CDI treatment. Testing and education bundles can help advance antimicrobial and diagnostic stewardship by improving detection, treatment, and tracking of CDI.

"The results showed that the hospital-onset CDI standardised infection rates reduced significantly."

Q5 How can the penicillin-binding protein 2a assay be used to improve antimicrobial stewardship?

The penicillin-binding protein 2a assay is a fast, precise, and relatively inexpensive test for determining methicillin susceptibility in *Staphylococcus aureus* (methicillin-resistant *S. aureus*). Our team conducted a study using this assay, with and without stewardship intervention, and our results showed that there was a significantly improved time to optimum therapy. The simple assay can be used as a part of stewardship practices, especially in places with limited resources.[2]

Q6 What effects have COVID-19 and the ongoing mpox (formerly monkeypox) outbreak had on antimicrobial resistance? What opportunities may arise from the pandemic that could help tackle antimicrobial resistance in the future?

The pandemic had a huge impact on antimicrobial resistance. According to the Centers for Disease Control and Prevention (CDC), the rates on resistant hospital-acquired infections and deaths increased by at least 15% during the first year of the pandemic.[3] The causes of this were multifactorial. The pandemic led to severe staffing shortages, clinic and laboratory closures, supply chain issues, and overuse of antimicrobials among hospitalised patients, all of which helped set back the efforts made by healthcare teams to combat antimicrobial resistance. We hope that the losses are only temporary, and most infection prevention and infectious diseases teams will be able to regain their full resources to be able to get back to work on this important topic.

Q7 How can a One Health approach be leveraged to combat the rise of drug-resistant infections?

Antimicrobial resistance is a multifaceted problem that requires a multipronged approach to combat it. There is a lot of antibiotic use in our food supply, from farming to meat production, which leads to increased colonisation of resistant organisms, as well as outbreaks of zoonotic diseases. The World Health Organization (WHO) and the CDC have made the multifaceted approach a priority in their effort to promote public health. Education and collaboration between the public, government, private companies, and medical community is needed to combat the threat of antimicrobial resistance.

> **"Antimicrobial resistance is a multifaceted problem that requires a multipronged approach to combat it."**

Q8 You co-authored 'Standardizing a Centralized Allocation Process for Rarely Used Anti-Infective Medications Across a Health System', which was presented at Infectious Diseases Week (IDWeek) 2022. Please highlight the value of this study and its implications for clinical practice.

Our health system created a centralised allocation process for rare-use anti-infectives so that supply of these medications is easily available to all sites within the system and inventory is maintained at the academic medical centre by the clinical pharmacy team. For example, in two cases of severe malaria, the centralised process prevented delay in obtaining the medication and reduced the time to medication administration significantly. We are considering expanding this process to high-cost, infrequent-use antibiotics as well. This process is more efficient and promotes fiscal stewardship whilst optimising patient care.

Q9 What advances in research and policy are necessary to address antimicrobial resistance knowledge gaps? Going forward, how will you continue to promote appropriate prescribing of antimicrobials, both at national and international levels?

Data show that in the USA most antimicrobial prescribing happens in the outpatient setting,[4] where the antimicrobial stewardship efforts are difficult to implement due to logistical barriers. There is a lot of potential to expand the use of rapid diagnostic testing and point of care testing in our outpatient clinics and emergency rooms, where they can be used to determine the cause of infection quickly and hopefully prevent the prescription of unnecessary antibiotics. As an infectious diseases pharmacist, I will keep working on educating our providers and the public, and do my part to make sure that our patients are not being prescribed antimicrobials unnecessarily. ●

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A Perfect Storm: COVID-19 and Antimicrobial Resistance

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INTRODUCTION

For decades, infectious disease and public health experts have recognised antimicrobial resistance (AMR) and resulting infections due to multidrug resistant organisms as a persistent and increasingly urgent threat to public health at the local, national, and global level. The years leading up to the COVID-19 pandemic were marked by important victories in the battle against AMR, including a surge in scientific inquiry on the topic, the development of multinational best practice consensus statements, the establishment of regional and global venues to share information, and a partially-funded commitment by world leaders to address the topic in a serious and sustained manner. In the USA and many other countries, there were some data to suggest these efforts may be generating positive results. The Center for Disease Dynamics, Economics & Policy (CDDEP), a USA-based not-for-profit that tracks antimicrobial resistance for the USA, Canada, and over 30 European countries, observed a recent plateau and even decrease in antimicrobial resistance across some nations for certain key organism-drug combinations like *Escherichia coli*-fluoroquinolones and *Streptococcus pneumoniae*-penicillins.[1] However, a more recent global analysis of bacterial

antimicrobial resistance-projected AMR will become the leading global cause of death by 2050 if existing trends continue.[2]

In late 2019 the world changed, as did the landscape for addressing the global threat of AMR. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and its associated clinical syndrome (COVID-19) continue to drive a global pandemic of viral respiratory illness that has subjected the world's population to unprecedented morbidity and mortality that is ongoing, and will not be fully understood for years to come. Although the latest COVID-19 wave related to the Omicron BA.5 subvariant is receding, the resolution of the pandemic is not yet in sight, and may never be. Nevertheless, it is a critical moment to consider potential collateral damage to the global fight against AMR resulting from the pandemic. Although data is sparse, the available reports from several countries indicate acceleration of AMR in the post-COVID era.[3-8] Here, the authors highlight key factors that contributed to the impact of the COVID-19 pandemic on AMR, with an eye towards lessons learned and next steps.

IMPACT OF HEALTHCARE SYSTEM STRAIN ON INFECTION CONTROL AND ANTIMICROBIAL STEWARDSHIP

As the operational capacity of healthcare systems across the globe was severely strained during the pandemic, disruptions to standard infection control (IC) and antimicrobial stewardship (AMS) were inevitable. These practices are key to keeping AMR in check by limiting the spread of multidrug resistant organisms (MDRO) within healthcare settings (e.g., long-term care facilities, inpatient wards), and decreasing selective pressure related to inappropriate or unnecessary antimicrobial therapy respectively. The Centers for Disease Control and Prevention (CDC) has tracked sporadic outbreaks of MDROs, including *Acinetobacter* and *Candida*, during the pandemic.[9] These outbreaks were linked to a breakdown of IC procedures (e.g., less personal protective equipment use and decreased screening) during COVID-19 surges.[10]

Disruptions to standard IC measures designed to mitigate AMS are compounded by the disproportionately long hospital and intensive care unit lengths of stay observed in patients with COVID-19 compared with patients infected with other viral pathogens. Critically ill patients usually require lines, tubes, and/or drains, which pose an increased risk of secondary bacterial infections the longer they are in place. These infections are notoriously difficult to treat due to the lack of blood flow combined with a plastic matrix that facilitates bacterial growth and creates conditions ripe for the development of AMR. This results in a negative feedback loop, with MDRO concern increasing broad spectrum antimicrobial use (e.g., carbapenems), which in turn drives resistance at the unit and hospital level.[11] While this synergistic challenge to IC and AMS is formidable, established programmes manage it as part of routine operations. However, the pandemic introduced system level disruptions to IC and AMS, including shortages in personal protective equipment, increased workload, staffing issues, and units operating beyond typical capacity limits. Two surveys of AMS pharmacists identified significant disruptions to routine AMS activities, such as auditing and quality improvement initiatives, during the pandemic.[12,13]

LACK OF AVAILABLE THERAPEUTIC OPTIONS EARLY IN THE PANDEMIC

Given the novel nature of SARS-CoV-2, researchers, public health officials, clinicians, and even patients have searched frantically for therapeutic interventions to mitigate the morbidity and mortality related to COVID-19. Among the potential candidates for therapeutic intervention, several antimicrobial agents were identified and investigated, some scientifically, and others in an *ad hoc* manner. For instance, a comparison of antibiotic use in 1,944 nursing homes and long-term care facilities in the USA between January 2019–October 2019 and January 2020–October 2020, respectively, observed a 563% increase in antiparasitic hydroxychloroquine use in April 2020.[14] Unsurprisingly, this spike corresponds with a U.S. Food and Drug Administration (FDA) emergency use authorisation for the use of hydroxychloroquine for treatment of COVID-19 on 28th March 2020. The subsequent drop

back to baseline of both hydroxychloroquine use in long-term care facilities in May 2020 likewise corresponds with a subsequent FDA release on 24th April 2020, warning of heart arrhythmias in patients with COVID-19 treated with the combination of hydroxychloroquine and azithromycin. These sudden and dramatic swings in prescribing habits during this period, and continued public debate over the efficacy of azithromycin and ivermectin despite the absence of supporting evidence, highlights the persistent, detrimental effects stemming from the dearth of therapeutic options early in the pandemic.

ANTIBIOTIC UTILISATION IN PATIENTS WITH SUSPECTED AND CONFIRMED COVID-19

Perhaps the most striking example of COVID-19-related collateral damage to the fight against AMR is the widespread utilisation of antibiotic therapy in patients with suspected and confirmed COVID-19.[15-17] Although reports related to the most recent waves are notably lacking, the available pooled data indicate that a significant percentage of patients admitted with COVID-19 receive antibiotic therapy.[16] For example, Rose et al.[15] compared antibiotic use in 716 hospitals in the USA between 2019–2020, and found that although total antibiotic use during 2020 was lower, nearly 80% of inpatients hospitalised with COVID-19 received empiric antibiotic. This was most prominent during the first pandemic wave, despite identification of bacterial co-infections in only 3.5% of patients at admission, and secondary bacterial infections developing in only 14.0% of patients during hospitalisation.[15] Given the already noted disruptions in AMS, overuse of empiric antibiotics appears to have gone relatively unchecked during at least the first year of the pandemic. Ceftriaxone and azithromycin, a combination frequently used to treat bacterial lower respiratory tract infection in patients who have been hospitalised, made up the vast majority of antibiotic use in this population.[15] Ongoing surveillance in the coming years will be critical to determining if this unnecessary prescribing accelerated resistance patterns in clinically important bacterial pathogens such as *S. pneumoniae*, *Chlamydia trachomatis*, and *Neisseria gonorrhoeae*. Understanding the primary drivers of the massive discordance between bacterial co-infection and antibiotic utilisation is key to preventing this behaviour going forward.

Diagnostic uncertainty related to the substantial overlap in clinical presentation between patients with COVID-19 and bacterial pneumonia was potentiated by limitations in the availability of rapid diagnostic tests.[18] Frontline clinicians were frequently forced to make a decision to initiate empiric antibiotic therapy without the benefit of knowing the result of the SARS-CoV-2 assay.[19] While rapid identification of a viral pathogen can assist frontline clinicians faced with choosing therapeutic options for a respiratory infection, these assays are most effective when paired with information about host response.[20] Although procalcitonin is a biomarker approved by the FDA to assist clinicians differentiate bacterial and viral pulmonary infections, reports related to the utility of procalcitonin to reduce unnecessary antibiotic prescribing in patients with COVID-19 have been mixed.[21-23]

Beyond the clinical conundrum, frontline providers in the USA are also subject to a regulatory mandate that creates significant time pressure to initiate antibiotic therapy. In 2015, Centers for Medicare & Medicaid Services (CMS) instituted an all-or-nothing Severe Sepsis and Septic Shock Management Bundle (SEP-1), with the goal of encouraging high-quality, cost-effective care, and ultimately improving sepsis mortality. Performance on the measure is considered a marker of hospital quality of care, and health systems receive financial incentives for high performance. The SEP-1 bundle calls for, among other interventions, early administration (<3 hours) of parenteral antibiotics, typically broad spectrum, for patients who meet sepsis criteria. This definition of sepsis is built upon the suspicion of an infection and presence of systemic inflammatory response syndrome criteria. Of course, due to the non-specific nature of systemic inflammatory response syndrome, many patients with COVID-19 presented to acute care settings meeting these criteria presumably received broad-spectrum antibiotics by providers looking to adhere to the SEP-1 measure. While CMS was relatively quick to exclude COVID-19 patients from the SEP-1 measure, it remains unclear how aware frontline providers are of this exception and to what extent delays in confirmatory SARS-CoV-2 testing render this guidance moot.

Finally, the recent Omicron wave revealed significant ongoing deficiencies in the availability of both rapid testing and genomic sequencing, which remain functionally unavailable for large portions of the population. Until significant improvements in the availability of rapid and accurate tests for identification of SARS-CoV-2 for both the public and healthcare providers, and incorporation of host response biomarkers into care pathways involving empiric antibiotics, are made, uncertainty of diagnosis will continue to be the primary driver of antibiotic overuse.

SILVER LININGS: POTENTIAL POSITIVE EFFECTS OF THE PANDEMIC ON ANTIMICROBIAL RESISTANCE

In all likelihood, not every consequence of the COVID-19 pandemic will be synergistic with AMR. There is a compelling argument that AMR could show a downtrend in well-resourced countries based on decreased frequency of human travel and general improvement in IC practices. It has been hypothesised that AMR is proportionally driven more by close human interaction and poor IC than by antimicrobial overuse. Given the societal level shifts in hand hygiene, mask wearing, and physical distancing, it begs to reason we may see a decline in AMR in some settings.

It is also important to note that the pandemic had some positive effects on AMS at a systems level. For instance, significant drops in antibiotic prescribing in ambulatory care settings were observed; there are accelerating efforts to develop rapid biomarkers that can help frontline providers differentiate viral from bacterial respiratory infections; and an increased recognition that systems engineering approaches are necessary to build resiliency into IC and AMS processes during times of operational upheaval.[9,12,24]

CONCLUSION

In summary, the COVID-19 pandemic has posed a significant threat to the longstanding fight against AMR. Whether or not future variant-driven waves disrupt society and healthcare operations, the fallout from what we have already experienced will continue to play out in the years to come. While the available reports examining AMR pre- and post-pandemic demonstrate an alarming trend, it is unlikely that a causal mechanism will ever be clear, given the snarling and pervasive nature of this global natural experiment. Moving forward, emphasis should be placed on bolstering IC and AMS infrastructure and programmes, as they represent the most potent interventions to mitigate AMR in healthcare settings. During the current relative lull in the pandemic, it is important to reflect on missteps and lessons learned so that we can be better prepared for future, inevitable AMR-related threats to patient safety and public health.

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Overcoming COVID-19 Misinformation: Lessons Learned at the Epicentre of the Outbreak in the USA

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THE COVID-19 pandemic has highlighted the dire need to foster increased public confidence in mitigation and prevention strategies through more and better health literacy. More than 2 years into the worst public health crisis of the 21st century, we continue to be consumed by the most basic health questions: should I get tested for COVID-19, should I get vaccinated and boosted against COVID-19, and should I wear a mask? In many countries, the tension between personal freedoms and public good helps to fuel a global threat, with continued transmission of severe acute respiratory syndrome coronavirus 2 and its evolving, more infectious variants. This short essay discusses the negative effects of misinformation and disinformation, and shares recommendations based on lessons learned.

New York City, New York, USA, was an early epicentre of the COVID-19 pandemic in the USA. Based on the authors' experiences as healthcare professionals in New York City, epidemiologists, and one who directly treats patients, they found the crux of these questions can boil down to three factors: there is low health literacy, which is defined as the degree to which individuals have the capacity to obtain, process, and understand basic health information needed to make appropriate health decisions;[1] there is not enough clear and unambiguous risk communication and outreach to the community for the public to better understand their respective risk and the role of preventative and mitigation measures; and, finally, there is misinformation and disinformation that can adversely impact a person's judgment, perception of risk, and level of trust in the various preventative and mitigation measures.

The rapid and frequent changes in public health guidelines due to the evolving biomedical knowledge about COVID-19 led to much confusion, even among those with high levels of health literacy. During the COVID-19 pandemic, as you look at the rampant rise of disinformation, which is to deliberately mislead, as well as the spread of misinformation, which is to share false information regardless of whether there is intent to mislead,[2] it is clear that these are

not new concepts. For example, during the Ebola outbreak of 2014–2016 in West Africa, misinformation was one of the largest hurdles, requiring responders to enlist the help of trusted messengers within the community to share safe burial practices and ways to prevent the spread of and contracting Ebola virus disease.[3] There was also widespread misinformation that generated undue fear and anxiety based on the threat posed by Ebola in the USA.[4]

The authors' work in the field of infectious diseases has highlighted parallels to how people may react to new and novel threats. Maurice Policar has learned much about dealing with misinformation from working with patients during their first pandemic, HIV. Many similarities are striking, particularly the impact on marginalised and underserved communities; the popularity of unproven and 'unappreciated' medications, such as compound Q, apricot pits for HIV, and ivermectin for COVID-19; and the rejection of effective interventions, including antiretroviral medications for HIV and vaccines for COVID-19. Although it has been many years since Policar has seen a patient refuse HIV medication, this was not rare in the early decades of effective HIV treatment.

For Syra Madad, due to their work helping respond to the Ebola cases in Texas in 2014, and the public perception of the risk of Ebola spreading, their dentist refused to see them for routine dental care, citing fear of contracting the virus.[5] Combatting misinformation was difficult during the early AIDS epidemic, but no comparison to the current COVID-19 era. One study found that nearly 70% of adults have been exposed to COVID-19-related misinformation through social networking services or instant messaging.[6] People can find both misinformation and disinformation freely on the internet, social media, and even on the news. In the USA, it is so widespread that the Surgeon General issued an advisory in 2021 to warn the American public about the urgent threat of health misinformation.[7]

As healthcare professionals who have been working to combat misinformation and disinformation over the course of multiple different epidemics, here are three lessons the authors have learnt that can help increase health literacy before, during, and after any health crisis; build trust in public health and healthcare response; and address the growing issue of misinformation and disinformation.

LEVERAGE ALREADY BUILT TRUST AND PARTNERSHIPS

When it comes to asking people to change their behaviour, like wearing a mask or avoiding crowded, indoor spaces because of the threat of viral spread, facts are not always enough. There are plenty of so-called facts circulating that dispute whatever can be said to prove that masks work, physical distancing helps prevent exposure, or even that available vaccinations are safe and effective. People may not always trust scientific institutions or government authorities; however, since patients already rely on providers to look out for their health,[8] a personal expression of concern for their wellbeing may be the most powerful tool we have. One review mentions interpersonal-level interventions (interactions between clinicians and patients) as an evidence-based strategy to address vaccine hesitancy. Healthcare professionals have historically been the most important drivers of vaccine uptake.[9] Studies suggest beliefs about health risk are affected by who communicates the risk message. The source must be trusted, and trust is associated with believing that the source is expert, knowledgeable, and unbiased.[10] Doctors remain the most trusted source for health information.[11] Making strong recommendations using presumptive language ("I strongly recommend you get the COVID vaccine. The nurse will give you the vaccine on your way out") has been shown to result in higher vaccine uptake.[12]

In addition, using other trusted voices in a person's community, like a pastor, imam, or rabbi, can play a vital role in influencing people's behaviour and health-related decisions.[13] A review of responses to the Health Information National Trends Survey (HINTS) suggests that although religious organisations were trusted less than other sources, the non-Hispanic Black population and those with lower education both reported higher trust in religious organisations.[11] What works is using the already established relationships we have, specifically the trust we have built with peers, patients, colleagues, friends, and community members, and leveraging these to advance public health goals.

TALKING TO PEOPLE

When COVID-19 vaccines first became widely available to hospital staff in December 2020, hospital leadership suggested discussing vaccination with colleagues and staff who might be hesitant to get the vaccine. Many of us were sceptical about the impact we could have. Since they are healthcare workers, shouldn't they already know the benefits of vaccination? And if not, how could we convince them if they did not want to get vaccinated? The nature and extent of COVID-19 vaccination hesitancy in healthcare workers has been addressed in various studies. In one large review (76,471 participants), the prevalence of COVID-19 vaccination hesitancy in healthcare workers worldwide was on average approximately 22.0%, but ranged from 4.3% to 72.0%.[14] Many people just needed a trusted voice, and wanted to talk about the science, the facts, and the benefits.

After getting this information, it was encouraging to see that they either changed their minds, or at least agreed to reconsider getting vaccinated. Individual-level educational interventions have also been proven to empower healthcare teams to promote vaccination and optimise efforts to address vaccine hesitancy among patients.[8] This approach holds true for the general public as well. As Policar noted, it was clear that they could have a major impact on patients by using the basic tools they use every day, namely listening to patients' concerns and discussing the risks and benefits of any intervention with them. Policar was pleasantly surprised to see how many reluctant patients quickly agreed to vaccination after a discussion. Many just needed a 'nudge'. Tailoring messages to patients to address common barriers and concerns may improve vaccine uptake. Framing messages in a positive way and addressing barriers with affirming dialogue can be helpful ("These are good ways to protect you and your family, and stay healthy. I understand your concerns given the situation").[8]

LISTENING WITH COMPASSION

It is easy to listen to what a person is saying; however, it is challenging to listen with compassion, which is to be nonjudgmental, empathetic, and willing to put aside your own feelings. Research has revealed that direct efforts to counter misperceptions may backfire, resulting in an increase in misperceptions or a decrease in intention to be vaccinated.[8]

It is not difficult to imagine that many are sceptical about the government and the scientific community. Despite this, it is not our place to argue or defend these institutions. Instead, the focus should be on a person's wellbeing and the measures to keep them safe. What has worked for the authors is to share what they know; provide the science in a way that can be readily understand; and share their personal experiences, which can resonate more with people. As noted by the Centers for Disease Control and Prevention (CDC), one of the guiding principles when talking with patients about vaccination is to be compassionate and show empathy.[15]

CONCLUSION

A core principle in all three of these lessons learned is that they are rooted in trust. Trust is not something that is fostered overnight. Nor is it something that can be gained immediately during a health crisis. Rather, it takes time to build trust between and among people, providers, public health professionals, healthcare systems, and public health agencies. We cannot tell someone to just trust this agency or person. Instead, we must acknowledge this imperative core principle in outbreak response and begin to work actively to foster it.

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The Aftermath of Widal Positivity on the Diagnosis of Tuberculosis and Fluoroquinolone Resistance in India

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INTRODUCTION

Drug resistance among patients with tuberculosis (TB) is an emergent topic that requires intervention. According to a survey of TB drug resistance in India,[1] resistance to any fluoroquinolones (FQ) reported in cases of multidrug resistance (MDR) to TB was 21.82%. On the other hand, in a developing country like India, amongst the commonest causes of pyrexia of unknown origin, TB and typhoid fever are two important differentials, and it is a standard practice to order a Widal test when evaluating fever, especially at the primary health centre. The Widal test has very low specificity and sensitivity, and is confusing and difficult to interpret when diagnosing typhoid fever because cross agglutinating antibodies remain from previous infection with related *Salmonella* serotypes, which give a false-positive result. There are equal chances of getting false-positive results for the Widal test in non-typhoid infectious fever and in healthy individuals.[2] In typhoid-endemic areas, it has been demonstrated that a number of illnesses caused by non-*Salmonella* organisms (such as malaria, dengue, miliary TB, endocarditis, chronic liver disease, brucellosis, etc.) exhibit this cross-reactivity, and this increases the error rate of the Widal test result.[3]

DISCUSSION

In a cohort study by Roberts et al.,[4] it was found that the most common cause of delay in initiating TB treatment was a delay in making the decision to refer a patient to TB specialists, with a median delay of around 88 days. In fact, evidence suggests that each infectious case of TB in areas with a high frequency of the disease can cause up to 20 secondary infections before being discovered.[5]

Here, the authors discuss one cause for the same scenario (i.e., a false-positive interpretation of a Widal test). To date, there is no consensus concerning diagnostic criteria for interpreting the test. Serological diagnosis classically relies on the demonstration of a rising titre of antibodies in paired samples that are 10–14 days apart, which is usually not demonstrable in most blood culture-positive typhoid cases.[6]

As initial titres in endemic areas such as India may be higher than anticipated, a single positive Widal test is never considered to be diagnostic. Thus, a rising titre has to be seen, and each titre has to have been taken 2 weeks apart.[7] As laboratories lack the qualified, experienced personnel and infrastructure needed to detect and serotype *Salmonella* isolates, clinicians in India rely primarily on the Widal test for the diagnosis of typhoid fever, which has been used without determining the locally appropriate threshold titre.

If the Widal test is positive, a course of FQs is usually given as monotherapy at primary centres. But in a time of increasing resistance to FQs, it is necessary to rule out TB before treating the patient as a case of typhoid fever. A positive Widal test leads to a delay in the diagnosis of TB, and FQs resistance in mycobacteria may develop in the short exposure to quinolone monotherapy.[8] The Widal test has a sensitivity rate varying from 61.0–81.5%,[9] 18.3% for specificity, 10.1% for positive predictive value, and 89.7% for negative predictive value.[10]

Furthermore, a positive Widal test leads to the prescription of FQs, causing an increase in the emergence of FQ resistance in patients with TB. According to reports, 36% of MDR TB infections have extra FQ resistance in India.[11,12] Here, the authors emphasise the need to rule out TB at primary health centres in patients with persistent fever and prevent the delay in diagnosis, which is one of the causes of increased morbidity and mortality in TB.

In a case report studied by Skoutelis et al.,[13] very high titres of typhoid and paratyphoid agglutinins were obtained with the Widal test, and these titres returned to normal after successful anti-TB treatment. Both TB and typhoid fever are common in India. In a case series including 31 patients with TB and 29 patients without TB, the Widal test was found positive in 22.58% of TB cases and 37.50% in non-TB cases, indicating a very high rate of false-positives from the Widal test in both groups. A total of 22.58% of patients with TB had already received a course of quinolone monotherapy based on the positive test result.[14]

In one study, 11% of patients had *Mycobacterium tuberculosis* isolates, with a decreased susceptibility to FQs amongst those who received FQ therapy; however, no FQ-resistant *M. tuberculosis* isolates were recovered from patients who had not received previous FQ therapy.[3]

In the past, a true agglutination reaction from a case of typhoid fever, along with a false partial reaction in TB, has been pictured. The microscopic Widal test has been found to give positive results in many cases of TB with no history of typhoid fever. The TB fixation and microscopic Widal test corresponded in 62% of the cases.[15]

The Widal test is no longer being used in developed countries due to its poor performance.[16] Hence, its use and interpretation should be done cautiously and in accordance with the clinical context, which is an arduous job.[6] Even in non-endemic countries, the diagnostic value of the Widal test is too low. Due to its limited value, it is recommended for use in patients who experience negative repeated cultures.[17]

In India, healthcare professionals can adopt a strategy that prevents delay in typhoid or TB, as both have dreaded complications. Therefore, the authors propose that, at the primary care centre, a patient presenting for persistent pyrexia should be evaluated with a limited number of investigations such as a chest X-ray, ultrasonography of the whole abdomen, the Widal test, and other routine tests, including a complete blood count and inflammatory markers. In light of clinical history and screening investigation positivity, healthcare professionals can then proceed further (i.e., if the chest X-ray shows lesions, a sputum for Ziehl–Neelsen stain can be completed and, in case of a positive result from the Widal test, blood cultures can be sent).

CONCLUSION

Throughout this feature, the authors point to a situation where the clinician feels justified in using quinolone monotherapy based on a positive Widal test in India. This can lead to resistance of mycobacteria to quinolones, and thus facilitate the emergence of MDR or extensively drug-resistant TB. Along with it, there is an emerging need to rule out TB at primary

health centres in patients with persistent fever to prevent the delay in diagnosis, which is one of the causes of increased morbidity and mortality in TB. Although data related to this issue in the literature is scarce, more studies are required for understanding the pathophysiology of a positive Widal test in various other diseases and the exact duration of FQ therapy, which can cause resistance. Although more trials and research are needed, healthcare professionals should interpret the results of the Widal test cautiously, as misdiagnosis and improper patient management may come from incorrect interpretation of the test results, which may cause significant morbidity and mortality.

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Antibiotic Stewardship Attitudes and Beliefs Among Frontline Staff Nurses: Impact of Virtual Education

Editor's Pick

This research article by Polisetty et al. describes antimicrobial stewardship beliefs in frontline nursing staff, and evaluates the impact of virtual education on awareness and participation in antimicrobial stewardship strategies. Antimicrobial stewardship is of emerging importance globally. The lack of novel antibiotic development, and increasing prevalence of antimicrobial resistance, are key concerns, which have prompted a drive in the development and implementation of antimicrobial stewardship strategies and awareness promotion amongst healthcare professionals and the wider community.

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Abstract

Background: Nurses are vital healthcare team members and are often underutilised in antimicrobial stewardship (AS) activities. Several nursing responsibilities, such as taking allergy history and obtaining cultures, already overlap with AS activities. Nurses can play a crucial role in promoting AS in resource-limited settings. This study was conducted to assess frontline staff nurses' baseline attitudes and beliefs towards AS, and see if a virtual education campaign consisting of newsletters and tip sheets would affect those attitudes and beliefs.

Methods: An online survey (pre-survey) was conducted of all in-patient nurses employed in the authors' hospital on their attitudes and beliefs regarding AS. The survey consisted of 24 questions divided into three domains: demographic and practice information, nursing roles, and beliefs and attitudes towards AS programmes (ASP). After obtaining the results of the pre-survey, the authors started distributing monthly newsletters on various AS topics via email and posting them on a resource page. Topics included how to obtain an accurate allergy history, how to use microbiology results to help guide decisions, and stop therapy in cases of colonisation. The authors also distributed the same survey as a follow-up 6-month survey (post-survey) in March 2021 to gauge the impact of their virtual education efforts.

Results: In total, 109 nurses working in the adult in-patient setting of the authors' institution completed the pre-survey in September 2020, and 64 nurses completed the post-survey in March 2021. Overall, most nurses had a positive attitude towards AS tasks, and over 90% of those who responded in the pre-survey and post-survey agreed with the statement that nurses are antibiotic stewards, thought it was important or very important to obtain appropriate cultures, and understood the relationship between *Clostridioides difficile* and antibiotics. Most pre-survey respondents listed knowledge gaps in microbiology (47 out of 64 [86%]) and antibiotics (53 out of 64 [84%]) as well as scope of practice concerns (48 out of 64 [75%]) as barriers to nurse participation. The virtual education helped raise the familiarity with the ASP and more nurses in the post-survey said they were familiar with the stewardship programme compared with the pre-survey (48.4% versus 23.2%; p=0.001).

Conclusions: This study showed that most nurses consider themselves antibiotic stewards and want to participate in AS activities; however, barriers to nursing involvement, such as lack of knowledge, scope of practice concerns, and time constraints, persist. Virtual education may be an option to increase nursing awareness and participation on ASPs in resource-limited settings.

Key Points

1. Nurses are vital healthcare team members who can play an important role in establishing or expanding antimicrobial stewardship (AS) programmes (ASP). However, nurses are often underutilised in AS activities. In addition, barriers to nursing involvement, such as lack of knowledge, scope of practice concerns, and time constraints, persist. This is partly because of the paucity of data on nursing attitudes and barriers towards AS, and because of the limited number of educational training programmes regarding AS that are designed specifically for nurses.

2. This study was conducted to assess frontline staff nurses' baseline attitudes and beliefs towards AS and to see if a virtual education campaign consisting of newsletters and tip sheets would affect those attitudes and beliefs.

3. In this study, over 90% of the nurses surveyed considered themselves to be antibiotic stewards and wanted to participate in AS activities, such as assessing adverse drug reactions and educating patients. The authors also found that virtual education was effective in increasing the familiarity of frontline nurses with the hospital ASP. A virtual education may be an option to increase nursing awareness and participation in ASP, especially in resource-limited settings.

INTRODUCTION

The World Health Organization (WHO) has designated antimicrobial resistance as one of the top 10 public health threats facing the global community, which requires an urgent and multipronged response.[1] Surveillance reports from around the world have shown a rise in multidrug-resistant or pandrug-resistant pathogens, and a decline in the number of effective agents to combat them.[2] There are multiple drivers of increasing antimicrobial resistance; however, excessive and inappropriate antimicrobial use has been identified in several studies as a major factor behind the proliferation of drug-resistant bacteria.[3-5] According to the Centers for Diseases Control and Prevention (CDC), approximately 30% of the antibiotic prescribing in the USA hospitals is inappropriate.[6] In response to the growing threat of drug-resistant bacteria and dwindling number of anti-infective agents available in the market to combat these pathogens, the CDC and the Infectious Diseases Society of America (IDSA) have published several guidelines to encourage the implementation of comprehensive antimicrobial stewardship (AS) programmes (ASP) in healthcare settings;[7-9] however, lack of monetary resources and inadequate work force make it a challenge to establish a robust ASP, especially outside the in-patient hospital setting.[10]

Nurses are vital healthcare team members who can play an important role in establishing or expanding ASPs; however, they are often underutilised.[11-14] Although ASPs have been around for the past few decades, most programmes are traditionally made up of infectious diseases physicians, pharmacists, infection prevention specialists, microbiologists, and information technology experts. The nurse role in ASPs has typically been limited to infection prevention nurses or hospital epidemiologists, and their role in ASP is not well-defined.[11-15] This is surprising considering the number of studies showing the positive role of nurses in pain management, prescription use, surgical prophylaxis, and sepsis management.[16-19] Several nursing responsibilities already overlap with AS activities and they could have a significant impact in promoting AS in settings where monetary and labour resources are scarce.[12,13,20] Nurses play a vital role in obtaining allergy history, procuring cultures, reviewing microbiology results, and counselling patients.[21,22] Several studies demonstrate the positive impact of collaborating with nurses in order to optimise antibiotic use.[21-24] Whether it is reducing the number of unnecessary urine and respiratory cultures, which prevents inappropriate prescribing of antibiotics, or reducing the unnecessary testing for *Clostridioides difficile*, there are several ways in which nurses have shown to complement existing AS activities.[25-27] Nursing involvement in AS is now required by

regulatory bodies such as The Joint Commission (TJC) to help combat the threat of antimicrobial resistance in the USA.[28]

Integrating nurses into the fight against antimicrobial resistance is not without its challenges. Several factors, such as workflow, regulatory requirements, education of healthcare workers, and improvements in clinical practice, need to be considered.[29] Benefits of a multidisciplinary team-based approach in improving patient outcomes need to be defined clearly in order to increase nursing participation.[30-31] Surveys of nursing attitudes towards AS show that nurses, except for advanced nurse practitioners, do not perceive themselves as antibiotic prescribers and hence are not integral to stewardship efforts.[32] Also, studies have shown that 35–62% of nurses were unfamiliar with AS and ASPs at their sites. A survey of 159 nurses in a tertiary medical centre showed gaps in education and identified time constraints and concerns over physician pushback as significant barriers to nurse participation in AS activities.[33,34] Studies highlighting the role of nurses as antimicrobial stewards show encouraging signs but also demonstrate the need for formal education or training among the nurses in order to be successful.[35-42]

Although providing educational content thorough virtual reality or virtual cases is not a novel concept in medical or surgical training, the unexpected crisis of the COVID-19 global pandemic has forced several programmes to quickly adapt to virtual teaching methods in the absence of traditional training.[43-46] While some of these programmes have shown promising results, the concept of teaching principles of AS via an online, virtual format is relatively new, especially when it comes to nurses, and there is a paucity of data on this subject.[47-51]

The authors' study was conducted to evaluate frontline, bedside staff nurses' baseline attitudes and beliefs towards AS, and to understand if a virtual education campaign consisting of newsletters and tip sheets would positively impact those attitudes and beliefs.

METHODS

Description of the Education Programme

As multidisciplinary rounds expanded in various units in the authors' 390-bed community hospital, a programme to integrate frontline nurses into AS was made with help from nursing leadership to utilise a valuable resource already at the patient's bedside and to reduce duplication of efforts. The AS team at the authors' institution, consisting of infectious diseases pharmacists, physicians, microbiologists, nurse managers, and infection prevention nurses, wanted to educate bedside nurses regarding AS and incorporate some AS best practices in their standard day-to-day workflow. The authors conducted a baseline survey (pre-survey) of in-patient hospital nurses in September 2020. In this study, face-to-face, live presentations, and professional education were originally planned via in-person conferences and continuing nursing education activities; however, the global COVID-19 pandemic forced the authors to conduct their education strictly via a virtual format. After the results were collected, the nurse managers distributed monthly tip sheets and newsletters (created by the AS pharmacists) via email to all the frontline, in-patient nursing staff. The newsletters focused on various AS topics, such as how and when to collect blood and urine cultures, screen for *C. difficile*, take an accurate allergy history, differentiate between colonisation and true infections, identify sepsis, and use culture results to help guide decisions to de-escalate or stop therapy in cases of colonisation. After the monthly emails were sent, the nurse managers posted various AS topic content on the nursing homepage. The content of the newsletters was re-enforced during CDC Antibiotic Awareness Week in November 2020 and during the various continuing nursing education accredited webinars and virtual professional development educational activities.

Survey

The authors conducted a baseline survey (pre-survey) of in-patient hospital nurses in September 2020 to assess their beliefs and attitudes towards AS. After a review of the literature on nursing roles relating to AS, a questionnaire draft was created by the principal investigator.

The draft was then reviewed by members of the research team with expertise on survey research. Finally, the survey was reviewed for content validity by two members of the nurse leadership and professional development team at the authors' institution. The review established that the wording of the survey would be understood by potential respondents and verified that the survey questions fell within the nursing scope of practice.

The survey was sent to 800 nurses who work in the adult in-patient departments at the authors' institution. These nurses belonged to 10 different clinical departments covering intensive care units, medical, surgical, preoperative, and oncology floors. The survey was not randomised but sent to all the in-patient nurses who cover adult wards. Participation in the survey was voluntary, confidential, and anonymous. The survey consisted of 24 questions broken down into three broad domains: demographic and practice information, questions on nursing roles in AS, and nursing attitudes and barriers towards AS.

Demographic questions included where the nurses practised, their level of education, and their years of experience. Assessment of the role of nurses in AS included comfort level in participation in various AS activities, such as obtaining cultures properly and understanding the relationship between antibiotics and *C. difficile*. The concluding section focused on assessing the beliefs and attitudes towards AS.

The survey was distributed to all in-patient nurses via Microsoft Office Forms (Microsoft, Redmond, Washington, USA), which is the format that has been used in the past by the nursing leadership at the authors' institution. The authors provided the respondents with 2 weeks to complete the survey and sent a weekly and final reminder before it was closed. A subsequent post-survey was sent to the same group of in-patient frontline nurses in March 2021.

The survey results were not matched since the data were deidentified and anonymous. No incentives were provided for the completion of the survey. The hospital's institutional review board approved the study in August 2020, before the first survey was sent out.

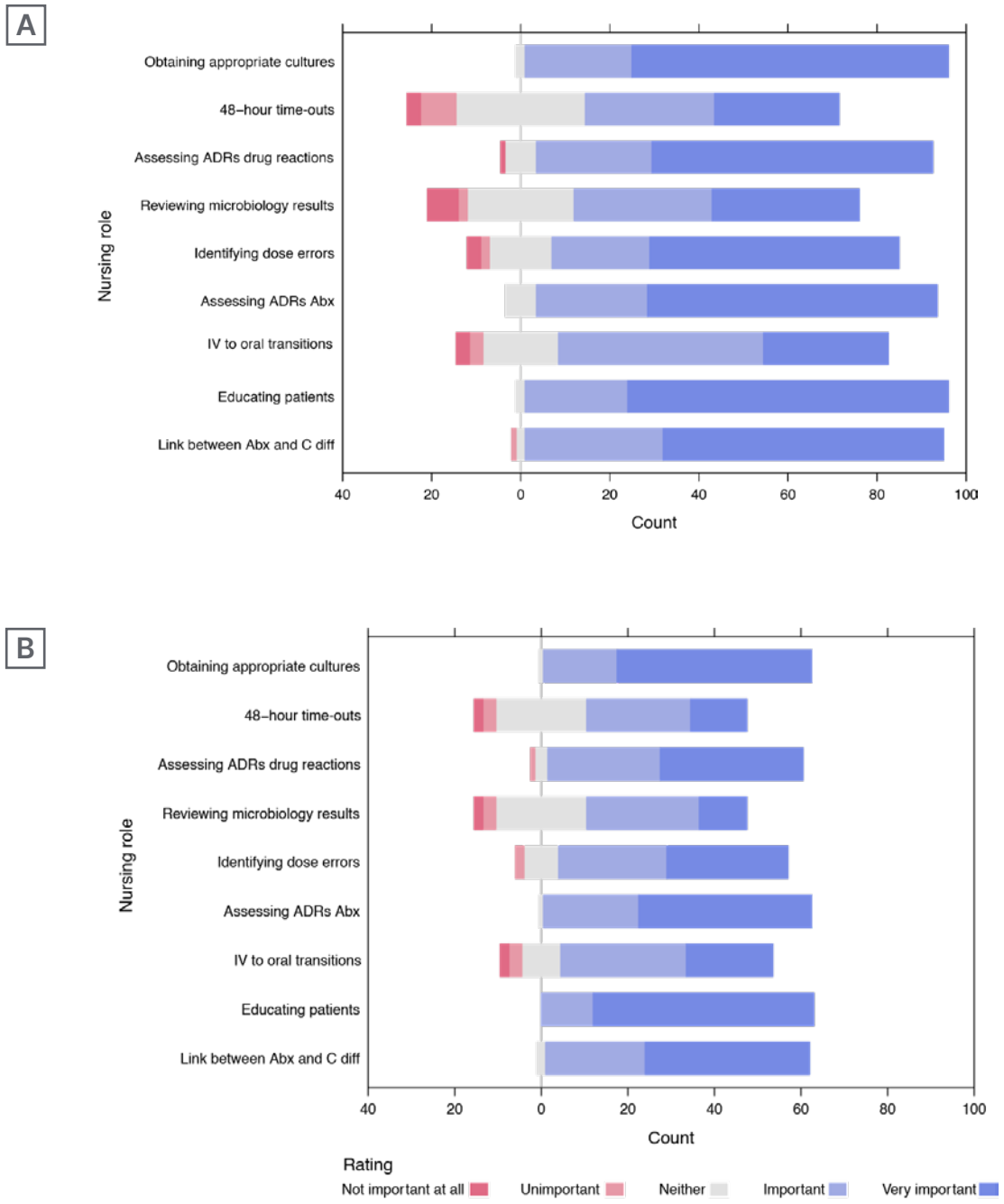
Statistics

Summary statistics for the pre- and post-survey data were calculated as counts and percentages. The demographic factors were compared between the pre- and post-survey to assess whether there was a relationship between the factors and survey groups. Given the small counts in some of the categories of the demographic factors, Fisher's exact test was utilised for the comparisons. A p value of >0.05 indicates that the participants for the pre- and post-survey were similar based on the demographics. The questions regarding nursing attitudes towards AS tasks and nursing awareness of AS, with responses to the Likert scale of 'Not important at all' to 'Very important', were compared between the pre- and post-survey using the Wilcoxon rank sum test. Each of the Likert scale questions were coded in the following way for analysis: 'Not Important at All'=1, 'Unimportant'=2, 'Neither'=3, 'Important'=4, and 'Very Important'=5. Summary statistics for each question were calculated using median, minimum, and maximum values. The Wilcoxon rank sum test was used to ascertain whether there was a significant difference in participants' view of the importance of the tasks between the pre- versus post-survey as responses were not paired across both surveys. The significance level was $\alpha=0.05$. All statistical analyses were conducted using R version 4.1.1 (R Foundation for Statistical Computing, Vienna, Austria).[52]

RESULTS

In total, 109 nurses responded to the baseline pre-survey, which was distributed in September 2020, and 64 nurses responded for the post-survey, which was conducted in March 2021. The baseline demographics were similar between the pre-and post-survey group regarding education and primary responsibility as a nurse. All specialties, such as the intensive care unit, medical, and surgical nurses, were well-represented, with the majority of survey respondents being frontline staff nurses holding a bachelor's degree. Overall, most nurses demonstrated a positive attitude towards antimicrobial stewardship tasks. For example, 97.9% of those who responded in the pre-survey thought it was 'Important' (Likert scale: 4) or 'Very Important' (Likert scale: 5) to obtain

Figure 1: A) Pre-survey nursing attitudes towards antimicrobial stewardship and B) a post-survey nursing attitudes towards antimicrobial stewardship.

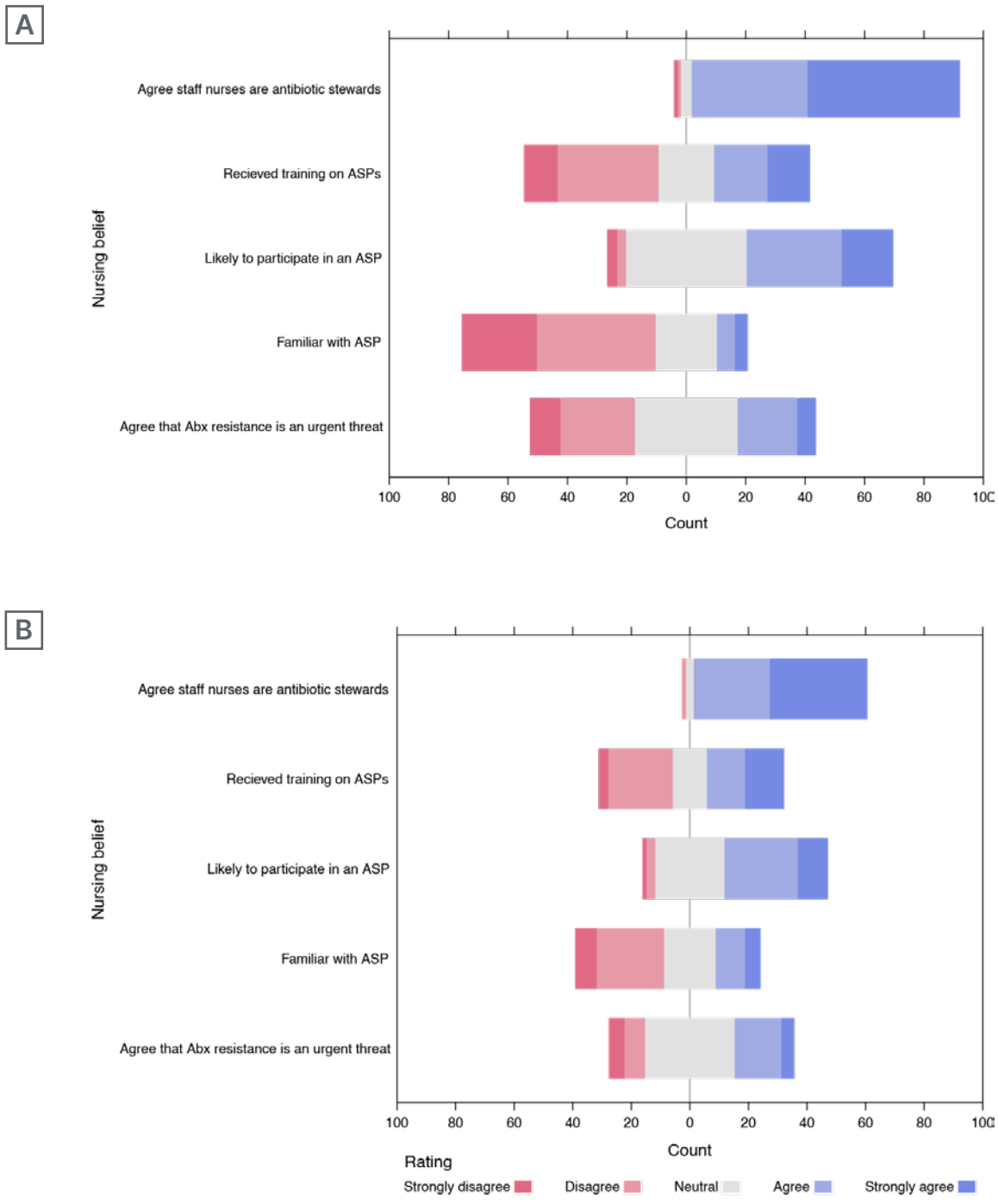


Abx: antibiotics; ADRs: adverse drug reactions; C diff: Clostridium difficile; IV: intravenous.

appropriate cultures. Other AS tasks, such as assessing adverse drug reactions, educating patients, and understanding the relationship between *C. difficile* and antibiotics, were also viewed as 'Important' (Likert scale: 4) or 'Very Important' (Likert scale: 5) by over 90% of the nurses in both the pre- and post-survey (Figure 1).

In addition, 93.8% of the nurses surveyed agreed with the statement that nurses are antibiotic stewards (Figure 2). Most pre-survey respondents listed knowledge gaps in microbiology (47 out of 64 [86%]) and antibiotics (53 out of 64 [84%]) as well as scope of practice concerns (48 out of 64 [75%]) as barriers to nurse participation.

Figure 2: A) Pre-survey nursing awareness of antimicrobial stewardship and B) post-survey nursing awareness of antimicrobial stewardship.



Abx: antibiotics; ASP: antibiotic stewardship programme.

The nontraditional, virtual education was effective in increasing the familiarity of the frontline nurses with the hospital ASP, as significantly more nurses in the post-survey reported that they were familiar with the stewardship programme compared with the pre-survey (31 out of 64 [48.4%] versus 24 out of 109 [23%]; p=0.001). Although not statistically significant, there was also a slight improvement in nursing attitudes towards evaluating a history of adverse drug reactions

Table 1: Comparison of nursing attitudes towards antimicrobial stewardship tasks between the pre- and post-survey.

Question	Pre-survey* (N=99)	Post-survey* (N=64)	p
Assuring cultures are obtained appropriately	5 (3–5)	5 (3–5)	0.8137
Evaluating continued antibiotic and performing 48-hour time-outs	4 (1–5)	4 (1–5)	0.5858
Assessing for a history of adverse drug reactions	5 (1–5)	5 (2–5)	0.1726
Reviewing microbiology results to antibiotic orders	4 (1–5)	4 (1–5)	0.1043
Identifying a wrong antibiotic dose	5 (1–5)	4 (2–5)	0.2878
Assessing for potential adverse events associated with antibiotics	5 (3–5)	5 (3–5)	0.9122
Collaborating with providers about transitioning from IV to oral	4 (1–5)	4 (1–5)	0.9942
Educating patients and families about the importance of taking antibiotics	5 (3–5)	5 (4–5)	0.3092
Understanding the relationship between antibiotics and *Clostridium difficile*	5 (2–5)	5 (3–5)	0.5090

*Likert scale responses were coded in the following way: 'Not Important at All'=1; 'Unimportant'=2; 'Neither'=3; 'Important'=4; and 'Very Important'=5. Data are presented as median (minimum–maximum).

IV: intravenous.

(median Likert score=5; p=0.17) and learning about the relationship between antibiotics and *C. difficile* (median Likert score=5; p=0.5) (Table 1). In terms of awareness of AS issues, a majority of nurses (51% in pre-survey versus 55.6 % in the post-survey) were likely to participate in ASP (Figure 2); however, despite education on the rationale and how to apply the recommended tasks to nursing practice, no change was demonstrated in perceived barriers to nurse participation in AS activities.

DISCUSSION

The results of the authors' survey showed that most nurses were aware of the urgent threat of antimicrobial resistance and want to contribute to a stewardship programme. Nevertheless, lack of education (especially in microbiology and antimicrobial spectrum of activity) and range of practice concerns were perceived as the most common impediments to participation. While not statistically significant, the virtual education campaign showed a positive impact on nurses' attitudes and beliefs on AS, and may be used as a blueprint moving forward in resource-limited settings.

Data on education for nurses on the topic of AS remains limited. Abbas et al.[33] surveyed 159 nurses in an 860-bed tertiary medical centre with an established ASP. They showed that while 62% of the respondents knew about the programme, time constraints, and concerns over physician pushback were identified as significant barriers to nurse participation. Bouchoucha et al.[53] surveyed 321 nursing students who were enrolled in an Australian university on their awareness and attitudes regarding the nurse's role in AS. Their survey found that while 45% of participants were familiar with the terminology of AS, 71% believed they had very little knowledge of the principles of AS. Merrill et al.[54]

also found comparable results when conducting an online survey of 316 staff nurses from three hospitals. A total of 52% of nurses did not recognise the term AS; however, almost 40% of nurses in the survey suggested that an ASP was moderately or extremely important in their healthcare setting.[54]

In the UK, efforts are underway to develop national consensus-based competencies to integrate AS education in undergraduate healthcare education. Courtney et al.[55,56] identified that only 67% of nursing programmes in the UK incorporate any AS teaching and only a handful (12%) actually go over all the recommended AS principles. A modified Delphi methodology involving two surveys (conducted online), delivered to an international panel of 15 experts, was utilised. These experts in education and AS provided the international consensus on the AS proficiency curriculum appropriate for nursing education.[57] The competencies developed will be used to guide future curricula in nursing schools. A total of 95% of the nurses in this study believed they should participate in AS interventions. Results from various previous studies and the authors' own study make it clear that nurses are aware of the urgent peril of antimicrobial resistance and would like to participate in AS actions but the main barrier is a lack of education.[35-42] The format of education is also important.[58-60] Interestingly, data shows that nurses learn better by researching questions themselves or asking their peers rather than formal education.[61] Hands-on training, using a collaborative approach, involving nursing leaders, and empowering nurses to be a member of the antimicrobial team, may help improve nurse confidence in AS activities and lead to improved outcomes.[62-68]

The advantages of a virtual structure are that it is much easier to disseminate and it is a lower-cost option. For example, study materials can be made available in a virtual format (self-study modules, pre-recorded webinars, newsletters, or tip sheets). These can be disseminated with minimal effort to all incoming staff upon hiring and continuing education of nurses. In addition, nurses can receive the same AS training as other healthcare workers, such as physicians and pharmacists, to be effective stewards in their day-to-day practice. Manning et al.[50] evaluated the impact of a virtual case-based simulation experience on nursing students and found improvement in knowledge domains regarding antibiotic use and resistance.

According to the USA healthcare workforce statistics, nurses practise in every healthcare setting, including nursing homes, clinics, dialysis centres, and long-term care facilities.[14] Therefore, empowering nurses to fight antimicrobial resistance will likely have a significant impact on reducing the inappropriate overutilisation of antibiotics and lessen the burden of antimicrobial resistance.

Limitations

This was a single-centre study conducted during a global pandemic. During this time, there was significant turnover in staff, and several staff nurses were cross-covering several units during the study period. Furthermore, since the survey was anonymous, the authors could not match data to look at the impact of education on an individual level or collect any feedback related to education.

CONCLUSION

In the authors' study, more than 90% of nurses agreed with the statement that staff nurses are antibiotic stewards, and more than 50% said they are likely to participate in some activities pertaining to AS; however, many barriers to nursing involvement, such as lack of education, range of practice concerns, and time constraints, persist. When real-time, face-to-face instruction is not feasible, as was the case in the COVID-19 era, virtual education via email and newsletters may be used instead of conventional methods to advance participation of nurses in AS projects in a resource limited setting.

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Epidemiological Features of the Molecular Surveillance of SARS-CoV-2 in Northern Greece: The Experience of a Regional Hospital

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Abstract

The COVID-19 pandemic has been a huge challenge for the Greek National Health System. Real-time reverse transcription PCR (rtRT-PCR) remains the reference method for early diagnosis, contact tracing, and containment of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The aim of this study is the documentation of the epidemiological features of SARS-CoV-2 laboratory surveillance with rtRT-PCR in the population residing in the Pieria province of Greece. Of the 15,486 nasopharyngeal and oropharyngeal samples tested with real-time reverse transcription PCR for the presence of SARS-CoV-2 RNA, 8,051 (52%) were from females and 7,435 (48%) from males, aged 7 days–103 years, with 69.9% coming from the age group of >40 years. The 4,616 out of 15,486 (29.8%) samples came from hospitalised patients. There were 3,771 positive samples out of 15,486 (24.3%); 1,890 (50.8%) males and 1,881 (49.2%) females, with the age group of 40–59 years being dominant (29.9%). Those diagnosed for the first time made up 3,352 out of 3,771 (88.9%) of positive samples. The monthly positivity rate ranged from 6.24–15.69% during the B.1.1.7 variant wave, 17.38–52.89% during the B.1.617.2 variant wave, and 59.76% during the first month of the B.1.1.529 variant wave. Absence of detection of the spike protein gene target was observed in 1,371 (36.4%) of positive samples. Cycle threshold values <20, indicative of higher viral load, had 43.2% of positive samples during the B.1.1.7, 70.0% during the B.1.617, and 92.0% during the first month of the B.1.1.529 wave. The positivity and distribution of variants in the study population was in accordance with the respective results announced by official government authorities for the Pieria region.

Key Points

1. This is the first analytical study that provides useful information regarding the prevalence and spread of different severe acute respiratory syndrome coronavirus 2 variants to the Greek National Health System, thus assisting in better health surveillance.

2. Real-time reverse transcription PCR was used to outline the landscape of positive severe acute respiratory syndrome coronavirus 2 samples, as well as the distribution of different variants in 15,486 people attending local healthcare facilities.

3. Variant identification can assist in the clinical management of patients in healthcare settings and also provide critical information for public health strategies.

INTRODUCTION

COVID-19, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was declared a pandemic by the World Health Organization (WHO) in March 2020.[1] The health and diagnostic services of all countries were faced with the urgent need for rapid and reliable detection of the virus from appropriate samples. Mass screening and early diagnosis were, and still are, important factors in identifying and isolating carriers, ideally before symptom onset, contact tracing, limiting the spread of the disease, and effectively confronting the pandemic.[2-4]

Since the onset of the SARS-CoV-2 pandemic, several variants of the virus have been identified. Depending on their ability to transmit, evade immunity, and disease severity, they have been characterised as variants of concern or variants of interest. Therefore, it is important for public health responses that variants can be identified and specified as accurately as possible regarding their epidemiological characteristics, and impact on the population.[5-7]

The reference method proposed by the WHO for the diagnosis of SARS-CoV-2 infection is the detection of viral RNA with real-time reverse transcription PCR (rtRT-PCR) in respiratory samples such as nasopharyngeal and oropharyngeal swabs, bronchial aspiration samples, oral swabs (saliva), and sputum.[8] Studies have reported the detection of SARS-CoV-2 RNA in blood, serum, and plasma.[9,10] However, nasopharyngeal, oropharyngeal, combination (naso-oro-pharyngeal) and oral swabs, and saliva samples, are the most appropriate for molecular diagnosis,[11,12] and are recommended by the Centers for Disease Control and Prevention (CDC) and the European Centre for Disease Prevention and Control (ECDC).[13,14]

From the beginning of the pandemic until the end of 2020, the molecular testing carried out by the Microbiology Laboratory of General Hospital of Katerini (GHK) in Greece was aimed at the diagnosis of emergencies, symptomatic and asymptomatic, and the prevention of inpatient viral dispersion. The largest volume of samples was tested by the microbiology laboratory of the Medical School, Aristotle University of Thessaloniki, Greece. However, during the second pandemic wave, in the autumn of 2020, several factors such as the large number of patients with COVID-19 increased hospitalisation needs in the COVID-19 clinic and intensive care unit, as well as the need for epidemiological surveillance of the regional population for disease dispersion, mandated the development and operation of the Laboratory for the Molecular Diagnosis of COVID-19 at GHK.

In this study, the authors aim to examine and present the demographic characteristics and results of the molecular testing from the laboratory surveillance of SARS-CoV-2 with rtRT-PCR, in order to assess the spread and prevalence of the SARS-CoV-2 variants in Pieria in northern Greece between January 2021–January 2022. However, the study includes only samples from residents who underwent molecular testing at the Molecular Testing Laboratory for Diagnosis of COVID-19 at GHK, and does not include those tested by the mobile units of the National Public Health Organisation (EODY) and private diagnostic laboratories, nor the results of the Ag Rapid tests. To the

authors' knowledge, this is the first analytical documentation of SARS-CoV-2 demographic and laboratory findings concerning a regional population attending the primary public health care facilities in Greece.

MATERIALS AND METHODS

From January 2021–January 2022, the Laboratory for the Molecular Diagnosis of COVID-19 at GHK conducted molecular testing with rtRT-PCR for the presence of SARS-CoV-2 RNA on 15,486 nasopharyngeal and oropharyngeal swab samples from citizens attending regional primary health facilities in Pieria. Demographics, sampling unit, testing reason, and SARS-CoV-2 contact information were obtained from the sample reference document to the degree they were provided. The provided information and rtRT-PCR results were used to assess the spread and prevalence of the SARS-CoV-2 variants in the above population in comparison to national results.

Samples were collected in IMPROVIRAL NAT Medium (Improve Medical Instruments Co. Ltd., Guangzhou, China) or Disposable Virus Sampling Tube (Zybio Inc., Chongqing, China), maintained at 4 °C and examined within 24–48 hours of sampling. Samples examined after 48 hours were preserved at -30 °C until the day of processing.

Viral RNA was extracted from 200 µL of each sample with the MagMAX™ Viral/Pathogen II (MVP II) Nucleic Acid Isolation Kit (Thermo Fisher Scientific, Waltham, Massachusetts, USA), amplified with TaqPath COVID-19 CE-IVDRT-PCR Kit (Thermo Fisher Scientific) in the QuantStudio™ 5 Dx Real-Time PCR System (Thermo Fisher Scientific). Results were obtained after data analysis by the COVID-19 Interpretive Software CE-IVD editions v2.3 and v2.5 (Thermo Fisher Scientific), according to the manufacturer's directions. All of the above are In Vitro Diagnostic (IVD) certified for *in vitro* diagnostic use.

The viral load of the samples was estimated by the cycle threshold (Ct) values of the positive samples.

RESULTS

During a 13-month period, 15,486 nasopharyngeal and oropharyngeal samples were tested for the presence of SARS-CoV-2 RNA. Samples were collected from residents of the regional province of Pieria, who attended or were hospitalised at GHK; the psychiatric clinic and its outpatient units; regional primary healthcare units, including the health centres of Aiginio, Litochoro, and Katerini; and the regional clinics for preventive or diagnostic testing. The demographic characteristics of the examinees, the origin of the samples, the reason for examination, and the positivity distribution per group are presented in Table 1, while the monthly percentage positivity distribution per SARS-CoV-2 variant and sampling unit is presented in Table 2. Positivity distribution data per testing reason was not available.

The age of the examinees ranged from 7 days–103 years, and those >40 years old contributed 69.9% of the samples. While the majority of the samples were obtained at the hospital settings (12,185 out of 15,486; 78.7%), only 4,616 (29.8%) were taken from hospitalised patients, and the remaining 10,870 (70.2%) from non-hospitalised patients. Regarding contact with a confirmed COVID-19 case, 3,508 (22.7%) of the examinees answered 'yes', and 4,233 (27.3%) 'no', while 7,745 (50.0%) answered 'unknown' or no answer was given. Out of 15,486 samples, 13,828 were examined for the first time, while the remainder concerned follow-up testing of patients and repetitions for the investigation of inconclusive results. Preventive screening for SARS-CoV-2 was the main reason for testing (11,224; 72.5%). Other reasons for testing were re-examination after preventive domestic restriction or illness, screening before therapy or surgery, travelling, return to work, and patient chaperone (Table 1).

Overall, 3,771 (24.3%) samples were positive, with 2,342 (62.1%) of these obtained at the hospital outpatient unit (OU) and emergency care units (ECU; Table 1). Of the 3,771 positive samples, 3,331 (88.3%) were positive for the first time. Of the samples examined, 804 (5.2%) patients had a previous positive molecular result, without specifying the time or the laboratory of diagnosis. Of these 804 samples, 440 (54.7%) concerned hospitalised patients and outpatients who remained positive, while 364 (45.3%) were samples of patients with a negative follow-up result.

Table 1: Demographic and laboratory characteristics for the 15,486 samples tested at the General Hospital of Katerini, Greece, from January 2021–January 2022.

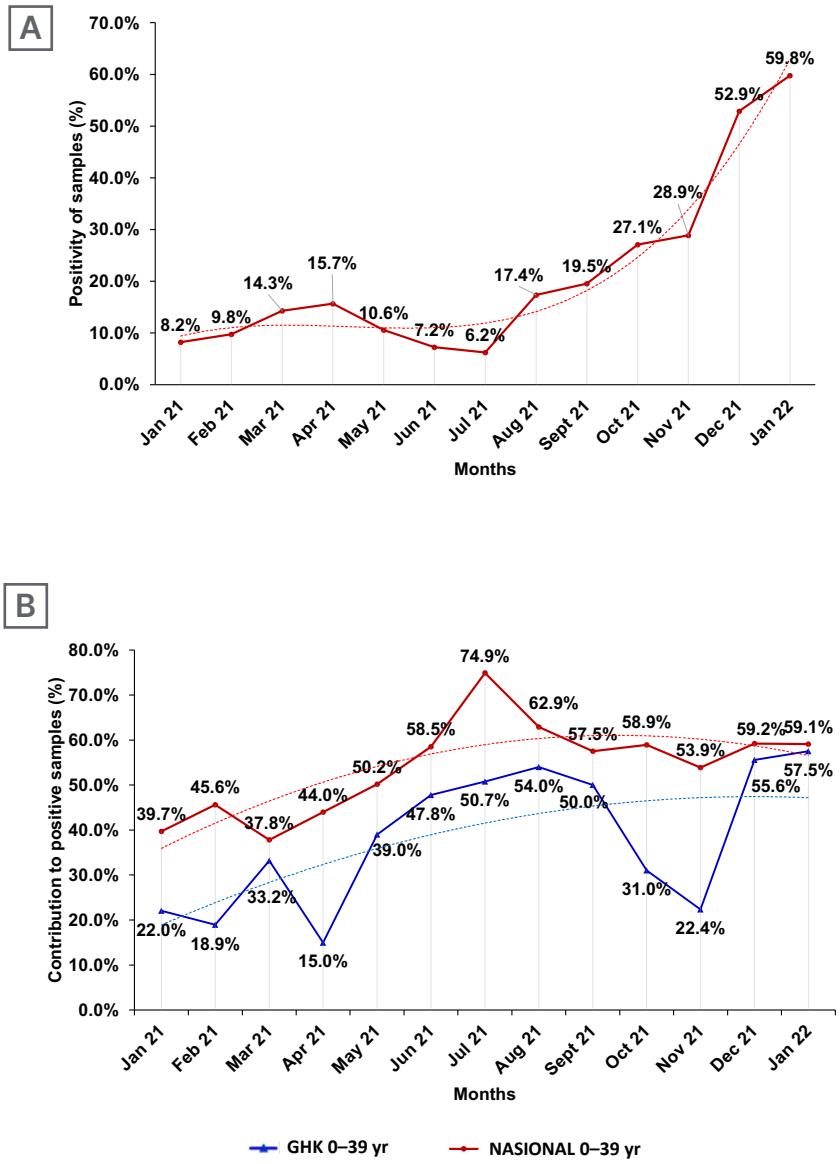
Variable	Number of samples (n=15,486)	Positive samples (n=3,771)
Gender		
Male	7,435 (48%)	1,890 (50.1%)
Female	8,051 (52%)	1,881 (49.9%)
Age group (years)		
0–19	1,917 (12.4%)	866 (23.0%)
20–39	2,780 (18.0%)	793 (21.0%)
40–59	4,823 (31.1%)	1,126 (29.9%)
60–79	3,921 (25.3%)	718 (19.0%)
≥80	2,045 (13.2%)	268 (7.1%)
Sampling unit		
Hospital clinics	3,915 (25.3%)	458 (12.2%)
Hospital outpatient unit	5,842 (37.7%)	1,437 (38.1%)
Hospital emergency care unit	1,727 (11.2%)	905 (24.0%)
Psychiatric department	701 (4.5%)	52 (1.4%)
Katerini Health Centre, Greece	1,645 (10.6%)	350 (9.3%)
Aiginio Health Centre, Greece	845 (5.5%)	286 (7.6%)
Litochoro Health Centre, Greece	811 (5.2%)	279 (7.4%)
Place of residence		
Provincial capital	9,002 (58.1%)	2,067 (54.8%)
Outside provincial capital	6,484 (41.9%)	1,074 (45.2%)
Contact with a known COVID-19 case		
Yes	3,508 (22.7%)	1,526 (40.5%)
No	4,233 (27.3%)	N/A
Unknown	7,745 (50.0%)	N/A
Testing reason		
Preventive testing	11,224 (72.5%)	N/A
Retest	1,658 (10.7%)	N/A
Therapy or surgery	2,028 (13.1%)	N/A
Travel	89 (0.6%)	N/A
Patient's chaperone	325 (2.1%)	N/A
Return to work	162 (1.0%)	N/A

N/A: no data available.

Table 2: Monthly positivity distribution per severe acute respiratory syndrome coronavirus 2 variant and sampling unit for the 15,486 samples tested at General Hospital of Katerini, Greece, from January 2021–January 2022.

Month	Hospital clinics		Hospital outpatient unit		Hospital emergency care unit		Psychiatric department		Regional primary health centres	
	Samples	Positivity	Samples	Positivity	Samples	Positivity	Samples	Positivity	Samples	Positivity
January 2021	173	2.90%	254	7.09%	17	17.65%	0	0.00%	275	12.00%
February 2021	319	10.00%	629	8.59%	55	0.00%	50	2.00%	300	15.00%
March 2021	281	10.30%	707	15.13%	42	2.38%	53	0.00%	412	18.69%
April 2021	311	17.00%	402	10.70%	26	11.54%	48	8.33%	405	20.74%
May 2021	332	11.10%	300	6.67%	37	13.51%	47	0.00%	230	16.52%
June 2021	342	4.40%	308	5.52%	50	28.00%	39	0.00%	187	11.23%
July 2021	361	4.40%	416	4.57%	81	16.05%	49	0.00%	167	11.38%
August 2021	364	6.30%	332	10.84%	161	42.86%	44	0.00%	250	28.80%
September 2021	165	10.30%	194	11.34%	101	53.47%	22	0.00%	153	20.26%
October 2021	249	12.40%	208	12.98%	247	60.73%	73	2.74%	116	27.59%
November 2021	446	22.65%	543	24.49%	313	5.63%	203	18.72%	230	23.48%
December 2021	306	15.00%	709	47.81%	381	74.02%	50	6.00%	507	71.40%
January 2022	266	19.50%	840	71.67%	216	64.81%	23	17.39%	69	68.12%
Total	3,915	11.70%	5,842	24.60%	1,727	52.40%	701	7.42%	3,301	27.72%

Figure 1: Monthly distribution of sample positivity from January 2021–January 2022.



A) Monthly percentage of the distribution of positivity for the samples tested at GHK.

B) Comparison of the monthly percentage contribution of the age group 0–39 years to positive samples of the province of Pieria, Greece, and at national level.

GHK: General Hospital of Katerini; NASIONAL: national level; yr: years.

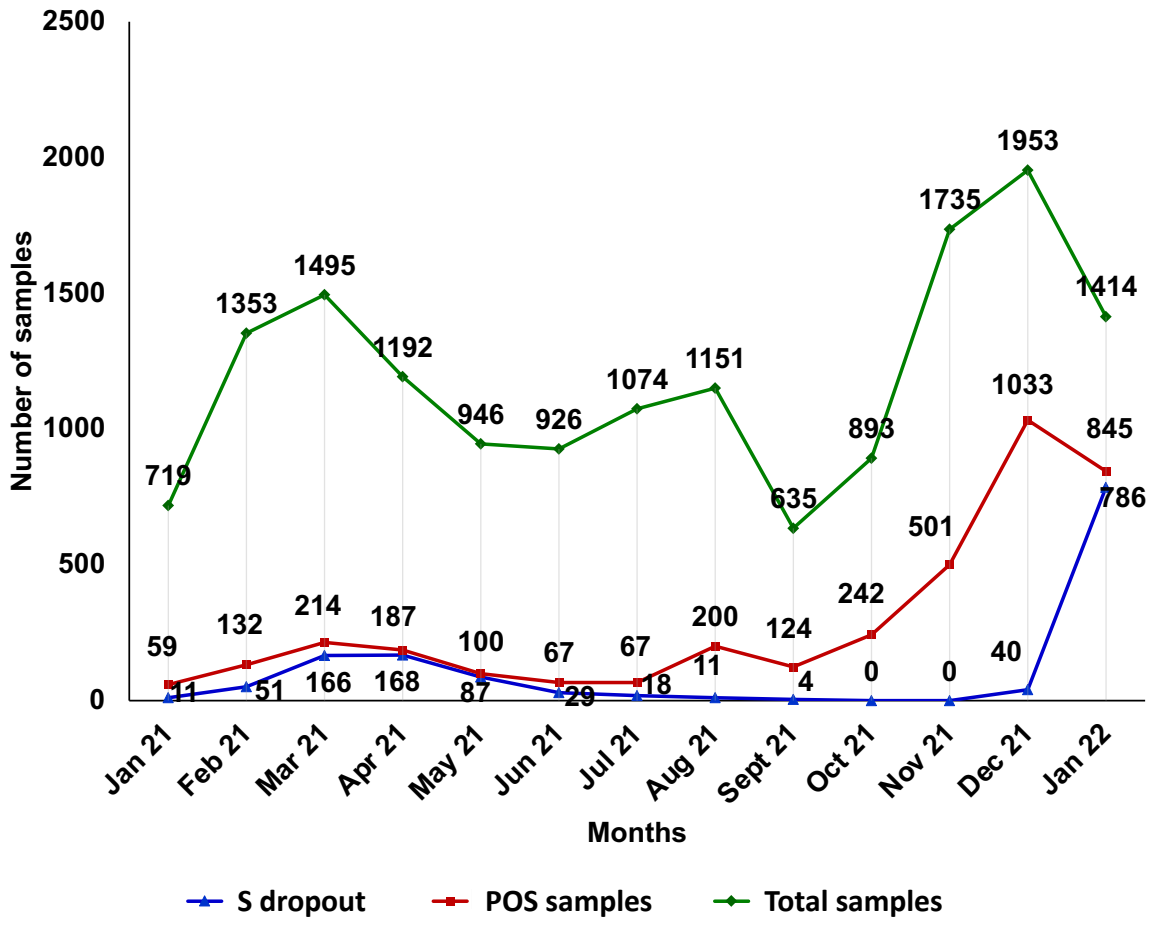
The monthly distribution of the sample positivity ranged from 6.2%–59.8% (Figure 1A). During the outbreak of the B.1.1.7 (Alpha) virus variant, positivity ranged from 6.2–15.7%, with maximum positivity (15.7%) in April 2021, and minimum positivity (6.2%) in July 2021. During the B.1.617.2 (Delta) variant wave, the positivity of the samples was much higher (17.4–52.9%), with the highest positivity observed in December 2021. Positivity reached 59.8% at the onset of the B.1.1.529 (Omicron) variant wave in January 2022 (Figure 1A). Regarding the 3,508 patients examined with a confirmed COVID-19 case contact, 1,526 (43.5%) had a positive PCR result. The monthly distribution of the positivity in this group ranged from 19.2% in January 2021 to 48.5% in January 2022.

Moreover, the monthly positivity distribution per SARS-CoV-2 variant data showed an increase of COVID-19 positive cases in hospitalised patients during the Delta and the onset of Omicron wave (this fluctuated from to 22.65% in November 2021 to 15.00% in December 2021 and 19.50% in January 2022) compared to the Alpha wave (17.00% in April 2021). Similar fluctuations in positivity were observed in patients attending the hospital OU, while the corresponding observation for the hospital ECU, as well as the local primary healthcare centres, show a steady increase of positivity during these periods (Table 2).

The presence of the 69-70del mutation in the spike protein gene (S gene), which prevents the amplification of this target, results in an S dropout effect, also known as the S gene target failure (SGTF). This phenomenon was detected in 1,371 out of 3,771 positive samples (36.4%). Higher SGTF rates were observed in April 2021 (168 out of 187; 89.8%) at the peak of the Alpha variant wave, and in January 2022 (786 out of 845; 93.0%) at the onset of the Omicron variant wave. The lowest (0.0%) were observed in October–November 2021 at the peak of the Delta variant wave (Figure 2).

Ct values of the positive samples in the present study ranged from 9–37. During the Alpha variant wave, 43.2% of positive samples had Ct <20, while during the Delta variant wave the corresponding value was 70.0%, and at the beginning of the Omicron variant wave was 92.0%.

Figure 2: Monthly distribution of samples tested at General Hospital of Katerini, Greece; samples with positive real-time reverse transcription PCR result and samples presenting the S dropout effect from January 2021–January 2022.



POS samples: positive samples.

DISCUSSION

There are two limitations in this study, concerning the samples and the provided information to the laboratory. First, the study population does not include those residents tested by the mobile units of the EODY and private diagnostic laboratories in the Pieria province, nor the results of the Ag Rapid tests, but only residents who underwent molecular testing at the Molecular Testing Laboratory for Diagnosis of COVID-19 at the GHK. Secondly, the demographic, contact, and clinical information provided to the laboratory was limited in some cases.

Due to the general data protection regulation, data concerning health facilities of coterminous northern Greece provinces was limited only to the sample positivity obtained from the EODY's official website.[15] As a result, and because of the diversity of rtRT-PCR kits used for the diagnosis of COVID-19 by other healthcare facilities, the authors' findings could not be analytically compared with data from coterminous health facilities in northern Greece.

The age distribution of the examined citizens, the origin of the samples, and the reason for examination are presented in Table 1. A small predominance of samples obtained from females (8,051 out of 15,486; 52%) was observed over males (7,435 out of 15,486; 48%); this is similar to statistics reported by the EODY for the region of Pieria, provinces adjacent to the Pieria region, and at the national level.[15,16] Almost half of the samples examined (7,569 out of 15,486; 48.9%) were obtained at the hospital OU and ECU, and only 4,616 out of 15,486 (29.8%) came from hospital clinics, with the main reason for testing (11,224 out of 15,486, 72.5%) being preventive screening for SARS-CoV-2 (Table 1).

Overall, 3,771 out of 15,486 (24.3%) of the samples were positive, with 3,331 (88.3%) of them obtained from those tested for the first time. Considering that access to hospital services during pandemic waves were restricted to mostly emergency cases, the majority of positive samples (2,342 out of 3,771; 62.1%) came from the hospital OU and ECU (Table 1). The combined positivity for the OU and ECU samples was high (2,343 out of 7,569; 30.9%), and the positivity of the samples obtained at the ECU alone were even higher (905 out of 1,727; 52.4%) as a result of testing mostly citizens with strongly suggestive COVID-19 symptoms. Although 4,616 out of 15,486 (29.8%) of the samples were obtained from hospitalised patients in both clinics and the psychiatric department, including the COVID-19 clinic, only 13.6% of the positive samples came from these patients for two reasons. Firstly, only patients with negative PCR negative results were admitted to non-COVID-19 clinics; secondly, the psychiatric department is comprised of closed and isolated facilities and, therefore, having the lowest positivity rate (Table 1). While in all samples examined there was a slight predominance of females over males, in all positive samples the difference was even smaller (1,890 [50.8%] of males and 1,881 [49.2%] of females), as it was at national level.[15,16]

Of the samples examined, 804 out of 15,486 (5.2%) had a previous positive molecular result, with 440 out of 804 (54.7%) samples concerning hospitalised patients and outpatients who remained positive, while the remaining 364 out of 804 (45.3%) had a follow-up negative result. The lack of information about the testing time and the testing laboratory for these 364 samples was a limitation to determine the positivity period for these patients. Although the provincial capital population represents the 43.0% of the regional population, it unproportionally contributed 58.1% of the examined and 54.8% of the positive samples (Table 1), probably due to easier access to public and private COVID-19 testing facilities.

While the group of patients <39 years old contributed only to 30.4% of the examined samples, it contributed 44.0% (1,659 out of 3,771) of the positive samples. The positivity rate within this age group was 1,659 out of 4,697 (35.3%), while in the group >40 years, the positivity rate was 2,112 out of 10,789 (19.6%). This difference could be explained as a result of the higher mobility of younger people, the beginning of in-person education after September 2021, and delayed admission to vaccination programmes.[15,16] Of those with contact to a confirmed COVID-19 case, 1,526 out of 3,508 (43.5%) had a positive PCR result. The monthly distribution of the positivity in this group of people ranged from 19.2% in January 2021 to 48.5% in January 2022. It gradually increased from 19.2% in January 2021 to 36.1% in

August 2021, reaching 47.4% in December 2021, and peaking at 48.5% in January 2022; this is indicative of the ease of viral transmission and increased spread of Delta and Omicron variants.

Another corroborating finding is the overall monthly percentage positivity distribution per SARS-CoV-2 variant and sampling unit, as presented in Table 2. As shown, a wider spread of the disease was observed during the Delta and the onset of Omicron waves compared to the Alpha wave. Moreover, the increase in the number of ECU samples tested, and the corresponding increase in their positivity should be noted, indicative of the increasing number of symptomatic patients attending the hospital ECU during this period. On the contrary, the number of the clinical samples tested and their positivity in January 2022, at the onset of the Omicron wave, were much lower than the respective ones from the OU and ECU, compared to those in April 2021 (Alpha wave) and November–December 2021 (Delta wave; Table 2).These observations are indicative of the increased transmissibility but milder symptoms, resulting in the decreased need for hospitalisation during the Omicron wave compared to the Alpha and Delta variant waves.[5,7]

The overall monthly distribution of the sample positivity ranged from 6.2–59.8% (Figure 1A). Despite any fluctuations, it showed a sharp increasing trend during the present study, and was much higher than the official reported by EODY for the region.[12,13] During the outbreak of the B.1.1.7 variant of the virus, positivity ranged from 6.2–15.7%, with maximum positivity (15.7%) in April 2021 and minimum (6.2%) in July 2021. During the pandemic wave of the B.1.617.2 variant, the positivity of the samples was much higher (17.4–52.9%), with the highest positivity observed in December 2021 at the peak of this pandemic wave. Positivity reached 59.8% on the onset of the pandemic wave of the B.1.1.529 variant in January 2022 (Figure 1A), confirming the higher infectivity and transmissibility of these two variants.[5-7] These values, although much higher than those officially announced by EODY for the region, show a similar increasing trend.[15,16]

Positive samples in the age group 40–59 years were the highest of all (1,126 out of 3,771; 29.9%), comprising 27.7% of males testing positive and 32.0% of females testing positive (Table 1). Positive samples in the age group <19 years were higher compared to the respective reported by EODY at the national level in March 2021 (14.5% versus 8.0%); July 2021 (29.9% versus 16.3%); December 2021 (26.7% versus 19.2%); and January 2022 (35.3% versus 24.6%). In contrast in this age group, during November 2021, while the pandemic wave of Delta variant was evolving, the participation rate was quite low (10.0%) compared to the previous periods, and to the respective at the national level (23.6%) for the same month. Nevertheless, this increase is in concordance with the increase of positivity both at regional and national level during these months.[15,16]

The above deviations of the positivity in the population and of the participation rates of the age groups in the positive samples are probably the result of two limitations. Firstly, the origin of the samples in the present study includes only the results of molecular testing carried out at GHK and does not represent the general population of Pieria; nor does it include the Ag Rapid test results which are included in the official data of EODY, and which were vastly increased over the PCR tests from April 2021 throughout this study.[15,16] Secondly, it does not take into consideration the possible differences in positivity due to quarantine restrictions and the different vaccination timetable for age subgroups. However, it should be noted that throughout the study period there has been an increasing trend in the participation of the age group <39 years in the positive samples commensurate to its participation at the national level and, therefore, a corresponding decrease in the participation of the >40 years age group,[15] even though the corresponding percentages in the regional population are lower (Figure 1B). This, in fact, indicates the spread of Delta and Omicron variants among the age group <39 years due to their increased transmissibility, but also due to the initiation of in-person teaching at all levels of education, the relaxation of restrictive measures, and the low vaccination coverage of young people.[5-7,15]

With the emergence of the B.1.1.7 variant, the mutation 69-70del S was observed in the S gene, which prevented the PCR amplification of the S target. The same mutation was subsequently identified in the B.1.1.529 variant.

The SGTF phenomenon, however, does not prevent the interpretation of the results, nor does it make a result negative. On the contrary, this pattern helped in the early recognition of Alpha and Omicron variants, as highlighted by the ECDC and CDC.[12,13,16] This pattern was detected in 1,371 out of 3,771 positive samples (36.4%). Higher SGTF rates, as expected, were observed in April 2021 (168 out of 187; 89.8%) at the peak of the Alpha variant wave, and in January 2022 (786 out of 845; 93.0%) at the onset of the Omicron variant wave. The lowest (0.0%) were observed in October and November 2021 at the peak of the pandemic wave of the Delta variant (Figure 2).[17]

The interpretation of the COVID-19 test results and the evaluation of the SARS-CoV-2 viral load of positive samples were carried out using the Ct value, a semi-quantitative parameter of rtRT-PCR. The Ct represents the lowest number of PCR amplification cycles required to produce a fluorescent signal greater than the background noise. It can approximately indicate the amount of the viral RNA present in the sample; the lower the Ct the higher the concentration, while a higher Ct indicates a low concentration of viral RNA. The Ct values of the positive samples in the present study ranged from 9–37. During the Alpha variant wave, 43.2% of positive samples had Ct <20, while during the Delta variant wave the corresponding value was 70.0%, and at the beginning of the Omicron variant wave it was 92.0%. This is an indication of higher viral loads in the samples of citizens infected with the Delta and Omicron variants, and reflects the higher infectivity of these variants.[6,18]

However, care should be taken when using Ct values as an indicator for viral RNA load for several reasons. Respiratory tract samples are not homogeneous, like those of blood, urine, or other body fluids. Thus, a nasopharyngeal or oropharyngeal sample with low Ct is usually associated with high infectivity or acute phase infection. On the contrary, a sample with high Ct could refer to a lower-risk, an early, or a rebound infection, but it also could refer to poor sampling procedure (e.g., insufficient collection and storage, or degradation of sample).[19-26] In addition, diagnostic laboratories use different reagent kits for RNA extraction and rtRT-PCR, and, therefore, Ct values are not comparable.[20,23] It is also known that in some patients a positive rtRT-PCR result is obtained for a long period after clinical symptoms subside (e.g., 60 to 100 days), indicating the shedding of SARS-CoV-2 RNA without necessarily the presence of infectious virus.[27-29]

Thus, it is obvious that, at present, no consensus exists concerning which Ct values relate to the level of severity of the disease, or to whether or not a person is infectious, unless repeated measures of Ct are taken for each patient throughout the course of the disease.[9,30] Therefore, appropriate caution should be taken to interpret the Ct values, and other laboratory and clinical findings should be considered in order to determine a true positive (viable viral shedding) or a true negative person with COVID-19, and the course of the disease.

CONCLUSIONS

In this study, the authors attempted to investigate and present the demographic characteristics and the laboratory molecular testing results of nasopharyngeal and oropharyngeal samples obtained from the residents of the Pieria region, who attended the regional public health care facilities for SARS-CoV-2 testing, and were examined by the COVID-19 Laboratory for the Molecular Diagnosis of COVID-19 at GHK from January 2021–January 2022 inclusive.

In this study, no difference in the positivity of males and females was observed. Sample positivity was lower (6.24–15.69%) during the pandemic wave of the Alpha variant, compared to that of Delta (17.38–52.89%) and the beginning of the Omicron waves (59.76%); this is in accordance with the transmissibility and infectivity of the variants, and the viral load of the samples. Positivity was higher compared to the daily COVID-19 reports of EODY for the Pieria region, as the study only includes the results of molecular testing carried out at the GHK, and does not include results from molecular testing carried out by the testing units of EODY and private laboratories, or the Ag Rapid test results.

In the age group <39 years, there is an increasing trend in positivity from January 2021–January 2022, similar to that at the national level, even though is lower than that. This is indicative

of the higher infectivity and transmissibility of Delta and Omicron variants combined with the initiation of in-person teaching at all educational levels, the relaxation of restrictive measures, the high mobility of this age group, and the low vaccination coverage of younger people.

Although the Ct value is a semi-quantitative parameter of rtRT-PCR, the lower Ct values of positive samples with the Omicron variant, compared to Alpha and Delta, are a strong indication of the high viral load, and confirm the increased infectivity and transmissibility of this strain.

The large number of samples tested, including confirmed, probable, and suspected cases of COVID-19, and the ability to distinguish viral variants due to the S dropout effect, could provide the clinicians with important information for the clinical management and hospitalisation of patients. In addition, the detailed documentation of demographic and contact data of the population at the sampling points, and the variant distinction by rtRT-PCR, would be beneficial in providing a comprehensive picture of the prevalence of the disease to public health policy officials, thus contributing effectively to establishing preventive and treatment strategies, to monitoring transmission clusters, contact tracing, and containment of COVID-19 in the Pieria region. Furthermore, the authors' findings in correlation with those from the primary health care facilities in neighbouring provinces of northern Greece could also provide accurate information of SARS-CoV-2 variant spread in the region during the pandemic phases.

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A Study on Surgical Site Infections and Associated Risk Factors in General Surgeries at a Tertiary Care Hospital: A Cross-Sectional Study

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Abstract

Background: Surgical site infections (SSI) remain a major cause of hospital-acquired infections, causing morbidity and mortality worldwide. In developing countries, 5.6% of surgical procedures will develop SSIs. These are further complicated by an increasing prevalence of multidrug-resistant organisms. Associated risk factors also play a role in contribution of SSIs. However, the identification of factors that cause or predict these SSIs remains an important area of research.

Objective: To investigate the risk factors for SSI together with the identification of the aetiological bacterial agents and their antimicrobial susceptibility.

Methods and Patients: A cross-sectional study was carried out on 143 patients who underwent surgery in a single tertiary care centre. Only the surgeries falling under clean and clean-contaminated categories were included in the study. SSI was determined by positive bacterial culture, and resistant pattern was determined by Kirby–Bauer disc diffusion method.

Results: Out of 25 different surgical procedures in 143 cases, four cases developed SSI due to *Escherichia coli* and *Staphylococcus aureus*. Diabetes, obesity, and smoking were the associated risk factors in these cases.

Conclusion: Prevention of SSI is complex and requires the integration of a range of preventive measures before, during, and after surgery.

Key Points

1. Surgical site infections (SSI) are a major cause of morbidity and mortality worldwide. These are further complicated by an increasing prevalence of multidrug resistant organisms, leading to increased average length of hospital stay among patients with SSI, which causes increased financial implications.

2. Patients with associated risk factors are at higher chances of developing SSIs. Modifiable patient factors such as glycaemic control in diabetes, managing hypertension, and ceasing of nicotine for weeks prior to surgery facilitates prevention of SSI and betters the management of patients.

3. Determining the antimicrobial patterns of the bacterial agents causing SSIs will help institutions to improve antibiotic policy and restrict the use of antimicrobials in preventing the spread of drug resistance in hospitals, which is needed as a part of antimicrobial stewardship.

INTRODUCTION

Surgical site infections (SSI) are a leading cause of morbidity and mortality around the world. Around 5.6% of surgical procedures develop SSIs, mostly in developing countries.[1] To track healthcare-associated infections, the National Healthcare Safety Network (NHSN) uses standardised infection ratio, which compares the number of observed infections with the number of predicted infections.[2]

Each year, a significant amount of morbidity and mortality is caused by infection at or around the surgical site, which occurs within 30–90 days of an operative procedure.[3] A Centers for Disease Control and Prevention (CDC) survey from 2015 estimated approximately 110,800 SSIs among inpatient surgeries.[4] There was 5% reduction in SSIs in 2020 when compared with 2015 data as baseline among all NHSN operative procedure categories combined.[5]

Despite improvements in surgical techniques, sterilisation of instruments, operation theatre practices, and the best efforts of infection prevention strategies, SSIs remain a major cause of hospital-acquired infections.[6] These are further complicated by an increasing prevalence of multidrug-resistant organisms.

Most of the time, it is the patient's endogenous flora that is responsible for many SSIs, and the commonly isolated pathogens include *Staphylococcus aureus, coagulase-negative staphylococci, Enterococcus* spp., and *Escherichia coli*.[7]

However, the identification of factors that cause or predict these SSIs remains an important area of research. The authors aim to investigate the risk factors for SSIs, together with the identification of the aetiological bacterial agents and their antimicrobial susceptibility in a tertiary hospital.

METHOD AND PATIENTS

A cross-sectional study was carried out on 143 patients who underwent surgery in a single tertiary care centre. The study was conducted over a period of 2 months between August–September 2021. The degree of surgical wound contamination was assessed by the operating team at the time of surgical procedure. The wounds were classified into clean, clean-contaminated, contaminated, and dirty/infected, based on NHSN wound classifications.[8] The study included patients who underwent elective surgeries falling under

clean and clean-contaminated cases with at least 7 days of hospital stay post-operatively, and who experienced signs and symptoms such as redness, pain, swelling around the surgical site, tenderness over the site, fever, delayed healing, etc. A written informed consent was obtained from the patients who agreed to participate in the study. Wounds falling under the categories of contaminated and dirty/infected were excluded from the study. Data was collected using a specially designed case report form.

Sample Collection and Processing of Specimens

Two swabs were collected from the surgical site taking aseptic precautions, and transported immediately to the laboratory in a sterile container to be added. One swab was subjected to Gram stain, for the presence of pus cells and organisms. Another swab was cultured on blood agar media, MacConkey agar media, and Thioglycolate broth. Interpretation of the cultures and identification of the organisms was done as per standard protocol, and antibiotic sensitivity was done using Kirby–Bauer disc diffusion method. Sensitivity pattern of the isolates are recorded as per Clinical and Laboratory Standard Institute (CLSI) guidelines.[9] Institutional Ethical Committee approval (letter number: RRMCH-IEC/77/2021; dated: 29.07.2021) was obtained for this particular study before commencing the study.

STATISTICAL ANALYSIS

Demographic variables were summarised using means for continuous variables. To know the significant difference between risk factor and presence or absence of SSI, analyses were done using the Z-test. A p value of <0.05 was considered statistically significant.

RESULTS

A total of 481 cases were admitted for surgery during the study period. Of these, 338 cases were excluded from the study group as the wound belonged to contaminated or dirty/infected classification.

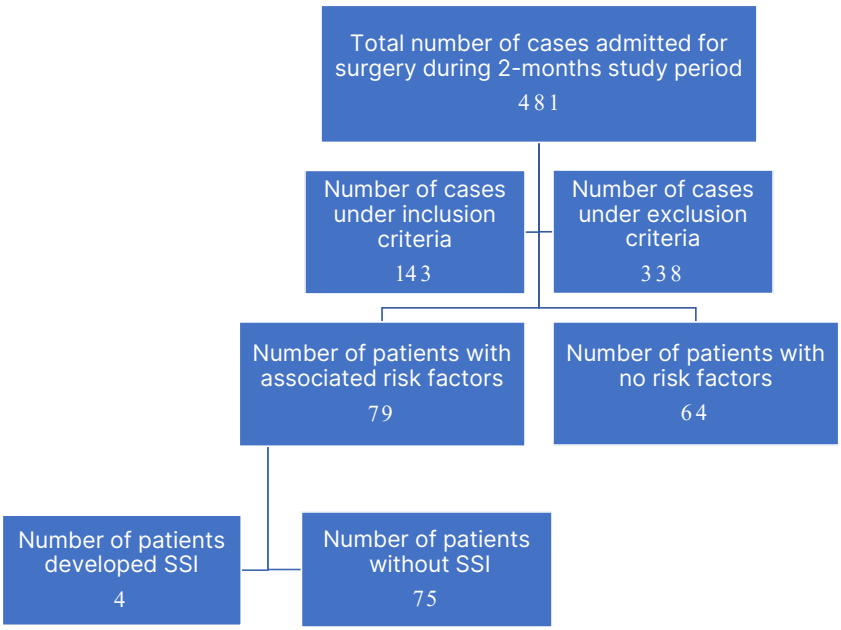
A total of 143 cases were included in the study as per inclusion criteria (Figure 1) The mean patient age ranged from 15–75 years; however, the majority (81.8%) of the study group were aged between 35–65 years. The majority of the patients were male (76%). Out of 143 cases, 79 (55.2%) had patient-related risk factors, and 64 (44.8%) cases did not have associated risk factors. Among the study group, 36.4% had uncontrolled diabetes, 17.8% had obesity, 10.2% were smokers, and 7.6% had associated hypertension (Figure 2).

Out of 79 cases with risk factors, four (5.06%) patients developed SSI. These four patients had diabetes with history of tobacco chewing, diabetes with chronic cough, and diabetes with obesity, and hypertension, respectively, as an associated risk factor. A significant result with $p<0.05$ was noted when analysis was done for the presence of risk factors, and their association with SSI, using Z-test for proportion (Table 1)

A total of 25 different surgical procedures were done under clean and contaminated wound category on 143 cases during the study period. Of these, nine cases showed symptoms of redness and discharge at the site of incision. Wound swabs collected showed culture positive in four (2.8%) cases. These cases had undergone hernioplasty, mastectomy, lower segment Caesarean section, fenestration, and discectomy surgeries, respectively. Out of four positive cultures, *E. coli* was isolated from two patients (2.5%) and *S. aureus* in the other two cases (2.5%). Out of the two strains of *S. aureus* one was Methicillin-resistant *S. aureus*. Antibiotic susceptibility pattern of *E. coli* isolates showed resistance to ampicillin (100%), β-lactams (50%), cephalosporins (50%), and ciprofloxacin (50%), and were sensitive to aminoglycosides (100%) and carbapenems (100%). Both isolates of *S. aureus* were sensitive to linezolid, vancomycin, and clindamycin (100%). A case-wise SSI summary is shown in Table 2.

All SSI cases were treated with appropriate antibiotic coverage and subsequently discharged home after wound healing and without any further complication.

Figure 1: Flowchart depicting study methodology.



Average inflow of cases per month is 250

SSI: surgical site infection.

Figure 2: Surgical cases with associated risk factors.

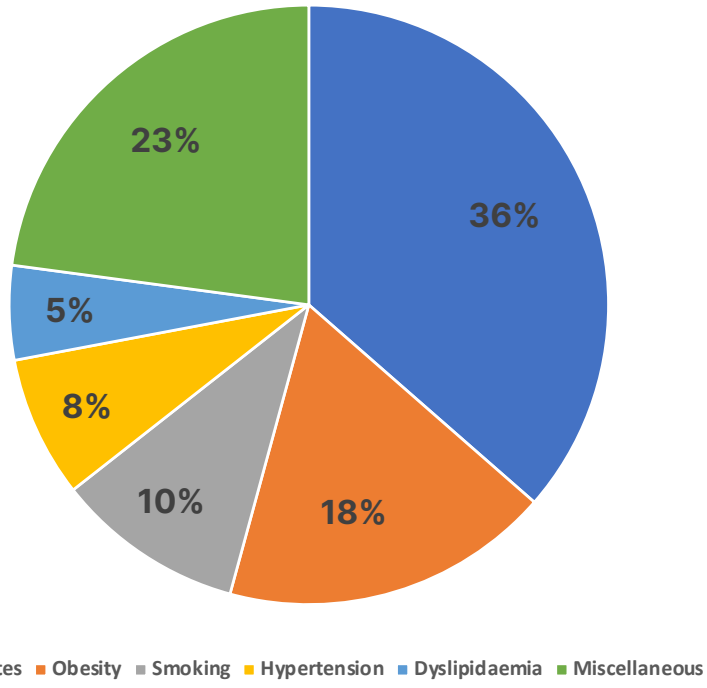


Table 1: Analysis using Z-test for proportion of risk factor present, associated with surgical site infection.

Risk Factor	SSI		Total	Z value	p
	Present	Absent			
	4	75	79	-11.29	<0.00001

SSI: surgical site infection.

DISCUSSION

SSIs are the infections that occur following a surgery at surgical site within 30–90 days of surgical procedure. Infection could be superficial, involving skin and subcutaneous tissue, or a serious infection involving deeper tissues, organs, or the implant itself.[10] In low-and middle-income countries, SSIs are the most surveyed and most common type of infection, with incidence rates ranging from 1.2–23.6 per 100 surgical procedures.[11]

Several studies from India have reported SSI rates ranging from 0.04–22.00%.[12,13] The authors' study reported 2.8% of SSIs, which is similar to a study by Satyanarayana et al.[14] SSIs are a major cause of morbidity and mortality worldwide, affecting 5.6% of surgical procedures, mostly in developing countries.[1]

Mangram et al.[15] have demonstrated several patient characteristics like diabetes, smoking, obesity, and remote site infections to have a significant, independent association for SSI prediction. A systemic review and meta-analysis by Martin et al.[16] reported significant association between diabetes and SSI that was consistent across multiple types of surgeries. Similar association was noted in the authors' study, where three out of four patients who had developed SSI had diabetes.

As the number and complexity of surgical procedures performed today are increasing, so does the cost of human life and financial burden if a person develops SSI, especially if there are associated comorbidities.[17-19] Numerous studies have proven increased financial implications and increased average length of hospital stay among patients with SSI when compared to non-infected patients with similar surgeries.[3,20-22]

In the present study, SSIs were recorded in the age group 22–65 years, which was also seen in the similar study by Keith et al.[23] However, gender was not a significant factor for prediction of SSI risk in the authors' study, which is similar to study by Berard et al.[24]

In the majority of SSIs, infections are mainly because of patients' endogenous flora. The aetiological agents will also depend on the type and location of the surgery. Various studies have reported *E. coli* and *S. aureus* as the frequent microbial flora associated with SSI, similar to the authors' study.[25,26]

In a hospital setting, various factors like poor infection control practices, inadequate sanitary conditions, and irrational use of antibiotics will favour development and spread of antimicrobial resistance. Hence, understanding the sensitivity pattern of the causative agent is crucial to initiate appropriate treatment. In the present study, *E. coli* was found to be 100% sensitive to aminoglycosides and carbapenems. *S. aureus* was sensitive to linezolid, vancomycin, and clindamycin (100%), which is comparable to studies by Mundhada et al.[22] and Dessie et al.[27]

High rates of methicillin-resistant *S. aureus* in clinical isolates from various studies in India have been documented, with rates as high as 41% reported.[28] In the authors' study, one of the two isolates of *S. aureus* was methicillin resistant.

Various factors influence the incidence of SSIs, such as pre-operative care, environment of operation theatre, efficient central sterile supply department, post-operative care, type of surgery, and associated host factors. Along with that, the factors that influence

Table 2: Case-wise surgical site infection summary.

SSI case number	Diagnosis	Surgery	Signs and symptoms	Organisms isolated	Sensitive to	Associated risk factors	Root cause analysis
1	Inguinal hernia (43 years; male)	Hernioplasty	Redness, wound discharge POD (4)	*E. coli*	Third generation cephalosporins, ciprofloxacin, aminoglycosides, carbapenems	Diabetes	High glycaemic index
2	Pre-eclampsia (23 years; female)	Lower segment Caesarean section	Redness, wound discharge, fever, tenderness, increased leucocyte count POD (2)	*E. coli*	Aminoglycosides, carbapenems	Hypertension	Pre-operative surgical prophylaxis was not given
3	Radiculopathy (24 years; male)	Fenestration and discectomy	Redness, wound discharge, fever, tenderness, increased leucocyte count POD (5)	*S. aureus*	β-lactams, cefoxitin, clindamycin, linezolid, vancomycin	Diabetes, obesity	Glycaemic monitoring was not done during surgery
4	Carcinoma breast (52 years; female)	Mastectomy	Redness, wound discharge, fever, tenderness, increased leucocyte count POD (4)	MRSA	Linezolid, vancomycin	Diabetes, chewing of tobacco	Immunocompromised state

E. coli: *Escherichia coli*; MRSA: methicillin-resistant *Staphylococcus aureus*; POD: post-operative day; *S. aureus*: *Staphylococcus aureus*; SSI: surgical site infection.

surgical wound healing and determine the potential for infection also play a vital role.

Numerous studies have estimated that approximately 60% of SSIs can be prevented by using evidence-based guidelines.[29,30] Berríos-Torres et al.[31] have recommended pre-operative full body bath; surgical prophylaxis as per clinical practice guidelines; surgical site skin preparation using alcohol-based agent; maintaining glycaemic levels less than 200 mg/dL; maintaining normothermia; and administration of increased fraction of inspired oxygen during surgery and after extubation in a patient undergoing surgery under general anaesthesia with endotracheal intubation and administration of blood products if required for preventing SSI. These guidelines can be incorporated into quality improvement programmes to have an holistic approach in prevention of SSIs and to improve patient safety.

In this regard, it is important that with multidisciplinary approaches and available preventive guidelines, improved antibiotic policy and addressing modifiable patient comorbidities before, during, and after surgery will help in prevention of SSI. Determining the antimicrobial patterns of the bacterial agents causing SSIs will help institutions to improvise antibiotic policy, and restrict the use of antimicrobials in preventing the spread of drug resistance in a hospital.

LIMITATION

The human behaviours in the form of hand hygiene and other complex nature of factors involved in development of SSI are the limitation of this study.

CONCLUSION

This study demonstrates significant association between patient-related risk factors and the development of SSIs.

Modifiable patient factors in the form of glycaemic control in diabetes, managing hypertension, and cessation of nicotine for weeks prior to surgery will facilitate the prevention of SSIs, which will assist in better management of surgical cases by reducing the length of stay in the hospital, and thereby reduce financial burden following surgery.

The early identification of SSI, supplemented with microbial cultures, will aid in initiation of early treatment with appropriate antibiotics, which will further prevent emergence of multidrug-resistant strains.

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First *Francisella novicida* Case Report in Argentina

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Abstract

The authors present a case report caused by *Francisella novicida*, a rare opportunistic human pathogen that may cause a tularemia-like disease in patients who are immunocompromised. The diagnosis is a challenge since it can be confused with *Pasteurella* or *Brucella*, and matrix-assisted laser desorption ionisation time-of-flight systems are limited due to its poor performance in identification.

Key Points

1. This manuscript describes a clinical case report caused by *Francisella novicida*, an uncommon bacteria that can cause opportunistic human infections in patients who are immunocompromised, with a range of symptoms from afebrile lymphadenopathy to pneumonia.

2. It is important that microbiologists bear this micro-organism in mind and to alert clinicians, since this disease is rare in the Southern hemisphere and occurs infrequently in patients.

3. The approach to the identification of this species is a challenge since the phenotypic identification cannot be achieved using biochemical tests and is not included in the matrix-assisted laser desorption ionisation time-of-flight mass spectra database.

CASE STUDY

A 38-year-old male with a right cervical mass, fever, and sweating was admitted to the authors' hospital in 2020. The patient had no weight loss or asthenia.

In 2015, the patient had presented with osteoarticular disseminated histoplasmosis, negative IgM, and negative anti-core antibodies (through a chemiluminescent microparticle immunoassay [CMIA]), which was treated with intravenous (IV) amphotericin B for 7 days, and then with itraconazole (200 mg/12 hour) for 1 year. In 2018, the patient also presented lymph node salmonellosis and was treated with IV ciprofloxacin for 11 days.

On admission a CT scan was performed, showing several enlarged lymph nodes visible on chest, abdominal, and cervical locations. The abdominal CT showed multiple lymph node images in the intercaval left lateral aortic, and coeliac regions, as well as in both external and retrocrural iliac chains.

Laboratory results revealed: haematocrit: 39.8%; white blood cells: 9.490×10^3 µL; lymphocytes: 8.0% (0.759×10^3 µL); platelets: 344.000×10^9 /L; urea: 28 mg/dL; and glucose: 92 mg/dL. The patient had normal transaminases (aspartate transaminase: 29 mg/dL; alanine transaminase: 14 mg/dL), and alkaline phosphatase (356 mg/dL).

In the context of prolonged febrile illness, serology tests were carried out and showed non-reactive protein 24 antigen; anti-HIV-1 and HIV-2 antibodies (CMIA); non-reactive anti-human T-lymphotropic virus 1 and 2 antibodies (ELISA); negative hepatitis B (HB) core antibodies and HB surface antibodies (CMIA); negative anti-hepatitis C antibodies (CMIA); positive IgG anti-cytomegalovirus and negative IgM (CMIA); negative IgM anti-capsid antibodies (enzyme-linked fluorescence assay [ELFA]); negative Epstein–Barr virus; positive anti-capsid IgG antibodies (ELFA); positive anti-Epstein–Barr virus antibodies (ELFA); negative anti-*Brucella abortus* antibody (Huddleston and Rose Bengal Tests); IgM negative anti *Bartonella henselae* antibodies; and negative PCR for severe acute respiratory syndrome coronavirus 2.

Because of the patient's history of infections, their immune status was studied with the following results: cluster of differentiation (CD) 4 cell counts in peripheral blood were 272 cells/mm^3 (normal value: 771–1,180 cells/mm^3), with a percent value of 30% (reference value: 10–38%); CD8 cell counts in peripheral blood was 229 cells/mm^3 (normal value 629–1,128 cells/mm^3), with a percent value of 73% (reference value: 55–83%); and CD3 cell counts in the peripheral blood of 550 cells/mm^3 (normal value: 1,543–2,484 cells/mm^3), with a percent value of 36% (reference value: 28–57%).

A scheduled lymph node biopsy was performed. In the following days, asthenia and temperature increased; therefore, the patient was hospitalised.

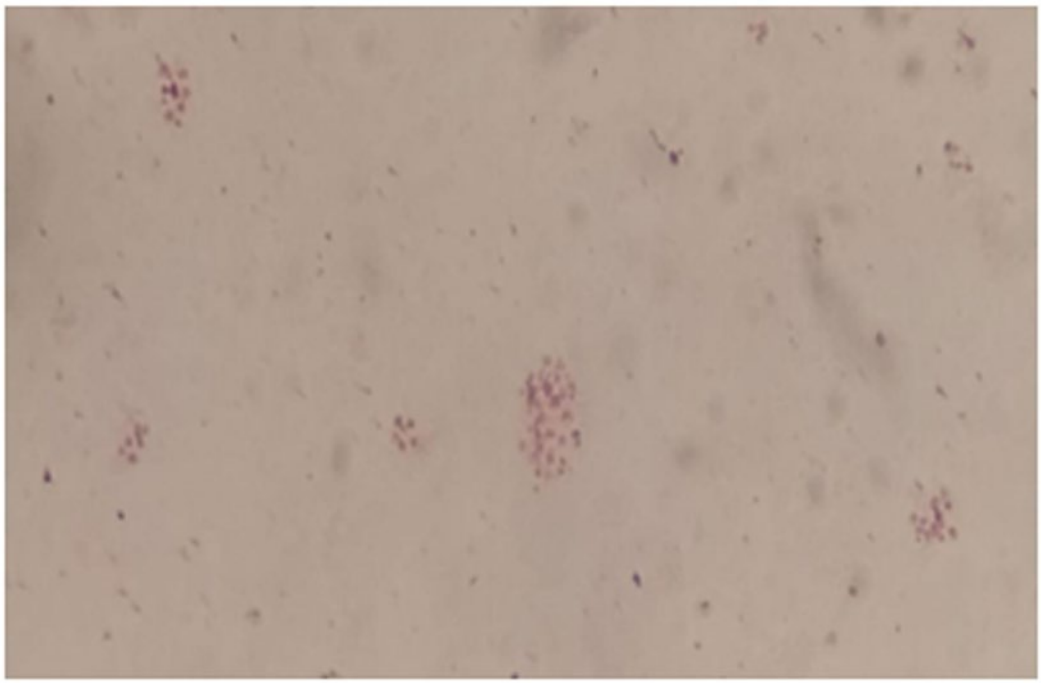
The biopsy sample was cultured for aerobic and anaerobic bacteria, as per the procedures of the microbiological laboratory. The conventional method was used in solid media (Löwenstein–Jensen [LJ] and Stonebrink mediums, with and without decontamination) to detect mycobacteria. The sample was also cultured for mycology diagnosis on Sabouraud agar and brain heart infusion blood agar (fungal formulation) at 28 °C and 35 °C.

Cultures for mycobacteria and fungi obtained from the lymph node biopsy were negative at 60 and 30 days of incubation, respectively.

At the bacteriology laboratory, the lymph node biopsy was cultured in chocolate (bioMérieux [Marcy-l'Étoile, France]) and Columbia blood agar. The biopsy was incubated in a 5% carbon dioxide atmosphere at 35 °C, and in *Brucella* blood agar, with and without antibiotics, in an anaerobic atmosphere. The microscopic examination on Giemsa stain did not show intracellular yeasts and Ziehl–Neelsen stain was negative. Gram stain using safranin was also negative, but the counterstain with fuchsin showed several short Gram-negative rods (Figure 1). Small colonies (1.0–1.5 mm) were observed after 72 hours of incubation on 5% sheep blood agar and on the chocolate agar plates. No differences in colony size were observed between both plates.

Since the patient continued to have a fever, three sets of blood culture were obtained, each set including one aerobic and one anaerobic bottle.

Figure 1: Gram stain of *Francisella novicida* isolate.



Morphology: very tiny gram-negative pleomorphic coccobacilli.

All sets, including aerobic blood bottles (BACTEC [Becton, Dickinson and Company, Sparks, Maryland, USA]) were positive at 44, 45, and 42 hours. Gram stain using fuchsin showed Gram-negative coccobacilli, identical to the Gram stain observed in the lymph node biopsy culture.

Matrix-assisted laser desorption ionisation time-of-flight (MALDI-TOF Biotyper; Bruker [Billerica, Bremen, Germany]) was also used to identify the micro-organism. Mass spectra were acquired using the MALDI-TOF MS in a linear positive mode (Microflex [Bruker]) in an m/z range of 2,000–20,000, using a Microflex LT controlled by FlexControl (software version: 3.4 [Bruker]).

The isolate could not be identified (not reliable identification) by MALDI-TOF MS. The phenotypic identification was performed by traditional biochemical tests, according to the Wauters and Vaneechoutte scheme.[1] The isolate was negative for oxidase, motility, nitrate reduction, urea, and indole; it also showed weak catalase activity. Acid production from carbohydrates glucose, glycerol, and sucrose in a cystine trypticase agar base with phenol red indicator was detected, but not from lactose. Pyrrolidonyl arylamidase and trypsin activity was detected. Gelatine hydrolysis was not detected. No inhibition halo (6 mm) to vancomycin (30 µg) and colistin (10 µg) were observed by disc diffusion on Mueller–Hinton blood agar. These results suggested that the isolate could belong to the *Francisella* genus (Table 1).[1,2]

Antibiotic susceptibility was determined by epsilometer test (Etest [bioMérieux]) on Mueller–Hinton agar, supplemented with 5% sheep blood agar, and incubated in a 5% carbon dioxide atmosphere at 35 °C for 24 hours. Minimum inhibitory concentration results were interpreted using the Clinical and Laboratory Standards Institute (CLSI) susceptibility breakpoints for *Francisella tularensis* with levofloxacin and doxycycline; *Pasteurella* with ceftriaxone and ampicillin/sulbactam; and *Haemophilus influenzae* breakpoints with clarithromycin.[3]

Minimum inhibitory concentration results showed that the isolate was susceptible to all the antibiotics: ampicillin/sulbactam (3.000 µg/mL); ceftriaxone (0.250 µg/mL); clarithromycin

Table 1: Biochemical tests to identify *Francisella* species.

Biochemical tests	Result
Oxidase	-
TSI agar	NG
Citrate	-
Mobility	-/-
PYR	+
Trypsin	+
Urea	-
DFO	S
Glucose (CTA)	+
Glycerol	+
Sucrose	+
Maltose	-
TM agar (base)	-
Gelatine hydrolysis	-
Van	R
Col	R

Col: colistin; CTA: cystine trypticase agar; DFO: deferoxamine; NG: no growth; PYR: pyrrolidonyl arylamidase; R: resistant; S: susceptible; TM: Thayer–Martin; TSI: triple sugar iron; Van: vancomycin; -: negative; +: positive.

(3.000 µg/mL); doxycycline (0.250 µg/mL); and levofloxacin (0.064 µg/mL).

The patient was treated empirically with IV ceftriaxone for 14 days. Doxycycline was initiated 2 days after starting ceftriaxone. At discharge, the patient's treatment was changed to ciprofloxacin 500 mg every 12 hours, plus doxycycline for up to 3 months.

In order to further identify the isolate, 16S ribosomal (r)RNA and *pgm* gene were amplified. Total DNA was extracted and used to perform PCR reactions according to the manufacturer's instructions (Inbio Highway® [Buenos Aires, Argentina]). Specific primers were designed for *pgm* amplification (*pgm*F: AGGCTTTTGGTGGGATTGTA; *pgm*R: AGTTGGTTCAGTCATTCCTGTT). By 16S rRNA gene sequencing, the strain was proven to be *F. tularensis*. The presence of the *F. tularensis* subspecies (subsp.) *Francisella novicida* was confirmed by *pgm* gene amplification, which showed a 99% identity with *F. novicida* U112 (AN CP009633). The *F. novicida pgm* gene sequence was deposited at GenBank (National Center for Biotechnology Information [NCBI; Bethesda, Maryland, USA]) under the accession number OQ122144.

DISCUSSION

The genus *Francisella*, a member of the γ-subclass of *Proteobacteria*, contains five valid species isolated in human sources: *F.*

hispaniensis, *F. opportunistica*, *F. salimarina*, *F. philomiragia*, and *F. tularensis*.[4,5] There are currently three proposed subsp., each of which displays several biochemical, epidemiological, and virulence characteristics: *F. tularensis* subsp. *holarctica*, *F. tularensis* subsp. *mediasiatica*, and *F. tularensis* subsp. *tularensis*.[6,7] In 2010, Huber et al.[8] validated the publication of the name *F. tularensis* subsp. *novicida* (herein *F. novicida*). However, according to the List of Prokaryotic names with Standing in Nomenclature (LPSN),[9] *F. novicida* has not been validly published. It is the correct name if this species is regarded as separate species; however, the appropriate nomenclature for *F. novicida* has been controversial.

F. tularensis is a Gram-negative coccobacillus and is the causative agent of the zoonotic disease tularemia in humans and animals.[7] The subsp. *tularensis* and *holarctica* are those most commonly associated with human disease.[7] However, *F. novicida* is considered a rare opportunistic human pathogen,[10,11] which may cause a tularemia-like disease in patients who are immunocompromised, similar to *F. philomiragia*.[12]

Kingry et al.[11] have highlighted clinical, ecological, genomic, virulence, and pathogenic differences between *F. novicida* and *F. tularensis*. *F. tularensis* causes the zoonotic vector-borne disease tularemia, while *F. novicida* does not. As determined by whole genome comparisons, *F. tularensis* evolved independently of *F. novicida*, which is consistent with its completely distinct ecological niche and mechanisms of transmission. Moreover, in relation to their intracellular lifestyle, they have different strategies to evade the immune response. The formation of the inflammasome, a multi-protein complex that is present in the host cell cytoplasm and can be activated by microbial components to induce maturation of cytokines, leading to death of infected cells, is present in *F. novicida* but not in *F. tularensis*. Therefore, *F. novicida* is unable to efficiently evade the host immune response in contrast to *F. tularensis*.

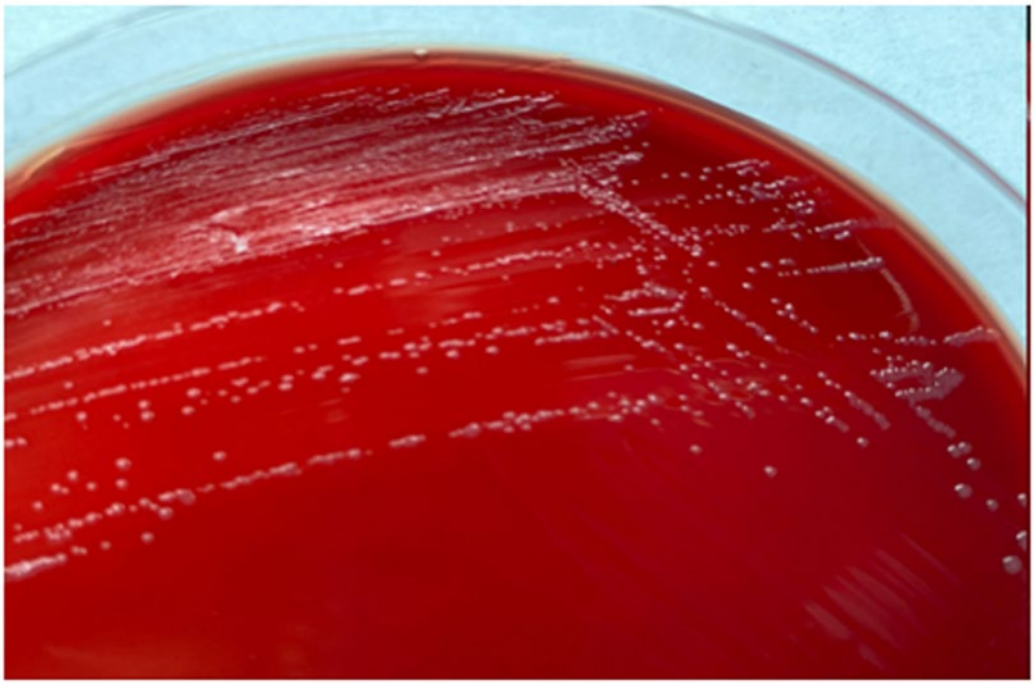
According to the authors, *F. novicida* encodes 84 genes that are inactivated in *F. tularensis*. The predicted function of these genes (carbohydrate metabolism, amino acid biosynthesis, metabolite transport, energy metabolism, transport, and DNA restriction or modification) is consistent with *F. novicida* maintaining the ability to exist in the environment, outside animal hosts. Genomic analyses of *F. tularensis* and *F. novicida* indicate a duplication of the 30 kb *Francisella* pathogenicity island (16–19 genes comprising a Type VI secretion system) in *F. tularensis* in comparison to *F. novicida*, which contains only a single copy. Furthermore, the virulence of *F. novicida* upon subcutaneous introduction appears to be less than *F. tularensis* in mice, guinea pigs, and rabbits. In addition, the cell surface, a critical pathogenicity determinant, is different between these two species. The structurally and antigenically unique O-antigens from both species appear to play different roles in the pathogenicity of each strain. Another difference has been observed in pulmonary infection in C57 black 6 mice, which demonstrated dissimilar cell types infected *in vivo*.[11]

The diagnosis of *F. novicida* is challenging. It is difficult to see the cells on Gram stain because they are very small, even smaller than the *Pasteurella* species, and safranin is not recommended (Figure 1) as a counterstain. On blood and chocolate agars, the colonies are also like *Pasteurella*, and there is no growth in Levine eosin methylene blue or MacConkey agar since they have nutritional requirements (Figure 2). However, the isolate differed from *Haemophilus influenzae* or *Pasteurella* since the colonies did not have the typical mouse-like odour that these genera usually have.[12] Besides, the isolate did not grow in triple sugar iron agar.

Using standard biochemical tests, the authors ruled out *Brucella* because the nitrate reduction, urease, and oxidase tests were negative. However, it should be noted that *Brucella canis* often oxidases negatively, and only the urease test is useful for the differentiation of both.[1,12]

The MALDI-TOF MS system is limited due to its poor performance in *Francisella* species identification. The main reason could be attributed to the fact that, in the authors' laboratory, none of the agents of bioterrorism are included in the database, and *F. tularensis* is known as a potential biological weapon due to its high virulence and low infective dose. When the authors performed the identification, a reliable identification could not be reached. Several spectra of *F. philomiragia* have been included in the database, but there is no other species

Figure 2: *Francisella novicida* colonies in blood agar after 72 hours of growth.



Small colonies (1.0–1.5mm) with entire margin, smooth, and moist.

of the genus included in the database. When the authors observed the top 10 identification scores, this species appeared among the options but not with a score that could at least suggest the genus identification.

Even though 16S rRNA gene sequencing is widely accepted as a method for species identification, there are some cases in which the amplification of alternative genes is more suitable for identification.[13,14]

The fact that *F. novicida* and *F. tularensis* share approximately 97% nucleotide identity, could lead to a misidentification between them. Therefore, it has been described that the amplification of different genes (such as *pdpD*, *sdhA*, *uup*, *aroA*, *atpA*, *pgm*, *tpiA*, *trpE*, and *parC*) would be useful for further resolution between *F. novicida* and *F. tularensis*.[15]

In the authors' case, the sequencing and subsequent analysis of the *pgm* gene allowed them to correctly identify the isolate.

Human infections caused by *F. novicida* are rare and considered opportunistic infections. Isolates were recovered from blood, lymph node tissue, and wounds.[2] Conversely *F. tularensis* causes tularemia in healthy individuals, which may be presented with any one of the clinical forms such as: ulceroglandular, glandular, oculo-glandular oropharyngeal, and pneumonic. The port of entry is via an infective arthropod bite (from ticks, flies, or mosquitoes), direct contact with infected animals, ingestion of water or food contaminated by infected animals, and inhalation of infective aerosols. In contrast, *F. novicida* is not a zoonotic pathogen and, due to its low virulence, infections are unusual. The few cases described occur in patients who are immunocompromised, so its accurate diagnosis is difficult.[11] *F. novicida* has never been identified in arthropod vectors in nature and the only source has been associated with salt water.[2,16]

Clinical information available of 11 reported cases indicate that nine of *the F. novicida* cases occurred in patients who were immunocompromised or had underlying health conditions. Clinical symptoms of

infection range from afebrile lymphadenopathy to pneumonia.[14,17-21] In the two healthy individuals with *F. novicida* infection, regional lymphadenopathy with no fever or other symptoms was reported.[17,19] In these cases, the route of infection was uncertain. Two cases were due to near-drowning events in salt water, and three cases were associated with environmental contamination of outdoor ice machines.[15,21]

The authors' patient developed idiopathic lymphocytopenia, multiple enlarged lymph nodes, and fever like glandular tularemia, while HIV was ruled out.

The clinical presentation in the authors' patient, which included fever and cervical lymphadenopathy, in addition to the above-mentioned opportunistic diseases, led the authors to study the immune system and perform a lymph node biopsy to dismiss a lymphoproliferative process. During the pandemic, the authors' patient worked in a rural area, helping patients with addictions to recover. Moreover, the authors' patient did not report any bite, and this was not observed during physical examination. The patient's medical record indicated they had visited a coastal city 3 months before the onset of symptoms, suggesting an environmental source of the infection.

There is no validated treatment for infections caused by this species, but antibiotics used for tularemia are usually effective for *F. novicida* infections. Aminoglycosides, tetracyclines, chloramphenicol, and quinolones are frequently used in the treatment and prophylaxis of tularemia.[7] Although they rapidly acquire resistance to fluoroquinolones, they have been demonstrated *in vitro* in both *F. tularensis* and *F. novicida*, while natural strains with acquired resistance have not been reported so far.[10,22]

Despite sensitivity tests being standardised by the CLSI for *F. tularensis*, for ciprofloxacin, doxycycline, chloramphenicol, and gentamycin the results of the antibiotics tested *in vitro* were active against this strain. The patient had a favourable progress after antimicrobial treatment.

To the best of the authors' knowledge, this clinical case is the first report of *F. novicida* described in Argentina. The approach to the identification of this species is a challenge. It is important that microbiologists bear this micro-organism in mind, since it is rare in the Southern hemisphere and uncommon in patients.

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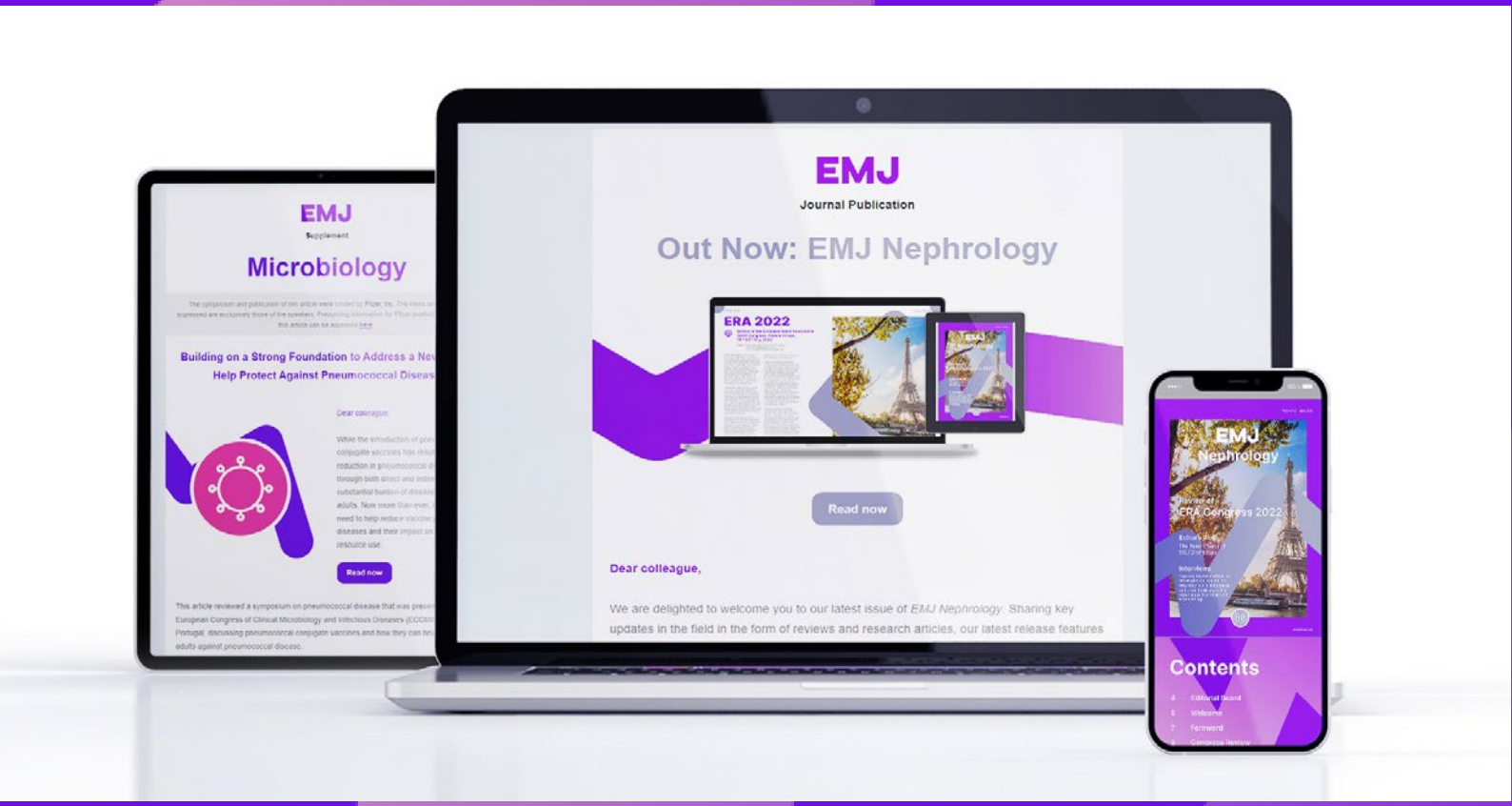

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