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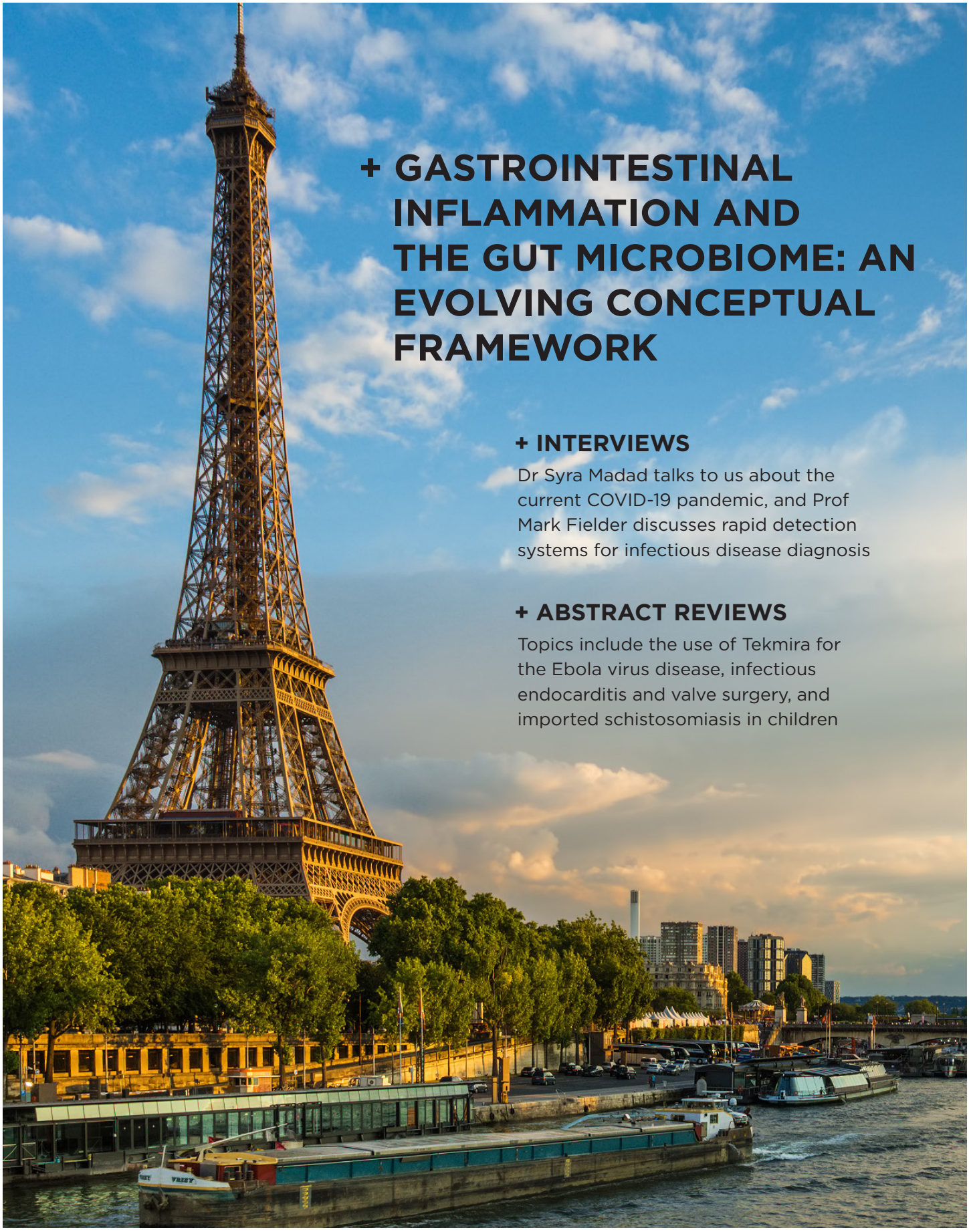

+ GASTROINTESTINAL INFLAMMATION AND THE GUT MICROBIOME: AN EVOLVING CONCEPTUAL FRAMEWORK

+ INTERVIEWS

Dr Syra Madad talks to us about the current COVID-19 pandemic, and Prof Mark Fielder discusses rapid detection systems for infectious disease diagnosis

+ ABSTRACT REVIEWS

Topics include the use of Tekmira for the Ebola virus disease, infectious endocarditis and valve surgery, and imported schistosomiasis in children

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"Launching a journal to disseminate research in this area of speciality could not be more relevant at this time, and EMJ is proud to be able to offer this open-access resource."

Spencer Gore, CEO

Editorial Board

VIEW IN FULL ←

Aims and Scope

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Welcome

Welcome to the first publication of *EMJ Microbiology and Infectious Diseases*, an eJournal for virologists, infectious diseases specialists, clinical microbiologists, and parasitologists. Launching a journal to disseminate research in this area of speciality could not be more relevant at this time, and *EMJ* is proud to be able to offer this open-access resource.

As always, this publication provides our readers with the latest research findings via our peer-reviewed articles, abstract summaries, in-house features, and interviews with key opinion leaders in the field. Our peer-reviewed articles feature a diverse range of topics, including gastrointestinal inflammation and the gut microbiome, urinary tract infection in children, and the association between the indoor microbiome and the rising prevalence of asthma. Our abstract summaries cover areas such as the use of Tekmira for the Ebola virus disease in Sierra Leone, the outcomes of drug injections leading to infectious endocarditis and valve surgery, and the prevalence and diagnostics of imported schistosomiasis in children.

Our peer-reviewed articles feature a diverse range of topics, including gastrointestinal inflammation and the gut microbiome, urinary tract infection in children, and the association between the indoor microbiome and the rising prevalence of asthma.

Our readers can also enjoy our exclusive interview with Dr Syra Madad, Senior Director of the System-wide Special Pathogens Program NYC Health + Hospitals, New York City. She talked to us about her views on the current COVID-19 pandemic and the biggest challenge facing our preparedness programmes in terms of pathogen control. Our editorial team also interviewed Prof Mark Fielder, Professor of Medical Microbiology at Kingston University London, London, who discussed rapid detection systems for infectious disease diagnosis and the use of artificial intelligence in the specialty, and how utilisation of such technologies could accelerate research and development.

I would like to offer my heartfelt thanks to our editorial board members, authors, peer-reviewers, and everyone here at EMJ for ensuring that publications such as this can continue to happen. So, without further ado, I hope you enjoy this issue, and that you are keeping safe and well.

Spencer Gore

Chief Executive Officer, EMG-Health

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Foreword

Warm Greetings!

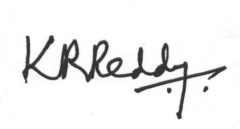
I welcome you all to this inaugural issue of *EMJ Microbiology and Infectious Diseases*. As the Editor-in-Chief of this promising ejournal, it gives me immense pleasure and pride to write a few words. This journal is a newly launched, international, rigorously peer-reviewed, open access, scientific and academic journal which comprises the outstanding achievements of the world's leading researchers and is dedicated to evolving and expanding the knowledge base of microbiology and infectious diseases. In this issue, readers will find a diverse group of articles; basic scientists and clinical specialists alike will be able to obtain valuable information and innovative ideas from these manuscripts.

Dr Oliver Grundmann describes the link between normal microbial flora of the gut and health and disease in an article pertaining to gastrointestinal inflammation and the gut microbiome. The gastrointestinal tract, particularly the large intestine, is the most heavily colonised portion of the body, with about 300 times as many anaerobic bacteria as facultative anaerobic bacteria. Dr Grundmann also discusses recent studies on antibiotic therapy. Injudicious use of broad-spectrum antimicrobial agents may suppress normal microbial flora, permitting the pathogen to predominate and cause infection.

Dr Kambiz Diba and colleagues, in their description of the molecular identification and antifungal susceptibility profiles of non-albicans Candida species, rightly point out that the incidence of fungal infections in recent years has been increasing rapidly. This has occurred alongside an emergence of newer fungal pathogens and anti-fungal drug resistance due to a multitude of predisposing reasons, including the prolonged and indiscriminate use of antibiotic therapy, immunosuppressive corticosteroid therapy, aggressive use of anti-cancer drugs, bone marrow and organ transplantation procedures, and underlying conditions such as immunodeficiency diseases (e.g., AIDS) and metabolic disorders such as diabetes.

A recent and most prominent clinical problem has been the emergence of the multi-drug resistant fungal pathogen *Candida auris*. Outbreaks have occurred world-wide in hospitalised patients in intensive care and many diagnostic laboratories do not identify Candida to the species level. The emergence of *Candida auris* as a nosocomial pathogen stresses the need for this level of identification. Molecular identification is needed for accurate recognition of organisms, as well as to find out the resistant strains.

I would like to thank all my editorial board members, reviewers, and authors for their excellent work and remarkable contributions in creating this first issue.



Prof. (Dr.) Rajeshwar Reddy Kasarla

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

Interviews

In the following interviews, EMJ speaks to two pre-eminent microbiology and infectious disease specialists, covering topics including their careers, research, and hopes for the future of this field.

Dr Syra Madad

Senior Director, System-wide Special Pathogens Program NYC Health + Hospitals, New York City, New York, USA

Q1

You started your career following completion of your degree in biotechnology. Why did you decide to pursue a career in special pathogen preparedness and response?

I've always had a passion for infectious diseases, highly infectious disease for that matter. My interest started very early on, after watching the movie "Outbreak" at the age of 13 and then in high school when my science teacher suggested I read the book "Hot Zone" by Richard Preston, and as they say, the rest is history. I decided to do my masters in biotechnology, with a specialisation in biodefence and biosecurity, to gain a better understanding of highly infectious agents. I then went on to pursue my doctorate in health science with a concentration in global health.

Special pathogens is a very niche area in the broad field of public health and there's not many people who are in this specific line of work. As we know, such pathogens can be more potent than conventional warfare. It's imperative to maintain readiness and critical that healthcare personnel have the training, knowledge, tools, and resources to be able to prepare for and respond to such events. It is because of the ever-present threat of special pathogens that I decided to pursue an active career towards it.

Q2

What are your main roles and responsibilities as the Senior Director of the System-Wide Special Pathogens Program? And what does the programme aim to achieve?

As the Senior Director I am responsible for the planning, organising, directing, leading, co-ordinating, and evaluating of emergency management activities for the NYC Health + Hospitals system-wide special pathogens programme efforts for all three NYC Health + Hospitals' service lines (acute, poste-acute, and ambulatory sites). I do so by developing policies and procedures for identification of patients

with highly communicable diseases (i.e., incident command/management, external notifications, clinical management, biohazard risks, personal protective equipment [PPE] management, staff training, and co-ordination with city, state, and federal agencies). I also develop, implement, and maintain effective training and education programmes on protocols and procedures to ensure the competency of healthcare workers to don/doff PPE, identify, and assess suspected or confirmed patients with special pathogens. The programme aims to achieve ongoing readiness for special pathogen events.

Q3 In 2015, you were awarded the Ebola Response Team Appreciation Award. Could you tell us about your contribution to the Ebola virus response effort that resulted in this award?

Working with the BioThreat and ChemThreat teams at the Texas Department of State Health Services, I performed senior-level consultative work in the planning, development, and implementation of the agency's public health preparedness and response initiatives. This included serving in the Ebola and Other Infectious Disease Surge Team to assist in a surge capacity, conducting bench work on select agents (i.e., Ebola, tularaemia, ricin, smallpox, etc.) for sample processing and proficiency test processing. This included working in the high containment BSL-3 laboratory with advanced PPE.

Q4 Currently, you are a Core Faculty and Exercise Resource Task Lead within the National Emerging Special Pathogens Training and Education Center (NETEC). Could you tell us about the centre and the services and resources it offers?

The NETEC is fully funded by the Centers for Disease Control and Prevention (CDC) and the Assistant Secretary for Preparedness and Response (ASPR) and is a collaboration between NYC Health + Hospitals, Emory University, and the University of Nebraska Medical Center. I lead the Exercise Resources Team as Task Lead of NETEC in: designing and developing Ebola and other special pathogens exercise materials; providing subject matter expertise on Ebola and special pathogens exercise design, development, conduct, and evaluation through technical assistance and site visits to healthcare facilities throughout the nation, as needed; co-teach the NETEC Emergency Management Workshop; and participate in conducting site visits to healthcare facilities throughout the nation. The combination of these different training resources contributes to the centre's goal to ensure national preparedness for Ebola and other special pathogens. More information about the centre can be found on their website.[1]

Q5 Pathogen control is important to the human population's health. What is the biggest challenge facing preparedness programmes, and pathogen control in general?

Preparedness programmes by large are funded by the government and are therefore a cost to the nation; however, a necessary one. A lot of the funding that we do receive is an emergency injection in response to an outbreak, and once the grant term ends the funding is basically at its end, meaning that we have to constantly advocate for funding to keep the programmes alive. So, the real challenge is obtaining sustained funding to keep these programmes ongoing on a long-term basis.

Q6 How does a seemingly simple infection then manifest as a pandemic? Are there any methods to predict and monitor pathogen threat level to prepare for an outbreak?

This goes to the concept of global health security. Outbreaks, regardless of origin and cause, can affect everyone. In an age of globalisation and rapid air travel, an outbreak that can start in a remote village can spread around the world in just 36 hours. To prevent outbreaks, there needs to be investment in zoonotic disease programmes that can catch a disease outbreak in its infancy, before it becomes a public health threat. In addition, there needs to be reliable surveillance systems, data sharing and transparency, international collaboration and response plans, and processes for prevention efforts.

Q7 There have been multiple serious outbreaks over the last decade; what are some of the main lessons that we have learnt from them? Have these already been implemented in the defence against new outbreaks?

Based on previous outbreaks, the lessons from those experiences suggest that through concerted efforts and partnerships at the local, national, and global levels, and greatly enhanced disease surveillance efforts, it is possible to continue making progress. This includes strengthening health systems, and training staff at all levels.

Q8

There have been outbreaks of pathogens that we believed to have been virtually eradicated. What reasons do you account for these resurgences?

There are a number of factors that contribute to the emergence and re-emergence of infectious diseases, including microbial adoption and change, human susceptibility, climate, changing ecosystems, economic development and land use, human demographics and behaviour, international travel and commerce, breakdown of public health measures, lack of political will, poverty, and social inequality etc. The threat of infectious diseases is continuous and is a perpetual challenge in any day and age.

There are a number of different external factors that influence an outbreak. Whether we are talking about surveillance or detection, or about the actual behavioural human response, antivaxxers are one of the factors that can influence an outbreak trajectory. So, this is always an issue, of which we may face again here in COVID-19 once a vaccine is available. We need to be conversant of these external factors, especially to the general public, and then work ahead of time to make sure that we are able to rectify or address any issues ahead.

Q9

You have spoken about the importance of public education before and during an outbreak. How does misinformation occur, and does it have an impact on the spread of disease?

The contagion of misinformation is real and is fuelling unwarranted fear and anxiety. The COVID-19 pandemic is constantly changing. It's important to get factual information from credible sources such as the CDC and World Health Organization (WHO), even your local health department. Due to misinformation, people may not know when it is appropriate to seek healthcare services or may not understand what the disease is itself or the signs and symptoms associated with it. Because there is a lot of misinformation, some sources are providing fake remedies or cures such as taking garlic or applying heat to the nostrils. These are unfortunately some of the myths out there that people believe and we want to make sure that people are going to credible, real sources and if they need to seek assistance that they know where to go to.

Q10

The recent pandemic of Wuhan Coronavirus spread from China to numerous countries worldwide. How was the initial pathogen control managed, and were there any additional steps that could have been taken to reduce the spread?

Infectious disease outbreaks are inevitable. Diseases such as the COVID-19 are very difficult to contain or control given the nature of respiratory viruses. Through the various factors that can influence or cause an outbreak as noted above, it's important to invest in epidemic surveillance at the local, national, and global level; strong public health and healthcare measures; and risk communication. It is vital to remember that for diseases that spread from human to human, like COVID-19, there may only be a limited window for action after an outbreak begins if a pandemic is to be averted. Transparency, sharing of information, and public trust, on top of public health measures, is critical.

Q11

During periods when a serious pandemic occurs, attention is drawn away from the influenza virus. Why is it important to not forget about flu season?

One might think of the flu as merely a seasonal inconvenience; the influenza virus is actually one of the deadliest pathogens ever known to science. It has a 1% mortality that results in 30,000–50,000 deaths annually, and places a significant burden on the health of the American population. At the same time, COVID-19 is not seasonal flu, and has a much higher transmissibility and fatality rate associated with it, in addition to the population being completely vulnerable due to having zero immunity to it.

References

1. National Emerging Special Pathogen Training and Education Center (NETEC). Available at: www.netec.org. Last accessed: 20 March 2020.

Professor Mark Fielder

Professor of Medical Microbiology, Kingston University London, London, UK

Q1 **You are the current President of the Society for Applied Microbiology (SfAM), the UK's longest-running microbiology society. What are the society's primary goals and how are you currently working towards these goals?**

At SfAM we have six main goals (or priority areas as we refer to them). These are, in no particular order: preserving and protecting our oceans; antimicrobial resistance (AMR); food safety and security; microbiomes; future applications of microbiology and microbial biotechnology; and finally, equality, diversity, and inclusion. Each of these areas is of great importance to our core aims in terms of protecting the natural environment, especially where microbes might be important in unlocking potential solutions to some of the problems we face, such as the biodegradation of plastics for example.

The AMR priority area is a must for a microbiological learned society. Antibiotics are possibly one of the most important medical inventions and interventions mankind has ever developed. They have facilitated improvements in human and animal health and support numerous medical interventions that might otherwise be difficult to undertake in their absence, such as cancer treatments, surgical procedures, and so on. The society takes an active role in areas that reduce selective pressure on microbes, help maintain the effectiveness of antimicrobials, and look to support the development of new technologies and tools that can aid in the fight against AMR. These approaches might also mitigate against the potential health, economic, and social impacts of AMR. Food safety and security remains a global challenge and microbiology is central to the production of certain common foods such as bread, cheese, and wine, as well as dietary additions such as probiotics and nutraceuticals. This particular goal links neatly with other priority areas such as AMR and microbiomes. The microbiome priority area encompasses applications of research that are vast and could transform aspects of healthcare, food production, and agriculture and environmental management. Understanding the natural biology of the microbiome is critical going forward.

Often the development of new drugs, diagnostics, and microbiome applications require aspects of applied microbiology and microbial biotechnology. The society recognises that these different technologies can be used and exploited for the global good and is at the heart of our organisation.

Finally: equality, diversity, and inclusion. Microbiology is without doubt a diverse specialty, as are the people that work in the area. The society passionately believes that all talented microbiologists, whoever they are, should be given the recognition, support, and opportunity they deserve. This approach, in our view, will ensure that the subject area will attract the best people. We are here to make sure we serve the needs of all citizens using microbiology to help solve some of the challenges the world currently faces.

Q2 **One of your research focusses is developing rapid detection systems for infectious disease diagnosis. Why is it important to diagnose these infections so rapidly?**

Rapid detection systems are of great importance to microbiology in a one-health context. We need to get targeted, effective treatment into our patients to eliminate infections and infectious organisms, wherever they manifest. However, microbiology is an intrinsically slow subject in that we must wait for the organism to grow and be identified, and then there is a further delay to

determine which antibiotics might be effective in treating the particular organism. This can take 36–48 hours, or even longer with some traditional culture methodologies. What is required is a much more rapid system that is either 'patient-side' or 'close to the point of decision making' that would take a much shorter time to provide a result. This means the attending clinician can have a clear view of whether the infecting agent might be bacterial or viral, perhaps whether the bacteria is Gram positive or negative, and even what the specific organism might be. This could then allow potential treatment to be targeted in a more accurate way to potentiate successful early treatment, killing the potential pathogen and limiting the chance for the development of antimicrobial resistance or treatment failure. So, in short, the quicker an infective agent can be identified then the better the chance of successfully treating that infection might be with a specific targeted treatment.

Q3

What are some of the current approaches to rapid infectious disease detection?

There are several different potential routes that are being examined as candidates for rapid diagnostics including microfluidics systems, loop-mediated isothermal amplification assays (LAMP), potentiometric immunological assays, and various molecular biological techniques. Many of these techniques can be specifically applied to a given diagnostic scenario for a particular pathogen or pathogen group. The issue can be getting the specificity and sensitivity of the new test to be acceptable as a usable diagnostic in the given setting. This is an important challenge to overcome to make sure that rapidity is not achieved at the cost of accuracy.

Q4

Infectious diseases can devastate populations in developing countries. What are examples of simple and low-tech rapid detection systems being developed for such environments, where high tech resources can be limited?

The need for usable tests in challenging, possibly resource-poor areas, is clear. However, tests still need to be both sensitive and specific. This can be a very difficult equation to balance. Sometimes the use of even potentially complex tests can be made simple for use in areas that are somewhat resource poor. One really good example I am aware of is the use of the LAMP assay for an important animal pathogen that can cause significant problems in developing countries. The standard test was a DNA-based test that required a clean laboratory with DNA preparation and amplification capability. In turn, tests have been developed using the LAMP platform that can amplify and identify the required gene marker whilst in the field, not just in a laboratory. One of the stages in the development of the test requires a period of incubation. Incubators can be problematic in the field; however, this test can be incubated under the arm of the operative (or assistant), a brilliant example of complexity being reduced to a simple, practical, pragmatic solution.

Q5

There has been interest from all areas of life science and healthcare towards artificial intelligence (AI). Is there room for AI in microbiology and infectious diseases, and will the utilisation of such technologies accelerate research and development?

The area of AI is quite exciting, and I believe there is a number of potential applications certainly in the direct diagnostics arena where AI could be used to identify patterns, pathology, or perhaps actually identify the organism itself. I certainly think that in time AI might well have a role here.

Already we have seen the potential identification of novel antimicrobials using AI; this is at an early stage but the use of AI in the development of new potential antibiotics is to be welcomed in my view.

Q6

Throughout medical history, we have discovered new antibiotics to then find that bacteria, some years later, have mutated against them. What does the future of antimicrobial drugs look like?

What does the new drug look like? That is hard to say. What we can say is that we now need to look to develop new potential antibiotics,

"We must ramp up the search for new antimicrobials and continue the work as time marches forward; we need to ensure we keep looking after future generations"

exploit existing ones, and use technologies such as AI to predict novel drugs from now on. We have done some preliminary work looking at potential antibiotic-producing bacteria from the soils of ancient and 'new' woodlands and we have found some potential candidates. This is really important work, it is a bit of a 'fishing trip' and we don't yet know how useful this could end up being; however, the search must be carried on. Initiatives such as Dr Adam Roberts' Swab and Send project is an excellent example of how we can find potentially useful antimicrobials and importantly include the public in the search. We must ramp up the search for new antimicrobials and continue the work as time marches forward; we need to ensure we keep looking after future generations.

"an integrated approach to biological and behavioural surveillance in healthy communities might be helpful in identifying possible zoonotic disease spill-over events"

Q7

The popularity of genetics has influenced many aspects of life sciences. How will it impact rapid detection of infectious diseases and the discovery of antibiotics?

The use of molecular biology in all realms of biology, including microbiology, has been revolutionary. Many detection systems utilise genetics and genetic variation to identify organisms and organism subtypes, as well as antibiotic resistance genes. The work to further employ genetics in the development of diagnostics and potentially identify novel antibacterial targets is exciting and must carry on at pace to ensure we can benefit from the findings going forward.

Q8

You are a co-author on the recently published paper titled 'Human-animal interactions and bat coronavirus spill-over potential among rural residents in Southern China'. What are the main take-away messages from this paper?

The paper reports on the indication that an integrated approach to biological and behavioural surveillance in healthy communities might be helpful in identifying possible zoonotic disease spill-over events or point surveillance towards at-risk populations. Using this approach might provide a form of early warning system for non-outbreak scenarios that might then be used to help identify possible emerging zoonotic diseases before large-scale outbreaks occur.

Abstract Reviews

Here EMJ introduces a collection of the abstracts that were to be presented at ECCMID 2020, prior to its cancellation.

The Case for Pharmacokinetic/ Pharmacodynamic Studies During Epidemics of High Consequence Pathogens: Tekmira For Ebola Virus Disease in Sierra Leone

Authors: *Janet T. Scott,[1,2] Raman Sharma,[3] Luke W. Meredith,[4,5] Jake Dunning,[6,7] Catrin E. Moore,[7] Foday Sahr,[8] Steve Ward,[3] Ian Goodfellow,[4,5] Peter Horby,[7] RAPIDE-TKM trial team[7]

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Acknowledgements: Dr Scott, Dr Sharma, and Dr Meredith all contributed equally. The authors would like to acknowledge the contribution of the Port Loko Public Health England (PHE) Laboratory in processing samples, the GOAL Ebola Treatment Unit staff who provided clinical care for the subjects and permitted the trial in their clinical facility. This work was supported by the Wellcome Trust of Great Britain (grant number 106491/Z/14/Z and 097997/Z/11/A and by the EU FP7 project PREPARE (602525). The PHE laboratory was funded by the UK Department for International Development. The funders had no role in trial design, data collection, or analysis. The views expressed are those of the authors and not necessarily those of PHE, the Department of Health, or the EU. Trial registration: Pan African Clinical Trials Registry PACTR201501000997429. Details on the RAPIDE-TKM trial can be found in the paper by Scott JT et al., titled 'Pharmacokinetics of TKM-130803 in Sierra Leonean patients with Ebola virus disease: plasma

concentrations exceed target levels, with accumulation in the most severe patients.'

Keywords: Ebola, epidemic, pharmacokinetics, Tekmira (TKM).

Citation: EMJ Microbiol Infect Dis. 2020;1[1]:16-17. Abstract No: AR01.

INTRODUCTION

Pharmacokinetic/pharmacodynamic (PK/PD) studies during epidemics pose substantial logistical and safety challenges. However, the data generated can be used in drug dose optimisation[1] and delineating toxicology thresholds (TT). It is possible to draw conclusions from a relatively small number of subjects. PK can alter substantially in the disease state, making information from patients invaluable. This is particularly true for the haemodynamic destruction caused by Ebola virus disease (EVD), culminating in multiorgan failure.[2] For example, isolated PK measurements were used to evaluate favipiravir concentrations in EVD patients, and an embedded PK/PD study of Tekmira (TKM-130803) (Arbutus Biopharma, Warminster, Pennsylvania, USA) in EVD patients yielded sufficient information to develop an *in silico* model, presented below.[3,4]

METHODS

TKM-130803 is a specific, anti-EVD therapeutic comprised of two small, interfering RNA (siRNA) siLpol-2 and siVP35-2. During the clinical trial in Sierra Leone in 2015, patients were given an intravenous infusion of 0.3 mg/kg of TKM-130803 over 2 hours daily for up to 7 days.[2,4] The trial was discontinued having reached a predefined statistical endpoint, which indicated a low probability of demonstrating overall therapeutic benefit compared to historic controls.[2] Plasma concentration of siRNA was compared to survival at 14 days. PK data were fitted to two-compartment models, after which Monte Carlo-simulated PK profiles were compared to efficacy thresholds (C_{max}: 0.04–0.57 ng/mL; mean concentration: 1.43 ng/mL), and TT (3,000 ng/mL).

RESULTS

siRNA was in quantitative excess of virus genomes throughout treatment: a level considered needed for efficacy, but the 95th percentile exceeded TT. The maximum area under the curve (AUC) for which the 95th percentile remained under TT was a continuous infusion of 0.15 mg/kg per day. Plasma concentration of both types of siRNA were higher in subjects who died, compared with subjects who survived ($p<0.025$ for both siRNA).

CONCLUSION

Subjects who died exhibited impaired drug clearance, justifying caution in dosing strategies for such patients. This analysis is the first PK model derived from patients with EVD and indicates that such studies are possible, though challenging. It has given a useful insight into the PK of the siRNA in the disease state and illustrates the value of designing PK/PD studies into future clinical trials in epidemic situations.[5]

References

1. Nguyen THT et al. Favipiravir pharmacokinetics in Ebola-infected patients of the JIKI trial reveals concentrations lower than targeted. PLoS Negl Trop Dis. 2017;11(2):18.
2. Malvy D et al. Ebola virus disease. Lancet. 2019;393(10174):936-48.
3. Scott JT et al. Pharmacokinetics of TKM-130803 in Sierra Leonean patients with Ebola virus disease: plasma concentrations exceed target levels, with accumulation in the most severe patients. EBioMedicine. 2020;52:102601.
4. Dunning J, Sahr F, Rojek A, et al. Experimental treatment of ebola virus disease with TKM-130803: a single-arm phase 2 clinical trial. Plos Medicine. 2016;13(4).
5. Rojek A et al. Insights from clinical research completed during the west Africa Ebola virus disease epidemic. Lancet Infect Dis. 2017;17(9):E280-92.

Bacteriophage Control the Prevalence of *Escherichia coli* ST131 in Different Countries

Authors: Jordan Mathias,[1] Abdulrahman Almusallam,[1] Dmitriy Babenko,[2] *Mark A. Toleman[1]

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Keywords: Bacteriophage, *Escherichia coli*, ST131.

Citation: EMJ Microbiol Infect Dis. 2020;1[1]:18-19. Abstract Review No:AR02.

BACKGROUND AND AIMS

Escherichia coli is the most important bacterial species in Europe, being the main cause of both urinary tract and bloodstream infections.[1] The *E. coli* species consists of at least 10,000 sequence types (ST),[2] with ST131 being the dominant cause of quinolone and β-lactam resistant infections throughout Europe.[3]

MATERIALS AND METHODS

In this study, 968 *E. coli* isolates from human bacteraemias, faeces, and sewage in 2014 were collected as part of a UK-wide study to assess the reservoirs and origins of *E. coli* that produce extended spectrum β-lactamase.[4] Of these *E. coli,* 332 belonged to ST131, and a subset of 193 of these isolates (all ST131) were used as representative of ST131 *E. coli* within the UK for the purpose of this study. Whole genome sequencing of these by Miseq (Illumina, inc., San Diego, California, USA) technology was performed. Core genome (CG) comparison of the ST131 isolates was achieved using blast 2.7.1 on 2,513 CG targets and CG tree generated using Ridom seqsphere (Ridon GmbH, Münster, Germany). The ST131 isolated were scattered throughout the CG phylogenetic tree displaying that the isolates were of diverse genetic makeup within the ST131 sequence type with maximum divergence of <1%.

Each ST131 isolate was tested for susceptibility to bacteriophage. The bacteriophages were isolated from human sewage collected from four countries during April–June 2019: the UK, Kazakhstan, Saudi Arabia, and Bangladesh. Each stock of bacteriophage was collected from similar sized waste-water treatment plants serving similar sized populations in each country. The bacteriophages were simply isolated by first removing the bacteria by centrifugation followed by filtration through a 0.45-micron filter. Bacteriophage were tested for activity by plaque assays on each ST131 isolate and characterised by electron microscopy and sequencing. The *E. coli* ST131 prevalence was also assessed in each country by random selection of 100 *E. coli* from the sewage samples and confirmation of the number of ST131 isolates by specific PCR.

RESULTS

The 193 *E. coli* ST131 isolates represented a broad range of CG multilocus sequence typing (cgMLST) types and were spread throughout the ST131 cgMLST tree. It was found that *E. coli* ST131 prevalence was dramatically different in the four countries: 11% in the UK, <1% in Saudi Arabia, 4% in Kazakhstan, and undetectable in Bangladesh. However, ST131-specific bacteriophages were highly prevalent in Bangladesh and Saudi Arabia (able to kill 71.75% and 68.20% of UK ST131 isolates, respectively), yet much less common in the UK (31.50%) and rare in Kazakhstan (5.8%) (Figure 1). Electron microscopy revealed that ST131 bacteriophage belonged to several different families including the *Siphoviridae*, *Podoviridae*, and *Myoviridae*.

CONCLUSION

The UK had the highest carriage rates of *E. coli* ST131, and Bangladesh the lowest.

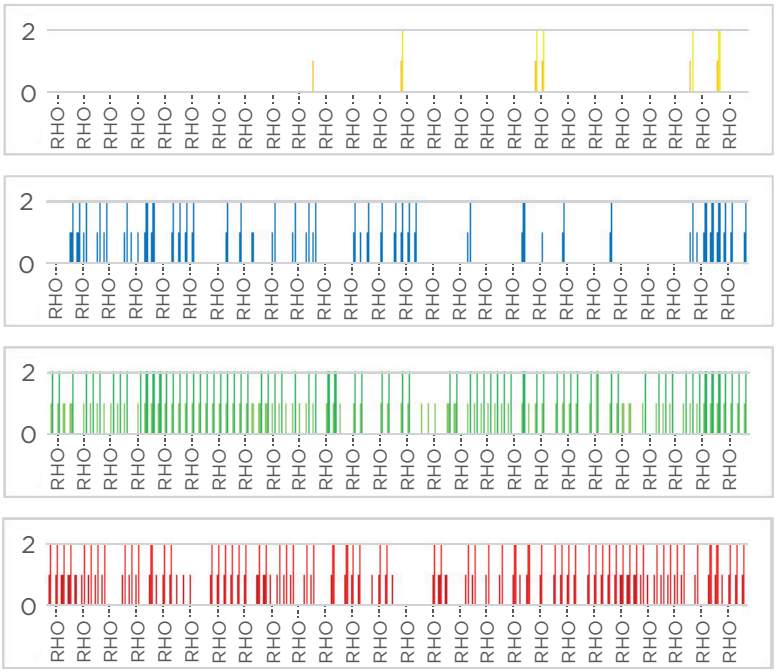

Figure 1: Availability of ST131 bacteriophages in sewage from four different countries.
The number of UK ST131 *Escherichia coli* strains that can be infected and killed by bacteriophage found in sewage isolated from Kazakhstan (yellow: 5.8%), the UK (blue: 31.5%), Saudi Arabia (green: 68.2%), and Bangladesh (red: 71.8%). Susceptible strains are indicated by vertical coloured lines for each country and assessed for high titre (long lines: >10^6 plaque forming units/mL) or lower titre (short lines: between 10^3 and 10^6 PFU/mL) by plaque assay on individual ST131 *E. coli* strains.

Thus, ST131 prevalence varies greatly by geographical location. Conversely, sewage from Bangladesh contained bacteriophage that could kill 72% of UK ST131, whereas sewage from the UK could only kill 32% of strains. This strongly suggests that prevalence of ST131 in different countries is controlled by bacteriophage. This difference can be manipulated to target problematic sequence types in one country using a pool of several countries' bacteriophages. Using a pool of all sites, bacteriophage activity against 91.3% of the 193 ST131 strains can be seen.

References

1. Russo TA, Johnson JR. Medical and economic impact of extraintestinal infections due to *Escherichia coli*: focus on an increasingly important endemic problem. Microbes Infect. 2003;5:449-56.
2. Nicolas-Chanoine M et al. *Escherichia coli*, an intriguing clonal group. J Clin Microbiol Rev. 2014;27(3):543-74.
3. PubMLST. *Escherichia coli* (Achtman) MLST locus/sequence definition database. Available at: https://pubmlst.org/bigsdb?db=pubmlst_ecoli_achtman_seqdef&page=query&scheme_id=4&submit=1. Last accessed: 17 April 2020.
4. Day MJ et al. Extended-spectrum β-lactamase-producing *Escherichia coli* in human-derived and foodchain-derived samples from England, Wales, and Scotland: an epidemiological surveillance and typing study. Lancet Infect Dis. 2019;19(12):1325-35.

SUSANA Project: Real-World Data Coming From The Use of New Antimicrobial Drugs

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Keywords: Adverse events (AE), antimicrobial drugs, multi-resistant infections, surveillance.

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BACKGROUND

Antimicrobial resistance is an increasingly serious threat to global public health.[1,2] In Italy, the issue of antimicrobial resistance is especially severe, as discussed in a report by the European Centre for Disease Prevention and Control (ECDC).[3] Although randomised clinical trials are considered the standard criterion for generating clinical evidence, real-world evidence evaluating efficacy and safety of new drugs is essential to improve their use. Herein is presented the preliminary data from the SUSANA Project: a study designed to evaluate the safety of new antibiotics in real-world use.

METHODS

This observational retrospective study started in April 2019 in a network of Italian infectious diseases units. Patients treated with ceftazidime/avibactam, ceftolozane/tazobactam, dalbavancin, and intravenous fosfomycin were included. The primary objective was the surveillance of adverse events (AE), to evaluate their frequency, type, and severity. The secondary objective was to investigate factors associated with AE onset. Causality was evaluated according to Jones' algorithm.

RESULTS

To date, 95 patients have entered the study (30.5% female). Median age was 68 years (range: 18–95). Infection sites were skin (n=27), genitourinary tract (n=23), respiratory tract (n=18), abdomen (n=11), osteoarticular tissue (n=10), cardiovascular system (n=7), central nervous system (n=2), and other or unidentified (n=9). Infection affected multiple sites in nine cases. Antibiotic treatment was empirical in 24 (25.6%) patients. Thirty-four subjects received ceftazidime/avibactam (15 with fosfomycin), 21 received ceftolozane/tazobactam (three with fosfomycin), 17 received dalbavancin (one with fosfomycin, one with ceftolozane/tazobactam), and 23 received fosfomycin.

Nineteen patients (20.0%) experienced at least one AE (causality). Three patients receiving ceftazidime/avibactam with fosfomycin had multi-organ failure (remote), one had an acute kidney injury (remote), and one experienced hypokalaemia (possible). One subject on ceftazidime/avibactam presented with a skin rash (probable), one had vomiting and peripheral ischaemia (both remote), one patient's platelet count decreased (remote), and one experienced bedsores (remote). Among the 15 on dalbavancin alone, one had a rash (possible). One patient on fosfomycin alone had hyponatraemia (remote), one experienced acute kidney injury (remote), and one had multiple events (remote). Four patients had AE possibly related to fosfomycin alone: oedema of the limbs, rash, nausea, and vertigo. One patient on ceftolozane/tazobactam with fosfomycin presented with atrial fibrillation (highly probable), and one on ceftolozane/tazobactam had a thromboembolic event, hypotension, and diarrhoea (all remote).

The outcome was known for 82 patients: 11 had a relapse of their infection, 63 recovered, one interrupted an empiric treatment, five died, and two were lost at follow-up.

CONCLUSION

Preliminary results of the SUSANA project show a high prevalence of AE in patients treated with the antimicrobials evaluated. However, most AE noted had low relationship of causality with the antimicrobials and were likely related to the clinical evolution of the primary conditions.

References

1. World Health Organization (WHO). WHO global strategy for containment of antimicrobial resistance. 2001. Available at: http://www.who.int/drugresistance/WHO_Global_Strategy_English.pdf. Last accessed: 19 March 2020.
2. World Health Organization (WHO). Antimicrobial resistance: global report on surveillance 2014. 2014. Available at: http://apps.who.int/iris/bitstream/10665/112642/1/9789241564748_eng.pdf. Last accessed: 19 March 2020.
3. European Centre for Disease Prevention and Control (ECDC), Cassini A et al. Mission report. ECDC country visit to Italy to discuss antimicrobial resistance issues 9-13 January 2017. Available at: https://ecdc.europa.eu/sites/portal/files/documents/AMR-country-visit-Italy.pdf. Last accessed: 19 March 2020.

Beyond Contact Precautions: What Do Surveillance Cultures Tell Us About Multidrug-Resistant Microorganisms in Critically Ill Patients? Data from a Public Hospital in São Paulo City, Brazil

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Keywords: Active surveillance culture, healthcare-associated infections (HAI), multidrug-resistant (MDR) colonisation, multidrug-resistant (MDR) infection.

Citation: EMJ Microbiol Infect Dis. 2020;1[1]:21-23. Abstract Review No: AR04.

BACKGROUND AND PURPOSE

In critically ill patients, colonisation by multidrug-resistant (MDR) bacteria is a significant risk factor for the development of subsequent infections.[1-3] Active surveillance cultures are routinely used to screen for MDR bacteria in selected scenarios.[4] The purpose of this study was to evaluate the epidemiological and molecular factors common between colonising bacteria and healthcare-associated infection (HAI) events caused by the same MDR bacteria in critically ill patients.

METHODS

The study was performed between January 2016 and May 2018 in two intensive care units (ICU), clinical and surgical, of a public tertiary hospital which specialises in cardiologic care. All patients admitted to these ICU had active surveillance cultures collected on entrance and then weekly, for the practice of contact precautions in cases of MDR bacteria detection.

The bacterial identification and susceptibility profiles were determined using Vitek® MS and Vitek® 2 Systems (bioMérieux, Marcy-l'Étoile, France), respectively; the detection of carbapenemases, *vanA*, and *vanB* genes were determined by real-time PCR. If a patient developed a HAI, comparison between antimicrobial susceptibility profiles of colonising and infecting strains was made. If available, genetic relatedness of the strains was characterised by pulsed-field gel electrophoresis. The HAI were characterised according to Centers for Disease Control and Prevention (CDC) criteria.

RESULTS

Thirty-six patients colonised by MDR bacteria who developed HAI by MDR were included in the study. Twenty-eight HAI episodes were caused by the same colonising bacteria with an identical antimicrobial susceptibility profile. The other eight HAI episodes were caused by non-concordant MDR microorganisms. The mean time between MDR colonisation and infection was 21.9 days. Carbapenemase-producing *Klebsiella pneumoniae* was the most frequently detected bacterium (n=24; 85.71%), followed by vancomycin-resistant *Enterococcus* spp. (n=2; 7.14%), carbapenem-resistant *Acinetobacter baumannii* (n=1; 3.57%), and *Pseudomonas aeruginosa* (n=1; 3.57%). The most prevalent HAI were central line-associated bloodstream infections in patients in the clinical ICU, and surgical site infections in patients in the surgical ICU. Fourteen episodes could be analysed by pulsed-field gel electrophoresis and the concordance rate was 100%. The colonising pathogens and HAI distribution are shown in Table 1.

CONCLUSION

MDR colonisation prevention measures are essential and must be performed in critically ill patients. Colonisation by MDR bacteria can be predictive of subsequent infection aetiology. An improvement in empiric antimicrobial treatment adequacy can be achieved if active surveillance culture is performed routinely.

References

1. Bhattacharya S. Early diagnosis of resistant pathogens. Virulence. 2013;4(2);172-84.
2. Papadomichelakis E et al. Screening for resistant Gram-negative microorganisms to guide empiric therapy of subsequent infection. Intensive Care Med. 2008;34(12):2169-75.
3. Tseng WP et al. Risk for subsequent infection and mortality after hospitalization among patients with multidrug-resistant Gram-negative bacteria colonization or infection. Antimicrob Resist Infect Control. 2018;7:93.
4. Centers for Disease Control and Prevention (CDC). Management of multidrug-resistant organisms in healthcare settings. 2006. Available at: https://www.cdc.gov/infectioncontrol/pdf/guidelines/mdro-guidelines.pdf. Last accessed: 29 March 2020.

Table 1: Colonising and infective bacteria concordance.

Microorganism	Colonised patients	Concordance by		PFGE - HAI topography
		ASP	PFGE	
	(N=36)	(n=28)	(n=14)	(n=14)
Cabapenemase-producing *Klebsiella pneumoniae*	28	24	10	CLABSI (n=6)
				VAP (n=2)
				UTI (n=1)
				SSI (n=1)
Vancomycin-resistant *Enterococcus* spp.	4	2	2	CLABSI (n=1)
				IE (n=1)
Carbapenem-resistant *Acinetobacter baumannii*	1	1	1	CLABSI (n=1)
Carbapenem-resistant *Pseudomonas aeruginosa*	1	1	1	CLABSI (n=1)
Carbapenem-resistant *Enterobacter cloacae*	2	0	0	0

ASP: antimicrobial susceptibility profile; CLABSI: central line-associated blood stream infection; HAI: healthcare-associated infection; IE: infective endocarditis; PFGE: pulsed-field gel electrophoresis; SSI: surgical site infection; UTI: urinary tract infection; VAP: ventilator-associated pneumonia.

Evaluation of A New Commercial Disc Susceptibility Kit for Detection and Differentiation of Carbapenemases Produced by *Enterobacterales*

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Keywords: Antimicrobial susceptibility, carbapenemase, *Enterobacterales*.

Citation: EMJ Microbiol Infect Dis. 2020;1[1]:23-24. Abstract No: AR05.

INTRODUCTION

Perhaps the most significant development in clinical bacteriology over the last decade has been the global proliferation of *Enterobacterales* with acquired carbapenemase enzymes (CPE).[1] Available data suggest that the vast majority of carbapenemases in *Enterobacterales* belong to one of the five major families: IMP, NDM, and VIM metallo-enzymes, and the *Klebsiella pneumoniae*

carbapenemase (KPC) and OXA-48-like enzymes.[2] CPE are frequently resistant to virtually all β-lactam antibiotics (including carbapenems) and carbapenemase genes are frequently transmissible via plasmids. Concomitant resistance to several other antimicrobial classes is common in CPE, dramatically reducing treatment options for infected patients.

Of particular concern among CPE are producers of OXA-48-like carbapenemase (hereafter referred to as 'OXA-48') that are already dominant among CPE in many European countries and are threatening to become dominant in the UK. Producers of OXA-48 can be particularly difficult to detect as they do not always confer resistance to carbapenems (particularly meropenem), and there is no specific inhibitor to assist in their detection. The MASTDISCS® Combi OXA (Mast Group Ltd., Bootle, UK) set for OXA-48 detection and carbapenemase screening is a kit comprising three discs. Disc A contains temocillin with KPC and metallo-β-lactamase (MBL) inhibitors, Disc B contains temocillin plus avibactam, and Disc C contains a penem antibiotic. The kit is intended to detect carbapenemase-producing *Enterobacterales* (CPE) and differentiate isolates with OXA-48-like enzymes from those with KPC or MBL. The authors describe here the first evaluation of this assay using standard European Committee on Antimicrobial Susceptibility Testing (EUCAST)/ Clinical and Laboratory Standards Institute (CLSI) methodology.

MATERIALS AND METHODS

The kit was evaluated using a diverse collection of 208 well characterised *Enterobacterales* including carbapenemase-producers (CPE: n=159), isolates with extended spectrum β-lactamase (ESBL) and/ or AmpC β-lactamase (n=47), and two control strains. Susceptibility testing was performed using EUCAST methodology on Mueller–Hinton agar. After overnight incubation, inhibition zone diameters were measured and the presence of carbapenemases was inferred following the kit instructions.

RESULTS

All of the CPE isolates (n=159) were correctly assigned as being carbapenemase-producers (sensitivity: 100%; specificity: 92%). Four out of 49 other isolates were incorrectly assigned as carbapenemase-producers, including isolates with TEM-10, LAT, and two isolates with DHA-1. Of the isolates with OXA-48-like enzymes, 61 out of 62 were correctly assigned as OXA-48-like producers with one isolate inferred to be a producer of KPC or MBL. Of 97 isolates with KPC or MBL, all but one were correctly assigned as KPC/MBL producers. A single isolate with a combination of carbapenemases (VIM and OXA-48) was assigned as an OXA-48 producer. Finally, one isolate of CPE with non-metallo-carbapenemase Class A (NMC-A) was falsely assigned as KPC/MBL.

CONCLUSIONS

The kit performed very well as a screening test with a challenging set of bacterial isolates. Most importantly, the presence of a carbapenemase was predicted with absolute sensitivity (100%) and high specificity (91.8%). Only 4 out of 49 non-CPE would require additional investigation as possible CPE, and the vast majority of these 49 isolates expressed ESBL or AmpC activity. This combination of three discs could be included with other antimicrobials for routine testing of *Enterobacterales* in clinical laboratories using EUCAST methodology.

References

1. Tängdén T, Giske CG. Global dissemination of extensively drug-resistant carbapenemase-producing *Enterobacteriaceae*: clinical perspectives on detection, treatment and infection control. J Intern Med. 2015;277(5)501-12.
2. Findlay J et al. Evaluation of three commercial assays for rapid detection of genes encoding clinically relevant carbapenemases in cultured bacteria. J Antimicrob Chemother. 2015;70(5)1338-42.

Outcomes of People Who Inject Drugs with Infectious Endocarditis and Valve Surgery

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Keywords: Cardiac surgery, infective endocarditis (IE), injection drug use, methicillin-resistant *Staphylococcus aureus*, people who inject drugs (PWID).

Citation: EMJ Microbiol Infect Dis. 2020;1[1]:25-26. Abstract Review No: AR06.

BACKGROUND AND AIMS

Infective endocarditis (IE) is a common disease state observed in people who inject drugs (PWID), with a 50–100 times higher incidence in PWID than the general population.[1] IE guidelines recommend valve surgery in patients who develop heart failure, have uncontrolled infection, large vegetation size, or present with systemic embolisation.[2-4] However, valve surgery may not always be feasible and is infrequently performed in PWID because of the perceived risk of prosthetic valve reinfection in recidivism.[1]

Valve surgery in PWID remains controversial, and data are conflicting when focussed on the short and long-term benefits of valve surgery in this population.[1,5-10] The timing of surgery and long-term outcomes depend on factors such as ongoing injection drug use and antimicrobial compliance;[7-8] reoperation rate in PWID is significantly higher than in individuals who do not inject drugs.[9-10] The objective of this study was to compare the outcomes of PWID who received valve surgery to those who did not receive valve surgery.

MATERIALS AND METHODS

This was a retrospective cohort study performed at a large academic medical centre in southeast USA and included hospitalised PWID with IE who received and did not receive valve surgery from January 2014 to October 2018. The primary outcome was all-cause 12-month mortality; secondary outcomes included short-term mortality and infection-related readmission.

RESULTS

The study included 178 patients: 41 (23%) received valve surgery and 137 (77%) did not. Patient demographics were similar between groups, except for that patients who received valve surgery were more likely to present with septic shock (73% versus 35%; p≤0.001); 103 (58%) patients were female, and the median age was 33 (interquartile range: 27–45) years. Native-valve IE was most common in both the valve surgery and non-surgery groups (91% valve surgery versus 93% non-surgery, p=1.0), and the most frequent IE types were left-sided (54% valve surgery versus 24% non-surgery, p=0.001), right-sided (32% valve surgery versus 64% non-surgery, p=0.001), both left and right-sided (15% valve surgery versus 6% non-surgery, p=0.1), and unknown (0% valve-surgery versus 5% non-surgery, p=0.4). From 176 patients, 216 organisms were identified; the most commonly identified organisms were methicillin-resistant *Staphylococcus aureus* (37%), methicillin-sensitive *S. aureus* (25%), streptococci (9%), enterococci (9%), *Pseudomonas aeruginosa* (5%), *Candida* spp. (1%), and other organisms (23%). Patients who received valve surgery had no significant differences in in-hospital (0% versus 8%; p=0.07) and all-cause 12-month mortality (15% versus 12%; p=0.6), or all-cause 12-month readmission (54% versus 53%; p=1.0) when compared to non-surgery patients, respectively. However, patients who received valve surgery were less likely to have an infection-related readmission at 90 days compared to non-surgery patients (26% versus 72%; p≤0.001). In multivariable regression, left-sided IE was the only variable associated with all-cause 12-month mortality (Table 1).

Table 1: Variables associated with all-cause 12-month mortality.

Variable	Total population n (%)	UnadjOR (95% CI)	AdjOR (95% CI)
Valve repair/replacement surgery	41 (23%)	1.3 (0.5–3.6)	Not tested
Left-sided IE	55 (31%)	6.2 (2.4–16.3)	6.1 (2.2–17.1)
Cardiovascular disease	11 (6%)	3.2 (0.8–13.0)	Not tested
Previous history of IE	36 (20%)	2.3 (0.8–6.2)	2.8 (0.9–8.3)
Septic shock on admission	65 (37%)	2.2 (0.9–5.4)	1.7 (0.6–4.7)
MRSA IE	80 (45%)	1.5 (0.6–3.8)	Not tested
Other concurrent infections	76 (43%)	0.5 (0.2–1.4)	Not tested

AdjOR: adjusted odds ratio; CI: confidence interval; IE: infective endocarditis; MRSA: methicillin-resistant *Staphylococcus aureus*; UnadjOR: unadjusted odds ratio.

DISCUSSION

Valve surgery in PWID was associated with fewer short-term infection-related readmissions but did not have any mortality benefit. These findings are significant because many previous reports showed improvement in early survival of PWID with IE after undergoing valve surgery, but subsequent poor long-term outcomes.[1,7-10] Valve surgery can address the embolic and haemodynamic complications of IE, but in PWID surgery should be a part of more comprehensive management approach that includes addiction and psychiatric assessment.

References

1. Straw S et al. Long-term outcomes are poor in intravenous drug users following infective endocarditis, even after surgery. Clin Infect Dis. 2019;ciz869. [Epub ahead of print].
2. Habib G et al. 2015 ESC guidelines for the management of infective endocarditis: the Task Force for the Management of Infective Endocarditis of the European Society of Cardiology (ESC). Endorsed by: European Association for Cardio-Thoracic Surgery (EACTS), the European Association of Nuclear Medicine (EANM). Eur Heart J. 2015;36(44):3075-128.
3. Baddour LM et al. Infective endocarditis in adults: diagnosis, antimicrobial therapy, and management of complications: a scientific statement for healthcare professionals from the American Heart Association. Circulation. 2015;132(15):1435-86.
4. Gould FK et al. Guidelines for the diagnosis and antibiotic treatment of endocarditis in adults: a report of the Working Party of the British Society for Antimicrobial Chemotherapy. J Antimicrob Chemother. 2012;67(2):269-89.
5. Lalani T et al.; International Collaboration on Endocarditis–Prospective Cohort Study Investigators. In-hospital and 1-year mortality in patients undergoing early surgery for prosthetic valve endocarditis. JAMA Intern Med. 2013;173(16):1495-504.
6. Habib G et al. Clinical presentation, aetiology and outcome of infective endocarditis. Results of the ESC-EORP EURO-ENDO (European Infective Endocarditis) registry: a prospective cohort study. Eur Heart J. 2019;40(39):3222-32.
7. Østerdal OB et al. Cardiac surgery for infective endocarditis in patients with intravenous drug use. Interact Cardiovasc Thorac Surg. 2016;22(5):633-40.
8. Wahba A, Nordhaug D. What are the long-term results of cardiac valve replacements in left sided endocarditis with a history of i.v. drug abuse? Interact Cardiovasc Thorac Surg. 2006;5(5):608-10.
9. Kim JB et al. Surgical outcomes of infective endocarditis among intravenous drug users. J Thorac Cardiovasc Surg. 2016;152(3):832-41.e1.
10. Kaiser SP et al. Long-term outcomes in valve replacement surgery for infective endocarditis. Ann Thorac Surg. 2007;83(1):30-5.

Analysis of *Escherichia Coli* Phylotypes and Known Sepsis Causing Sequence Types in UK Sewage Reveals a Direct Link Between Sepsis Rates and Carriage of Pathogenic Sequence Types in the Community

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Keywords: *Escherichia coli* (*E. coli*), sepsis, sewage, sequence type 73 (ST73), , ST95.

Citation: EMJ Microbiol & Infect Dis. 2020;1[1]:27-28. Abstract Review No: AR07.

BACKGROUND

UK *Escherichia coli* (*E. coli*) sepsis rates have been rising for the last 20 years, however the reason behind the consistent year on year increase is an enigma. Good information on the rates of increase are available from the Public Health England, Wales, and Scotland agencies. *E. coli* bacteraemia (blood infection) rates have been closely monitored since mandatory surveillance was initiated for acute NHS trusts in 2011. For example, rates have risen by 49% in Wales (60.3–89.8 per 100,000 population from 2010–2017),[1] 71% in England (45.0–77.7 per 100,000, 2009–2018),[2,3] and 31% in Scotland (66.6–87.3 per 100,000, 2009–2018).[4] However, the reason behind this increase is, to date, unknown. The sepsis rate also varies greatly between NHS geographic regions and considerably between London (64/100,000) and South Wales (85/100,000). The authors hypothesised that the different rates could be due to differing prevalence of pathogenic *E. coli* types in the different UK NHS regions.

METHODS

The authors investigated the common *E. coli* strains in the community at several UK locations by sampling sewage on entry to waste water treatment plants at multiple sites: Longreach (about 20 km east of London on the Thames), Marlow (Buckinghamshire), Reading (Berkshire), Bristol (Avon), Ponthir (South Wales), and Cardiff (South Wales) sewage works from 19.9.2019–26.9.2019. These samples were diluted and streaked on chromogenic UTI agar (no antibiotics) and approximately 100 *E. coli* isolates were chosen from each location. Species was confirmed by matrix-assisted laser desorption/ionisation-time-of-flight mass spectrometry and susceptibility determined by disc diffusion (European Committee on Antimicrobial Susceptibility Testing [EUCAST] methodology). The phylotype was determined by multiplex PCR (Clermont method). Each pathogenic B2 isolate was further tested for known common sepsis sequence types (ST): ST131, ST73, ST95, and ST69 by multiplex PCR (Doumith method).

RESULTS

The prevalence of pathogenic B2 phylotype *E. coli* was considerably higher in South Wales than in England (32.5% versus 17.8%), reflecting the differing sepsis rates between the two countries. B2 phylogenetic prevalence at each location was 33.0% (Ponthir), 32.0% (Cardiff), 24.0% (Bristol), 12.0% (Reading), 17.0% (Marlow), and 18.0% (Longreach). Prevalence was lowest in the London region (15.6%), an average of the sites that were all within 40 miles of central London, i.e., Reading, Marlow, and Longreach. The multiplex PCR for detecting known sepsis-causing pathogenic *E. coli* ST95, ST131, ST73, and ST69 detected one or more of these ST at all locations (Table 1).

Table 1: Sepsis-causing pathogenic *Escherichia coli* strain distribution across different geographical sites.

Location	*E. coli*	B2	ST95	ST131	ST73	ST69	Known ST	B2 other
Cardiff	60	19 (32%)	1	3	1	1	6 (10%)	13
Ponthir	117	39 (33%)	2	7	4	1	14 (12%)	25
Bristol	113	27 (24%)	10	4	2	1	17 (15%)	10
Reading	122	15 (12%)	-	2	5	-	7 (6%)	8
Marlow	95	16 (17%)	3	2	2	-	7 (7%)	9
Longreach	96	17 (18%)	2	2	2	-	6 (6%)	11

E. coli: *Escherichia coli*; ST: sequence type.

The prevalence of these specific ST was also higher in Wales than in England (11% versus 8.5%). The highest rate of specific sepsis *E. coli* ST was found in Bristol mostly due to a very high prevalence of ST95 (8.8%) in the community. However, overall Bristol had less of a diversity of sepsis causing ST as compared to the Welsh sites, with several additional sepsis-causing types commonly found in Wales.

CONCLUSION

This study demonstrates that: 1) human carriage of pathogenic B2 *E. coli* phylotypes is very high in the UK, especially in Wales; 2) specific ST within the B2 phylotype, known to be the cause of *E. coli* sepsis in hospitals across the nation, are commonly carried in the human gut; and 3) carriage rate is related to sepsis rate. Taken together, this information suggests a rational explanation of the rising sepsis rates in the UK, i.e., they are directly related to increasing carriage rates of virulent *E. coli* strains in the community.

References

1. Public Health Wales. All Wales *E. coli* bacteraemia surveillance 2018. Available at: http://www2.nphs.wales.nhs.uk:8080/WHAIPDocs.nsf/3dc04669c9e1eaa880257062003b246b/136795628878f1de8025838d003a6d1d/$FILE/Wales%20E.%20coli.pdf. Last accessed: 16 April 2020.
2. Public Health England. Laboratory surveillance of *Escherichia coli* bacteraemia in England, Wales and Northern Ireland: 2017. Available at: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/718820/hpr2218_ecoli.pdf. Last accessed: 16 April 2020.
3. Public Health England. Annual epidemiological commentary: gram-negative bacteraemia, MRSA bacteraemia, MSSA bacteraemia and *C. difficile* infections, up to and including financial year April 2018 to March 2019. Available at: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/843870/Annual_epidemiological_commentary_April_2018-March_2019.pdf. Last accessed: 16 April 2020.
4. Public Health Scotland. Gram negative bacteraemia infographic. Available at: https://hpspubsrepo.blob.core.windows.net/hps-website/nss/2776/documents/7_GNB_infographic_2019.pdf. Last accessed: 16 April 2020.

Direct Matrix-Assisted Laser Desorption Ionisation (MALDI) Identification from Positive Blood Cultures: Rapid Sepsityper®

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BACKGROUND AND AIMS

Sepsis remains one of the leading causes of death worldwide, with high morbidity and mortality rates.[1] Early and appropriate antimicrobial therapy is essential for the clinical outcome[2,3] because the survival rate of improperly treated patients decreases by each hour of treatment delay.[4] The rapid identification of the causative agent of sepsis is crucial for the patients' outcome. The Sepsityper® kit (Bruker Daltonik GmbH, Bremen, Germany) is a sample preparation method which enables direct application of matrix-assisted laser desorption ionisation–time of flight mass spectrometry (MALDI–TOF MS) to positive blood culture samples, and obtainment of a microbial identification at species level within 30 mins.[5] Steps include the lysis of blood cells, not disruptive for microorganisms, followed by centrifugation and washing steps to obtain a bacterial or fungal pellet. This microbial biomass is suitable for MALDI–TOF MS identification by either ethanol/formic acid extraction or direct smear (rapid Sepsityper), but also for further downstream applications, thus significantly shortening the time to reporting of results.[6-8]

MATERIALS AND METHODS

In this study, the authors evaluated the rapid Sepsityper procedure with a large collection of routine positive blood cultures (N=5,047) collected from February 2018 to October 2019. Further, the authors investigated the use of the residual bacterial pellet to perform susceptibility testing by the Microscan WalkAway microdilution panels (Beckman Coulter, Brea, California, USA). The Sepsityper method was performed following manufacturer's instruction.[9] Briefly, 200 µL of lysis buffer was added to 1 mL of positive blood culture broth. After vortexing, the sample was centrifuged for 2 mins at 14,000 rpm, and the supernatant was discarded. The pellet was resuspended in 1 mL of washing buffer and centrifuged for 2 mins at 14,000 rpm. The supernatant was removed, and the microbial pellet was used to prepare the MALDI Biotyper target for identification. For a subset of samples (1,648 overall: 1,370 enterobacteria; 98 nonfermenting Gram-negative rods; 83 *Staphylococcus aureus*; 83 coagulase-negative staphylococci; and 23 enterococci) the residual pellet was then used to prepare the inoculums for the Microscan panels (Neg Multidrug Resistant MIC 1, Pos MIC STA 36, and POS MIC E 37 panels).

RESULTS

Overall, the rapid Sepsityper enabled the direct identification of 4,304/4,848 (88.8%) monomicrobial samples, including 67 genera and 170 species. It showed a very good performance for enterobacteria (98.3%), nonfermenting Gram-negative rods (86.7%), *S. aureus* (96.2%), and enterococci (93.7%). The missed identifications were restricted mainly to a few groups of microorganisms (streptococci, *Corynebacteria*, *Bacteroides*, and yeasts) (Table 1). Among the 200 polymicrobial samples, both species were identified in 62 samples, only one species was identified in 73 samples, and none of the species were identified in 65 samples. Susceptibility testing was successful for 1,549/1,648 samples (94.0%), while for 99 samples the growth in the panel was insufficient, corresponding to the samples that delivered a pellet of poor quality.

Table 1: Efficacy of Sepsityper® for the identification of different groups of bacteria.

		ID	%	No ID	Total
Bacilli Gram negative	enterobacteria	1937	98.3	34	1971
	nonfermenting	221	86.7	34	255
Staphylococci	*S. aureus*	507	96.2	20	527
	CoNS	1152	85.1	201	1353
	Staph other	12	80.0	3	15
Streptococci	enterococi	192	93.7	13	205
	Streptococcus spp.	100	57.1	36	136
	S. pneumoniae	8	15.1	45	53
	others	6	54.5	5	11
Bacilli Gram positive	*corynebacteria*	24	55.8	19	43
	Bacillus spp.	12	66.6	6	18
	others	2	50.0	2	4
Anaerobes	Gram positive	44	67.7	21	65
	Gram negative	13	35.3	22	35
	yeasts	74	47.1	83	157
	Total	4304	88.8	544	4848

CoNS: coagulase negative staphylococci; ID: identification.

CONCLUSION

In this study, the rapid Sepsityper proved to be a reliable and robust method for bacterial identification directly from positive blood cultures, with an excellent efficacy for the most clinically relevant causative agents of sepsis. It enabled delivery of a result in a very short time, <1 hour for a batch of 10–15 samples, from the harvesting of the positive bottle to the MALDI result. Further, the same bacterial pellet used for the MALDI identification was suitable to set up the antibiotic susceptibility testing, simplifying and speeding up the routine workflow and the time-to-report.

References

1. Adhikari NK et al. Critical care and the global burden of critical illness in adults. Lancet. 2010;376(9749):1339-46.
2. Seifert H. The clinical importance of microbiological findings in the diagnosis and management of bloodstream infections. Clin Infect Dis. 2009;48(Suppl 4):S238-45.
3. Cattoir L et al. Improving timelines in reporting results from positive blood cultures: simulation of impact of rapid identification on therapy on a real-life cohort. Eur J Clin Microbiol Infect Dis. 2018;37(12):2253-60.
4. Kumar A et al. Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. Crit Care Med. 2006;34(6):1589-96.
5. Schubert S et al. Novel, improved sample preparation for rapid, direct identification from positive blood cultures using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry. J Mol Diagn. 2011;13(6):701-6.
6. Wimmer JL et al. Strategy for rapid identification and antibiotic susceptibility testing of Gram-negative bacteria directly recovered from positive blood cultures using the Bruker MALDI Biotyper and the BD Phoenix system. J Clin Microbiol. 2012;50(7):2452-4.
7. Hazelton B et al. Rapid and accurate direct antibiotic susceptibility testing of blood culture broths using MALDI Sepsityper combined with the BD Phoenix automated system. J Med Microbiol. 2014;63(Pt 12):1590-4.
8. Idelevich EA et al. Rapid identification and susceptibility testing of *Candida* spp. from positive blood cultures by combination of direct MALDI-TOF mass spectrometry and direct inoculation of Vitek 2. PLoS One. 2014;9(12):e114834.
9. Opota O et al. Microbial diagnosis of bloodstream infection: towards molecular diagnosis directly from blood. Clin Microbiol Infect. 2015;21(4):323-31.

Imported Schistosomiasis in Children: A Multicentre Study of Prevalence and Diagnostic Methods

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INTRODUCTION

Schistosomiasis is currently the second most common parasitosis in the world, following malaria, in terms of morbidity and mortality.[1] It is thought that, as a result of the increase in international travel and immigration from endemic regions, the incidence of imported schistosomiasis in France is rising. There are few data on imported schistosomiasis, especially in children. International guidelines recommend that adult migrants from endemic areas should be systematically screened for schistosomiasis, regardless of whether they are symptomatic or not.[2,3] However, the lack of studies on paediatric imported schistosomiasis means that there are no specific guidelines on screening children. The objectives of the present study were to estimate the prevalence of imported schistosomiasis in at-risk children in the greater Paris region of France and to compare diagnostic methods.

METHODS

All children at risk of schistosomiasis and who had undergone consultations in four hospitals in the greater Paris region between June 2017 and June 2018 were prospectively included. Clinical and laboratory data were collected after a consent form and an information leaflet were given to legal guardians, and to the child using a translator if needed. Unaccompanied minor refugees signed their consent in the absence of legal guardians (considered emancipated minors). This research was declared to the French Commission Nationale Informatique et Libertés according to the French law relating to computers, files, and freedoms (CNIL). Urine and faeces samples were screened using microscopy, a point-of-care circulating cathodic antigen,[4] and a real-time polymerase chain reaction assay.[5] Serum samples were screened using a Western blot assay, an ELISA, an indirect haemagglutination assay, and an immunochromatographic assay.[6] The Western blot assay and the microscopy analysis

were the reference methods used to estimate the prevalence of schistosomiasis. A latent class model was used to evaluate each method's diagnostic performance.[7]

RESULTS

A total of 114 children were included (male-to-female sex ratio: 2:9; mean age: 13.2 years). Most of the children were newly arrived migrants from sub-Saharan Africa. The prevalence of schistosomiasis was 26.3% and half of these positive patients were asymptomatic. In a latent class model analysis, the ELISA and the Western blot assay had the same sensitivity (83%) and specificity (99%). The serum immunochromatographic assay also performed well (sensitivity: 100%; specificity: 89%).

CONCLUSION

The high prevalence of imported schistosomiasis among at risk children in the greater Paris region confirms the need for systematic screening. A serum immunochromatographic assay appears to be the most cost-effective screening method.

References

1. World Health Organization (WHO). Schistosomiasis. 2019. Available at: https://www.who.int/schistosomiasis/en/. Last accessed: 21 August 2019.
2. Centers for Disease Control and Prevention (CDC). Schistosomiasis - Resources for Health Professionals. 2019. Available at: https://www.cdc.gov/parasites/schistosomiasis/health_professionals/index.html. Last accessed: 29 October 2019.
3. Gray DJ et al. Diagnosis and management of schistosomiasis. BMJ. 2011;342:d2651.
4. Danso-Appiah A et al. Accuracy of point-of-care testing for circulatory cathodic antigen in the detection of schistosome infection: systematic review and meta-analysis. Bull World Health Organ. 2016;94:522-33A.
5. Cnops L et al. A schistosoma haematobium-specific real-time PCR for diagnosis of urogenital schistosomiasis in serum samples of international travelers and migrants. PLoS Negl Trop Dis. 2013;7(8):e2413.
6. Beltrame A et al. Accuracy of parasitological and immunological tests for the screening of human schistosomiasis in immigrants and refugees from African countries: an approach with Latent Class Analysis. PLoS Negl Trop Dis. 2017;11(6):e0005593.
7. Rutjes AWS et al. Evaluation of diagnostic tests when there is no gold standard. A review of methods. Health Technol Assess. 2007;11(50):iii, ix-51.

Carrier Prevalence of Unknown Vancomycin-Resistant *Enterococcus Faecium* Carriers in The Capital Region of Denmark

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AIM

The aim of this study was to describe the prevalence of vancomycin-resistant *Enterococcus faecium* (VREfm)-carriers admitted to two of the hospitals in the capital region of Denmark during a 3-week screening period. The main outcome was to examine the prevalence of unknown VREfm-carriers and the secondary outcome was to compare this with the VREfm prevalence in the background population.

Table 1: Characteristics of the 172 patients screened during the study period.

	Non VRE-carriers	Unknown VRE-carriers	Known VRE-carriers
Number (%)	161 (93.6)	5 (2.9)	6 (3.5)
Median age	72	73	72
Admitted within last month (%)	36 (22.4)	5 (100.0)	5 (83.3)
Antibiotics within last month (%)	37 (23.0)	5 (100.0)	4 (66.7)
Antibiotics within last 6 months(%)	19 (11.8)	5 (100.0)	4 (66.7)

The red circle marks the characteristics of unknown VREfm carriers.

VRE: vancomycin-resistant *Enterococcus;* VREfm: vancomycin-resistant *Enterococcus faecium.*

BACKGROUND

Vancomycin is the first-line treatment of infections caused by *E. faecium*. VREfm infections are increasing, especially in hospital settings. Therefore, it was of great concern when VREfm began to increase in the capital region of Denmark towards the end of 2012.[1,2] Unidentified faecal carriers are a problem for infection control as they are a major reservoir of VREfm and a source for dissemination of VREfm.[3] With increased numbers of VREfm-infected or colonised patients, it is evident that new measures are needed to reduce the number of VREfm cases in hospitals.

MATERIALS AND METHODS

A rectal swab was obtained from all adult patients willing to participate, who were admitted to either the emergency department at Bispebjerg Hospital or Frederiksberg Hospital during a 3-week period in June to July 2019. All patients were screened for age, hospital admissions in the last 6 months, and antibiotic treatment within the last 6 months. The swabs were analysed for VREfm by culture and PCR for the *vanA* and *vanB* genes at the Department of Clinical Microbiology, Hvidovre Hospital. The 100 faecal samples sent to the department by general practitioners in the capital region of Denmark were examined for intestinal pathogens. The exclusion criteria consisted of age <50 years, VREfm and/or *Clostridioides difficile* positive samples within the last 6 months, hospital admission within the last 6 months, and travel outside the Nordic countries. They were subsequently screened for VREfm as mentioned above.

RESULTS

In the study, 172 patients were included and the median age was 72 years. Within this cohort, 11 (6.3%) were colonised with VREfm, 6 (3.4%) were known VREfm-carriers, and 5 (2.9%) were unknown VREfm-carriers. Of these unknown carriers, all had been hospitalised and received antibiotics within the last month (Table 1). Of the 100 faecal swabs sent by the general practitioners, one out of 100 (1%) had positive *vanA* and v*anB* PCR and none were culture positive.

CONCLUSION

Hospital admission and antibiotic use within the last month predisposed to colonisation with VREfm. The authors found a prevalence of unknown VREfm-carriers of 2.9%. In comparison, 1% of the patients without prior hospitalisation or antibiotic use were VREfm positive. Admission VRE screening could help relieve the burden of VREfm transmission within hospitals. As a result of this study, the authors recommend screening all patients admitted to the emergency department who have been hospitalised within the last month.

References

1. Pinholt M et al. Multiple hospital outbreaks of *vanA Enterococcus faecium* in Denmark, 2012-13, investigated by WGS, MLST and PFGE. J Antimicrob Chemother. 2015;70(9):2474-82.
2. Hammerum AM et al. Emergence of *vanA Enterococcus faecium* in Denmark, 2005-15. J Antimicrob Chemother. 2017;72(8):2184-90.
3. Christiansen KJ et al. Eradication of a large outbreak of a single strain of *vanB* vancomycin-resistant *Enterococcus faecium* at a major Australian teaching hospital. Infect Control Hosp Epidemiol. 2004;25(5):384-90.

Know Thine Enemy: Viral Genome Sequencing in Outbreaks

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CONTAINING a viral outbreak with public health measures firstly requires identification of the causative virus, followed by more detailed understanding of viral features. Genomic sequencing provides exhaustive insight into viral features that may help predict outbreak behaviours, assist in diagnosis and tracking, and shape treatment and vaccination strategies. When coupled with epidemiologic study of outbreak data, viral genomic sequencing can be used to direct public health measures and increase the speed of understanding compared to epidemiology alone.

Community spread of cases can be used to guide mathematic models and contact tracing of viral outbreaks for public health response. However, epidemiologic data alone better suits responses to low-prevalence and less-widespread outbreaks. Where pathogens have a longer latency period or spread affects rural and remote communities, features of the virus itself must be considered in determining the response. Genotypic and phenotypic characteristics, identified using molecular biology tools, can clarify the type and strain of a virus responsible for an outbreak, and inform and improve case diagnosis, treatment options, and vaccine development, as well as improve tracing accuracy.[1]

VIRAL GENOMIC SEQUENCING

Genomic analysis has developed to the point of near-real-time whole genome sequencing, evident in the identification and publication of the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) whole genome sequence on 11th January 2020, 12 days after the first announcement of the original cluster of cases in Wuhan, China, with a diagnostic test made available 2 days later.[2] This is a significant improvement in the speed of sequencing compared to the 2002–2003 SARS-CoV (SARS) outbreak, where the first case was identified on the 16th November 2002 and the viral genome sequence published on the 30th May 2003.[3]

Different sequencing methods provide specific insights into viral outbreaks. Amplicon-based sequencing rapidly duplicates viral nucleotide fragments via reverse transcriptase polymerase chain reaction (RT-PCR) and is helpful for the initial detection and study of viruses, as it can amplify even small fragments of viral material and provide fast results. However, its reliance on

Sanger sequencing means that it is unlikely to identify low-frequency variants, and amplification depends on the availability of PCR primers which requires pre-existing knowledge of the viral sequence, introduces bias, and limits the ability to undertake metagenomic analysis. It can also have limited utility in providing full genome sequences, as sample degradation prevents full-length amplicon production and high sequence variation in viruses means it is difficult to design primers that will produce full-length genomes.[1]

High-throughput sequencing, also referred to as second-generation sequencing, is more reliable for determining genome sequences of viral fragments or partially degraded samples. It can also detect low-frequency, within-host variants, can be used to sequence new or unknown pathogens, and can be used in metagenomic analysis. High-throughput sequencing combines selective RNase H-based digestion of contaminating RNA (mainly host ribosomal RNA) with sequence-independent primer amplification and was utilised during the 2014–2015 Ebola epidemic in western Africa.[1]

Features of a virus identified by genomic sequencing can determine the species responsible for an infection; clarify diversity within outbreaks, both genotypic and phenotypic; and provide understanding of the evolutional history of the virus, which may aid in treatment and vaccine development. Construction of a phylogenetic tree, that maps sequenced samples against one another by comparing nucleotide substitutions, is used to track the evolutional history of a new virus or outbreak. A phylogenetic tree was used in the 1997 avian flu outbreak in Hong Kong to identify the overlap between the human influenza A H5N1 virus in terrestrial poultry and a similar virus in quail.[1] Analysis of the phylogenetic tree mapping for the Ebola outbreak revealed high variation between outbreaks but low variation within outbreaks, which may suggest an animal reservoir and single zoonotic transmission for each outbreak of the virus.[1] The utility of a phylogenetic tree is affected by many factors, including accurate timestamping of collected samples and features of the virus itself, such as viral recombination during replication.[1]

EPIDEMIOLOGIC MEASURES AND MATHEMATICAL MODELLING

Epidemiology is the study of patient and community data to analyse population spread and behaviour of pathogens within populations. In the case of viral outbreaks, epidemiologic study provides insight into factors that affect distribution of infections within a community, including risk factors for contracting or transmitting a virus, and severity of infection; the prognosis of the viral illness; and the success of prevention and treatment strategies. Historically, epidemiology was dependent upon case-based data collection and analysis with deductive and inductive reasoning. However, the field has grown since the first application of mathematical modelling to population health by mathematician Daniel Bernoulli in 1760 tracking the effectiveness of an early smallpox vaccine. In modern epidemiology, use of statistical assessment and mathematical modelling has greatly expanded the reliability and applicability of epidemiological data.[3]

R_0 is the basic reproduction number of a virus, revealing the speed at which a virus spreads through a population by describing how many new cases can arise from one infected person. It is a theoretical parameter, in that it cannot be directly measured but instead is estimated based on epidemiological data including infection and recovery rates, viral transmissibility, and population size.[4] It is a valuable epidemiologic parameter but is imprecise, as factors contributing to R_0 can vary from person to person and the R_0 of a virus will change during the course of an outbreak. Pairing R_0 with genomic insights about viral evolution is part of a new field called phylodynamics, an emerging strategy for studying the activity and spread of viral outbreaks and helping to determine appropriate public health strategies.[1]

Contact tracing and mathematic analysis of case spread can provide insight into viral transmission and track success of public health measures. Contact tracing practices can clarify mode of transmission for new or unknown viruses, including whether a viral infection is vector-borne

"In the case of viral outbreaks, epidemiologic study provides insight into factors that affect distribution of infections within a community"

or capable of human-to-human transmission. Mathematical modelling to determine cost-effective and high-impact strategies to reduce viral prevalence during an outbreak are hindered by the fact that populations are heterogenous; population density, travel behaviours, and individual susceptibility or risk factors for illness vary throughout the population. Most epidemiologic mathematical modelling is undertaken as compartmental modelling to assess population subgroups, generally divided by risk factors such as age or by dividing populations into those at-risk, those infected, and those recovered from infection. This poses challenges for then attempting to scale insights into making public health recommendations across the full population.

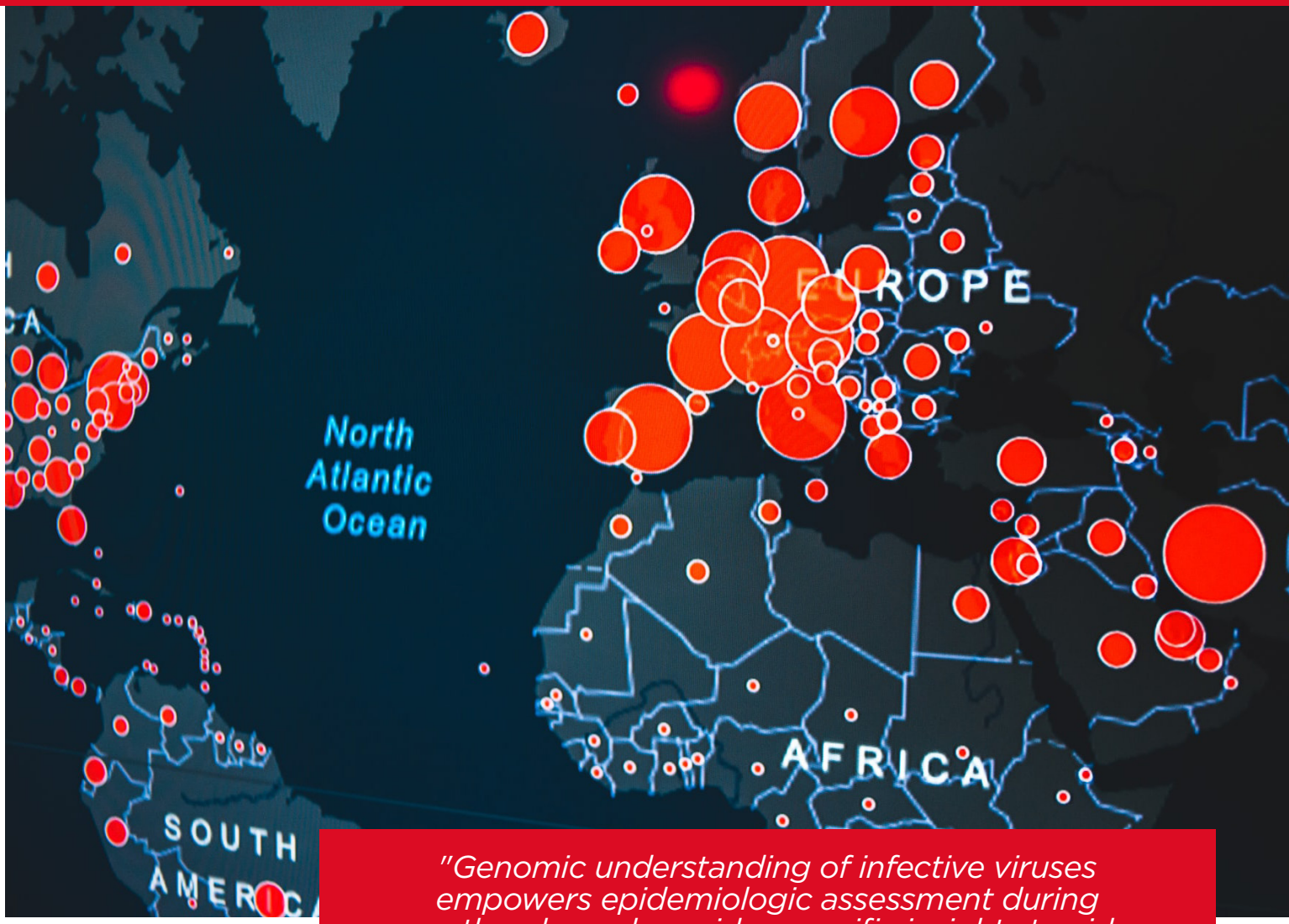
The incubation period of a virus describes the time from infection to displaying symptoms, while the latent period of a virus describes the time from infection to becoming infectious to others. These periods vary greatly between viruses, but both impact the infection rate during an outbreak. They also have a significant impact on public health measures, as interventions such as isolating infected individuals may be challenging to implement in cases with long incubation periods. The case fatality rate is another epidemiologic measure tracked during an outbreak. However, determining the percentage of infections that are fatal is difficult in the presence of heterogenous viral phenotypes, where some infected cases are asymptomatic or mild community cases.[3]

VIRAL MUTATION RATES AND VIRULENCE FACTORS

Other strategies for pairing epidemiologic and genomic data can provide further comprehension of viral outbreaks. Determining mutation rate of a virus and within-host substitution rate can further clarify transmissibility factors, direct treatment strategies, and determine viability of vaccine strategies. This makes use of both genetic sequencing and longitudinal case sampling, which requires many cases over longer-term periods and is currently most utilised in studying chronic viral infections such as HIV, although applications in acute outbreaks are developing.[1]

The mutation rate of a virus reflects the evolutionary changes occurring during and between outbreaks and is dependent on properties of the virus including whether it contains RNA or DNA, the fidelity of its polymerase, and the speed of replication of its own genome. Usually, RNA viruses mutate faster than DNA viruses. The nucleotide substitution rate is a measure of nucleotide mutation accumulation over a virus' lineage, and is determined by mutation rate, effective viral population size, and natural selection. The nucleotide substitution rate is most useful during an outbreak because it can develop understanding of the selection pressures and can be calculated from the phylogenetic tree and sampling dates. During outbreaks, the calculated substitution rate may be falsely elevated; the natural course of viral evolution includes deleterious substitutions that will be selectively removed from viral populations over time but are still present at the time of outbreak analysis. Overall, most models for analysing genetic changes in viruses, particularly to identify selection factors for host infection and viral survival, were developed to compare across viral species rather than for dynamic analysis of a single species during an outbreak, so this branch of genomic analysis is still limited in its application to outbreaks.[5]

Virulence factors specific to a virus are also important features for guiding the public health response to an outbreak. Virulence factors are features of the virus that determine the harm that it can do to the host, usually meant in terms of mortality. High virulence is often associated with high viral load, as the interaction of viral and host factors optimises virus survival and replication. However, in cases where viral features trigger an inappropriate immune response in the host, virulence may be high in the absence of high viral load. Vector-borne diseases often have higher virulence than viruses transmitted host-to-host. This is because, evolutionarily, host-to-host transmission and viral survival is dependent on host mobility and behaviour, although this virulence is increased in the case of viruses capable of prolonged environmental survival, i.e., survival outside of a host or vector carrier.[6,7] Virulence factors detected in laboratory assessment may be further understood with genomic analysis, such as cell surface receptor virion attachment, replication rate at different temperatures and in different inflammatory conditions, and virus tissue specificity or tropism.[8]



"Genomic understanding of infective viruses empowers epidemiologic assessment during outbreaks and provides specific insights to aid diagnosis, treatment, and vaccine strategies"

CONCLUSION

Genomic understanding of infective viruses empowers epidemiologic assessment during outbreaks and provides specific insights to aid diagnosis, treatment, and vaccine strategies. The improvement in genomic sequencing techniques has meant that public health action advances much more rapidly during an outbreak. The complexity of virus–host interactions and the heterogeneity of human populations make public health interventions difficult, but viral genomic sequencing provides a valuable contribution to planning whole-population and individual-level responses.

References

1. Wohl et al. Genomic analysis of viral outbreaks. Annu Rev Virol. 2016;3:173-195.
2. World Health Organization (WHO). Diagnostic detection of Wuhan coronavirus 2019 by real-time RTPCR. 2020. Available at: https://www.who.int/docs/default-source/coronaviruse/wuhan-virus-assay-v1991527e5122341d99287a1b17c111902.pdf. Last accessed: 1 May 2020.
3. White P, Enright M, "Chapter 5 - Mathematical models in infectious disease epidemiology," Cohen J et al. (eds.), Infectious Diseases: Volume 1 (2010) 3rd edition, Philadelphia: Mosby Elsevier, pp.70-5.
4. Ridenhour B et al. Unraveling R0: Considerations for public health applications. Am J Public Health. 2014;104(2):e32–41.
5. Morse SM, Khan AS. "CHAPTER 8 - Epidemiologic Investigation for Public Health, Biodefense, and Forensic Microbiology," Breeze RG et al (eds.), Microbial Forensics (2005), San Diego: Academic Press, pp.157-71.
6. Longdon B et al. The causes and consequences of changes in virulence following pathogen host shifts. PLoS Pathog. 2015;11(3): e1004728.
7. Brown NF et al. Crossing the line: selection and evolution of virulence traits. PLoS Pathog. 2006. Available at: https://doi.org/10.1371/journal.ppat.0020042. Last accessed: 1 May 2020.
8. Baron S et al. "Chapter 45 Viral Pathogenesis," Baron S (ed.), Medical Microbiology (1996) 4th edition, Galveston: University of Texas Medical Branch at Galveston. Available at: https://www.ncbi.nlm.nih.gov/books/NBK8149/. Last accessed: 1 May 2020.

Lessons Learned from a Global History of Pandemics

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PANDEMIC: the term making headlines across the world, instilling fear in many, and urging scientists across the world to unite and find a cure. For as long as the global population has exploited freedom of travel, so too have infectious diseases spread. Outbreaks have been nearly constant since the dawn of mankind; however, not all escalate to global levels. There have been many pandemics in history, the most recent being COVID-19 declared as such by the World Health Organization (WHO) on March 12th, 2020.[1] As the COVID-19 pandemic continues to disrupt our everyday lives, it is important to look back in history and reflect on what previous pandemics have taught us.

EPIDEMIOLOGY

Throughout the course of history, neither wars or natural disasters have killed as many humans as the viruses, bacteria, and parasites that cause disease.[2] Within our environment, a myriad of infectious pathogens perpetually surrounds us, some leading to mild or severe symptoms and others leading to none at all. The risk of infection is dependent on health, immunity, and in some cases even luck. When one of these diseases rapidly infects thousands within a community, population, or region, it is classified as an epidemic. When an epidemic spreads across multiple countries or continents it is referred to as a pandemic, a severe and worst-case scenario demanding immediate action.[3]

The introduction of agrarian communities led to an increased scale and spread of diseases. Widespread trade routes created new opportunities for zoonosis aiding the spread of epidemics such as malaria, influenza, and smallpox, all of which first appeared during this time.[4] As time progressed and humans settled in larger cities, established long-distance trade routes, and increased contact with various populations of people, animals, and ecosystems,[4] the likelihood of pandemics increased.

YERSINIA PESTIS: THE FIRST PLAGUE

A pinpoint in the history of pandemics is the first recorded outbreak of the plague, the plague of Justinian (541–542 AD, with recurrences until 750 AD), which was carried over the Mediterranean Sea from Egypt to Constantinople. Fleas carrying the bacteria *Yersinia pestis* infected rats, and these plague-infected rodents travelled aboard grain ships heading towards Constantinople. The rats thrived in the granaries, breeding rapidly and amplifying the contagion. This spread among the human population, and quickly dispersed across Europe, Asia, and North Africa, claiming the lives of an estimated 30–50 million people.[3] During this time, there was no understanding of the origin of

the plague and how to fight it other than to avoid those who were sick. Many ancient societies believed that spirits and gods inflicted disease and destruction upon those that deserved their wrath.[4] Even though the understanding of the pandemic at the time was limited, and factors such as poor sanitation and hygiene facilitated its spread, parallels can still be drawn to the COVID-19 pandemic as both originated from animal to human transmission. The fact that despite the above drawbacks humanity managed to overcome the plague, amid an enormous death toll, indicates the resilience of the human race, which could be an ally in overcoming COVID-19.

THE BLACK DEATH: QUARANTINE AS A MEANS TO PREVENT SPREAD

How the plague ended is yet to be defined, yet the consensus is that those that survive a pandemic have immunity.[3] Unfortunately, the first plaque never disappeared and returned 800 years later. During these years we learnt to travel further and faster; built more populous cities; and armies, colonisers, and traders all imported and exported the disease on ships and land,[5] all of which allowed far easier transmission of pathogens than during the previous plague. In just 4 years the Black Death was responsible for the death of one-third of the world population (200 million). There was still no understanding of how to stop the disease; however, it was understood that the spread of the disease was related to proximity.[3] Soon after the plague arrived in Europe, emergency public health measures were invoked that foreshadow today's best practices of social distancing.[6] The word quarantine stems from the Venetian word quarantena (40 days) referring to the 40 days ships were required to wait in the harbour before docking to ensure that no one aboard was carrying the plague.

"When an epidemic spreads across multiple countries or continents it is referred to as a pandemic, a severe and worst-case scenario demanding immediate action"

UNDERSTANDING THE SCIENCE: THE FIRST VACCINE

The efforts to tackle COVID-19 have primarily focussed on producing a vaccine against SARS-CoV-2. In what currently sounds like a best-case scenario, the ultimate goal would be to produce a vaccine that completely eradicates COVID-19, much like the smallpox vaccine led to the ultimate eradication of smallpox. For centuries, smallpox, caused by the virus *Variola major* or *Variola minor*, was endemic to Europe and Asia. Yet, the death rate paled in comparison to the devastation it brought to native populations when the virus arrived in the New World in the 15th century with the first European explorers.[7] Unfortunately, the indigenous population had no innate or acquired immunity to smallpox, and from 1520 onwards the disease has killed 56 million. However, as time had passed so had humanity's scientific understanding of pathogens grown. In many cases we have learnt from previous outbreaks to develop new techniques in treatment and prevention, such as the case for smallpox. In the 18th century, Edward Jenner discovered that those inoculated with a milder virus (cowpox) appeared immune to smallpox and thus smallpox became the first pandemic to be ended by a vaccine.[7]

Whether this will be the case for COVID-19 is something that remains to be determined.

AIRBORNE: PATHOGENS TAKING FLIGHT

Just as World War I was coming to an end, the world was struck by another pandemic in the form of Spanish flu (H1N1 [1918–1920]). The virus was airborne and spread through the sneezes and coughs of those infected. Unbeknownst to them, World War I troops that had worked in close quarters and then travelled around the world allowed the deadly influenza to spread rapidly. In 2 years, 40–50 million deaths were attributed to the virus.[3] Social isolation measures were invoked in many communities, including cancelling public events and closing schools, significantly reducing the spread of the disease. While most infectious diseases are particularly dangerous to the elderly, the very young, and those that are already immunocompromised, the Spanish flu bucked this trend by affecting the healthy and young to a similar degree. Although the jury is still out on whether COVID-19 is an airborne disease,[8] social distancing is the widely accepted course of action for slowing down its spread. However, unlike the Spanish flu, in the case of COVID-19 age seems to be a key determining factor of mortality, in addition to other less well-understood factors such as obesity.[9]

MODERN PROBLEMS: MISCONCEPTIONS AND DISEASE PREVENTION

Since the last pandemic, we have taken massive leaps forward in improved sanitation, hygiene, and nutrition, making the human population healthier and less vulnerable to illness than ever before. Despite this, we continue to face challenges. HIV is considered a pandemic by some; however, the WHO currently uses the term 'global epidemic.' To this day 35 million people worldwide have died of the disease and a cure is yet to be found.[3] These numbers are attributable to the spread of misinformation, especially amplified by homophobic attitudes in light of inaccurate scientific information during the initial outbreak in the 1980s. Prevention methods failed to reach people both within and beyond the targeted population as the public considered AIDS a disease of men who have sex with men. It was not until society acknowledged that AIDS can affect everyone that prevention increased and infection declined. This reinforces the view that dissemination of accurate information to the public, alongside medical progress is key in curbing a pandemic.

CONCLUSION

Despite the persistence of disease and pandemics throughout history, humanity has always continued to move forward and the gradual reduction in the death rate has been a consistent trend. Understanding the factors that nurture pandemics and improvements in healthcare have been powerful tools in mitigating their impact. It has been many years since humanity discerned the origin of these pandemics as being rooted in infectious microbes, as opposed to vengeful deities. This realisation foreshadowed continual learning and adaptation in the face

"Despite the continuous persistence of disease and pandemics throughout history, humanity has always continued to move forward "

of countless challenges. This adaptation has included quarantining the infected, enacting social distancing measures, and conducting geographical and statistical analysis in order to limit the spread, alongside the discovery of vaccines. Pandemics are more likely to occur if the threat has not been seen before and is easily transmissible, as is the case for COVID-19. However, the measures introduced by governments have enabled the pandemic to be better contained than those previously. The modern generation is experiencing the challenges of a pandemic first-hand and learning the importance of strict social distancing and prevention measures. Contact tracing, personal protective equipment, and testing have proven pivotal in the face of all pandemics. By implementing preparations for these events far earlier in the process, pandemics can be ended more quickly and met with better success, limiting disruption to life.

References

1. World Health Organization (WHO) Europe. 2020. Available at: http://www.euro.who.int/en/health-topics/health-emergencies/coronavirus-covid-19/news/news/2020/3/who-announces-covid-19-outbreak-a-pandemic. Last accessed: 29 April 2020.
2. BBC Future. 2020. Available at: https://www.bbc.com/future/article/20200325-covid-19-the-history-of-pandemics. Last accessed: 30 April 2020.
3. History. 2020. Available at: https://www.history.com/topics/middle-ages/pandemics-timeline. Last accessed: 30 April 2020.
4. Visual Capitalist. 2020. Available at: https://www.visualcapitalist.com/history-of-pandemics-deadliest/. Last accessed: 30 April 2020.
5. Science Museum. 2019. https://www.sciencemuseum.org.uk/objects-and-stories/medicine/bubonic-plague-first-pandemic. Last accessed: 30 April 2020.
6. History. 2020. Available at: https://www.history.com/news/quarantine-black-death-medieval. Last accessed: 30 April 2020.
7. History. 2020. Available at: https://www.history.com/news/pandemics-end-plague-cholera-black-death-smallpox. Last accessed: 30 April 2020.
8. Nature. Is the coronavirus airborne? Experts can't agree. 2020. Available at: https://www.nature.com/articles/d41586-020-00974-w. Last accessed: 27.05.2020.
9. Giacomelli A et al. 30-day mortality in patients hospitalized with COVID-19 during the first wave of the Italian epidemic: a prospective cohort study. Pharmacological Research. 2020; 104931. https://doi.org/10.1016/j.phrs.2020.104931.

Gastrointestinal Inflammation and the Gut Microbiome: An Evolving Conceptual Framework with Implications for Diagnosis and Therapy in Inflammatory Bowel Disorders

EDITOR'S PICK

The normal microbial flora of gut plays a very important beneficial role in the human body: it synthesises vitamins such as vitamin K and B complex, supplying some of the nutritional needs of the host; it prevents or interferes with colonisation or invasion of the body by pathogens through bacterial interference; it raises the overall immune status of the host against pathogens by presenting related or shared antigens; it kills or inhibits the growth of pathogens or other microorganisms by producing a variety of metabolic products, including bacteriocins; and the endotoxins liberated by Gram-negative bacteria may help defence mechanisms of the body by activating the alternate complement pathway. However, normal microbial flora may also have some harmful effects by helping opportunistic pathogen development when host immunity is compromised, and contributing to drug resistance through the production of penicillinase. I hope you enjoy this interesting article by Grundmann, my Editor's Pick for this issue.

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Abstract

The human gut microbiome has garnered much attention over the past two decades with important discoveries linking it to human health and disease. The commensal bacterial flora evolves due to the influence of a number of factors including diet, pathogen exposure, environmental toxicants, disease states, and a challenged microenvironment that requires balancing with the host itself. However, the composition of bacterial species can impact and contribute to the development of local and systemic inflammation. Among the factors attributed to intestinal inflammation are dysbiosis caused by pathogenic bacteria, following decreased host immunity or loss of intestinal barrier function. Dysbiosis can also be triggered by antibiotic therapy or the use of other medications that allow for colonisation of pathogenic bacteria, such as proton pump inhibitors. The imbalance with commensal bacteria leads to the generation of proinflammatory mediators and a reduction of host immune

defences, due to a lack of short-chain fatty acid generation needed for energy production to maintain barrier and immune function. The initially localised inflammation results in further dysbiosis as former commensal bacteria are able to breach the barrier and cause systemic immune responses. Low-grade systemic inflammation is a hallmark of inflammatory bowel disease. Because a specific dysbiosis is common in patients with inflammatory bowel disease, it can serve as an early diagnostic marker in its development. Furthermore, faecal microbiome transplants have shown promising benefits in patients with ulcerative colitis and Crohn's disease.

INTRODUCTION

In the past 10 years, the intestinal microbiome has been recognised as an important contributor to human health and disease. Scientific investigations have focussed on its maturation, interindividual differences, and interplay between the gut microbiome and a broad range of conditions. What was once perceived as a hypothesis with little science to back it up is now a well-regarded fact: the individual microbiome composition influences the host immune system, gastrointestinal health, and interplay with the central nervous system (the so-called 'gut–brain axis').[1]

This does not suggest that the intestinal microbiome is static from birth to death. While the mother primarily provides the initial gut microflora at birth, environmental and interindividual factors impact how the host–bacteria symbiotic interaction develops over time.[2] The influence of mode of birth delivery (vaginal versus caesarean) on microbiome composition and development remains controversial. Some research shows no correlation between microbiome diversity and mode of delivery,[3] while other trials indicate that specific groups such as preterm deliveries are at greater risk of opportunistic pathogens and stunted microbiota because of the expression of proinflammatory mediators.[4] Similarly, babies born via caesarean delivery were more likely to acquire pathogens commonly found in the hospital environment (*Enterococcus*, *Enterobacter,* and *Klebsiella* species), rendering them vulnerable to infections and dysbiosis in early life.[5]

The various aspects of symbiotic interplay have been established over millions of years as part of evolutionary and adaptive selection among mammals, and remain to be further explored.[6,7] In the past decades, the composition of the intestinal microbiome has been analysed to understand correlations between the levels of specific microbiota and the risk for development of chronic disorders such as diabetes,[8] obesity,[9] inflammatory bowel disease (IBD),[10] liver disorders,[11] cardiovascular disorders,[12] and systemic inflammatory disorders.[13] Although the composition of the intestinal microbiome is one contributing factor among many, its contribution may indicate a profound dysbiosis between the human host and detrimental bacterial strains. The complex nature of the microbial composition should be considered when evaluating the individual patient and the nature of their symptoms. For particular disorders, both qualitative and quantitative differentiation among microbial strains that may contribute to the underlying pathophysiology need to be considered.

The diversity of bacterial phylae differs among healthy children and adults with a predominant presence of Firmicutes (approximately 70% and 55%), Bacteroidetes (approximately 12% and 35%), Proteobacteria (approximately 5% and 6%), and Actinobacteria (approximately 5% and 4%) in adults and children, respectively.[14] For specific disease states, both the relative quantity of healthy bacterial phyla and invasion of pathogenic strains will contribute to dysbiosis, leading to local and systemic inflammation, disrupted enterocyte barrier function, and host immune responses.[15,16] While the phylum Firmicutes are primarily producing the short-chain fatty acid (SCFA) butyrate, and Bacteroidetes producing the SCFA propionate, it is Proteobacteria and Actinobacteria that appear to be primarily involved in health and disease states.[17] To date, three predominant healthy microbiome enterotypes have been proposed that can be differentiated by their quantitative composition of *Prevotella* (genus of Bacteroidetes), *Bacteroides* (genus of Bacteroidetes), and *Ruminococcus* (genus of Firmicutes).[18]

In recent years, the changes in microbiome composition and expression of specific proteins in special populations has been evaluated, indicating a role for the microbiome in the development of such disorders. One population are patients with colorectal cancer that present with abnormal levels of reactive oxygen species, facilitated through increased expression of bacterial superoxide dismutase, leading to oxidative stress in enterocytes and subsequent DNA damage.[19] Obesity is associated with the development of colorectal cancer and has also been linked to a dysbiosis of the gut microbiome.[20] A mediator for the development of obesity and insulin resistance appears to be the presence of toxic lipopolysaccharides secreted by mostly Gram-negative bacteria.[21] Lipopolysaccharides interfere with tight junctions, disrupt intestinal barrier integrity, and can passively diffuse through the enterocyte layer into the systemic circulation to trigger an inflammatory response.[22] Similarly, the overabundance in a dysbiotic intestinal microbiome of lipopolysaccharides leads to inflammation of pancreatic islet cells, contributing to the development of diabetes. In addition, a shift in the production of SCFA from propionate to acetate can contribute to reduced production of histone deacetylase inhibitors which are crucial in the regulation of inflammation and glucose sensitivity.

REGULATION OF INTESTINAL AND SYSTEMIC INFLAMMATION

Local intestinal and systemic inflammation are interconnected as blood biomarkers have been elucidated to help diagnose a range of intestinal inflammatory disorders, although additional symptom presentation and differential diagnosis is necessary.[23] The epithelial cells of the intestinal lining depend on metabolic products generated by intestinal bacteria, primarily resulting from both carbohydrate and lipid metabolism. Intestinal bacteria metabolise complex and simple carbohydrates and lipids into SCFA, primarily butyric and propionic acid.[24] Both are utilised by enterocytes and epithelial intestinal cells as energy sources in primary metabolism (Figure 1).

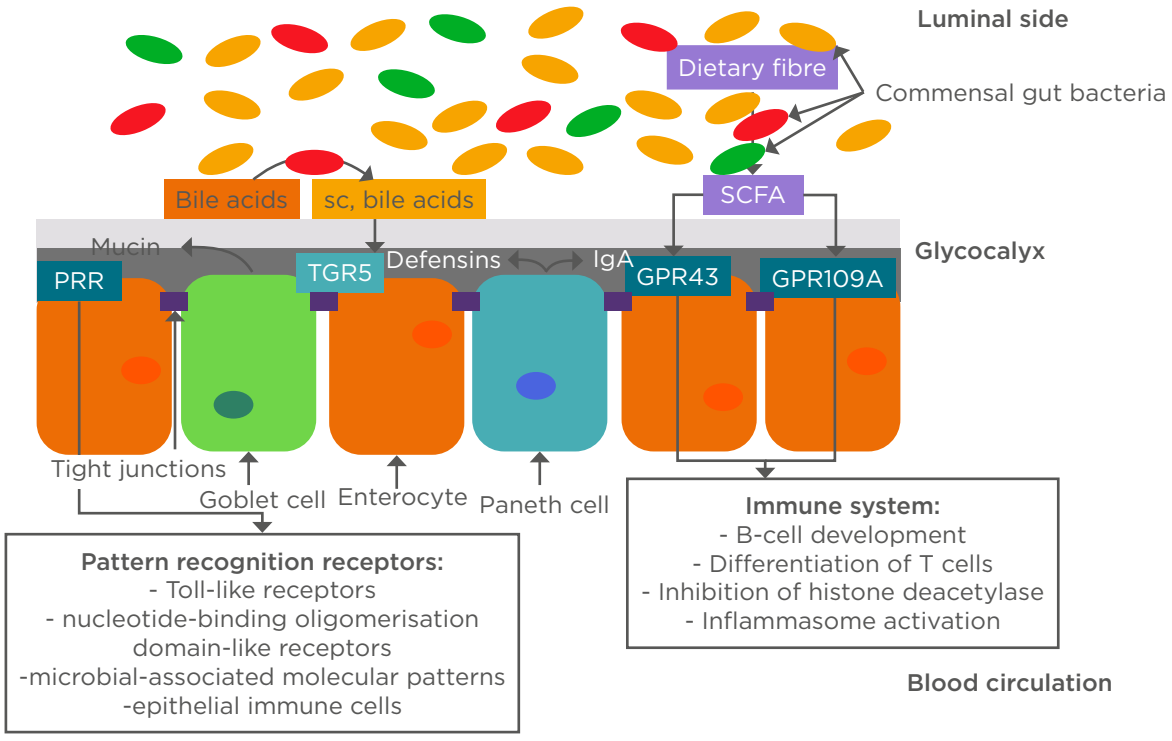

Figure 1: Physical and chemical intestinal barrier functions and their inter-relationship with commensal gut microbiome.

GPR43/109A: G protein-coupled receptor 43/109A; IgA: immunoglobulin A; PRR: pattern recognition receptor; sc: secretory component; SCFA: short-chain fatty acids; TGR5: transmembrane G protein-coupled receptor 5.

The diversity of intestinal bacteria is dominated by the phyla Firmicutes and Bacteroidetes that have been shown to interact with the intestinal barrier function and systemic immune system in a symbiotic manner.[2,6] The intestinal bacteria serve as the first defence against environmental disease factors such as viruses, bacteria, and other toxins. In that capacity, the intestinal commensal microbiome is challenged on a daily basis and maintains a highly competitive environment to protect against external bacterial strains.[25] Pattern recognition receptors are one component of the link between the intestinal microbiome and the host, and are associated with regulation of immune function.[26] Several receptors and immune cells are involved in distinguishing between symbiont and pathogen, and in regulating the microflora, including: toll-like receptors; nucleotide-binding oligomerisation domain-like receptors; microbial-associated molecular patterns, activated by microbial lipopolysaccharides, peptidoglycans, or formylated peptides; and epithelial immune cells (Figure 1).[26,27] Primary regulatory pathways utilised by the host to limit proinflammatory responses from the commensal bacteria include nuclear factor κB (NFκB), mitogen-activated protein kinase, and caspase-dependent signalling cascades.[26] These pathways lead to induction of apoptosis of pathogenic bacteria and limit the inflammatory response in the surrounding tissue.

A less specific approach to eliminating pathogenic bacteria is the involvement of both the innate and adaptive immune system of the host to release reactive oxygen species, that also leads to the localised loss of symbiotic bacteria.

A major aspect of recognition of commensal bacteria by the host is established through expression of intraepithelial lymphocytes early after colonisation, for differentiation between symbiont and pathogen. Because fucose serves as an energy source for many commensal bacteria, its expression on epithelial glycans favours early colonisation with Firmicutes and Bacteroidetes bacteria and thus helps to establish the early microbiome.[28] In order to restrict bacteria from penetrating through the epithelial layer of the intestinal lining, toll-like receptors located in the subepithelial layer and on the basolateral side of enterocytes serve to monitor and activate the immune system.[29] Microbial-associated molecular patterns are generated by commensal bacteria to stimulate the host's innate immune system, provide differentiation from pathogens, activate proinflammatory mediators in the intestinal lumen and the systemic circulation, and recruit T-helper cells to initiate a local inflammatory response.[30] Regulated by the adaptive immune system are a range of proinflammatory mediators that both function to maintain the microbiome equilibrium and play a role in the development of inflammatory disorders. The primary proinflammatory mediators involved are interleukins, neutrophils, and stress-mediators (noradrenaline and corticosterone).[31-33] This leads to a chronic suppression of commensal microbes in the gastrointestinal tract, which causes damage to mucosal barrier function that triggers an inflammatory and immune response.[34]

Intestinal barrier functions are maintained by epithelial cells that provide both physical and chemical barriers to protect against invasion of pathogens. At the same time, epithelial cells absorb nutrients and water from the lumen and interact with the microbiome to maintain homeostatic balance.[35] The physical barriers separate the gut bacteria from epithelial cells through secretion of a mucus layer, the glycocalyx covering absorptive epithelial cells, and tight junctions linking enterocytes to prevent passage of bacteria and pathogens into the bloodstream. The mucus produced by goblet cells in the large intestine consists of a thick inner and thinner outer layer of O-glycosylated Mucin-2 protein. This protein is utilised by the host in the inner layer to deter bacteria and by bacteria in the outer layer as an energy source after proteolysis to polysaccharides.[36] The inner mucus layer is hypothesised to be kept free of bacteria by secretion of IgA and defensin proteins that are part of the chemical barrier (Figure 1). Defensin peptides are antimicrobial peptides secreted by Paneth cells that require activation by matrix-metalloproteinases to protect against Gram-positive and Gram-negative bacteria.[37]

INTERPLAY BETWEEN MEDICATIONS AND THE INTESTINAL MICROBIOME AND HOST IMMUNE FUNCTION

The commensal microbiome plays a prominent role in both nutrient metabolism and defence against pathogens, and contributes to inter-individual variability in pharmacokinetic and

pharmacodynamic effects of drugs. The differences in expression of microbial phyla affect drug metabolism on an individual level. This has been shown for a range of drugs through metabolomics and genomics studies.[38] In an analysis of 271 orally administered drugs and 76 different human gut bacteria, the antiviral drug famciclovir was noted to have a significant correlation in altered metabolism in the presence of higher abundance of the bacterial phyla Bacteroidetes and the genera *Bacteroides*, *Parabacteroides*, and *Alistipes*.[38] In contrast, the corticosteroid norethisterone was not noted to have significant correlations on both the phylum and genus level. As a consequence, bioavailability of drugs and their respective active metabolites may be altered by individuals' microbiome, resulting in variable blood levels. Metabolism due to intestinal microbiota is currently given little consideration as a factor in drug bioavailability.

Other factors that can impact the symbiotic relationship between the intestinal microbiome and the host are xenobiotics that disrupt commensal bacterial growth (e.g., antibiotics), and physiological irregularities such as sleep–wake cycle abnormalities.[39,40] Antibiotic therapy intermittently disrupts the balance of commensal intestinal bacteria and increases the selection pressure towards potentially pathogenic bacteria. A study explored the expression of antibiotic-resistant genes in commensal bacteria under ciprofloxacin and cotrimoxazole therapy. It found distinct patterns between the two antibiotic therapies, causing either positive or negative selection pressure correlating to higher levels of specific bacterial strains. While ciprofloxacin therapy resulted in higher amounts of *Actinomyces meyeri*, *Acinetobacter johnsonii*, and *Staphylococcus saccharolyticus*, treatment with cotrimoxazole resulted in higher levels of *Proteus vulgaris*, *A. meyeri*, and *Acinetobacter lwoffi*. Ciprofloxacin also resulted in reduction of *Citrobacter europaeus*, *C. koseri*, and *C. werkmanii*, while the yeast *Candida tropicalis* was less abundant with cotrimoxazole treatment.[40] In a separate study with the antibiotic clindamycin using an *ex vivo* donor-simulated microbiome, a significant reduction in the phylum Bacteroidetes was observed primarily because clindamycin targets primarily anaerobic bacteria.[41] Because the Bacteroidetes are significantly contributing to the generation of SCFA such as acetate, propionate, and butyrate, clindamycin antibiotic therapy results in decreased generation and delayed recovery of SCFA synthesis. Given the importance of SCFA to maintain host immune function and intestinal barrier integrity, efforts to restore intestinal microbiome homeostasis as early as possible by administering probiotics or replacing specific mixtures of Bacteroidetes strains are under research.

Dysbiosis may also lead to pathogenic infections including *Clostridium difficile*, which have been linked with chronic disorders such as metabolic syndrome, IBD, diabetes, and obesity.[42] Chronic use of proton pump inhibitors for the treatment of gastric acid reflux disorders has also been shown to reduce the diversity and abundance of the intestinal microbiome.[43,44] In two separate clinical trials, the use of proton pump inhibitors led to a significant reduction in bacterial abundance and diversity that may increase the risk of subsequent pathogen infections such as *C. difficile*. However, the bacterial abundance and diversity was distinctly different from that of *C. difficile* patients, primarily displaying an increase in Bacteroidetes and Lactobacillales and a reduction in Cyanobacteria and Firmicutes.[44]

INFLAMMATORY BOWEL DISORDERS AND THE MICROBIOME

The underlying pathogenesis of IBD remains unknown but commonly includes morphological changes in brush border physiology with inflammatory infiltrates, and dysregulation of epithelial barrier function, that often leads to significant loss in quality of life for patients. A common change in intestinal barrier function is reduced expression of defensins and mucin that contributes to a weakened physical and chemical barrier against pathogenic bacteria. With decreased production of the protective mucus layer, even commensal flagellated bacteria such as *Escherichia* and *Proteus* species can lead to colitis with subsequent infiltration, due to loss of tight junction integrity.[45] The initiation phase leading to loss of barrier functions can be caused by environmental factors or genetic factors, relating to mucosal barrier-related genes including *FUT2*, *MUC19*, and *NOD2*, increasing the susceptibility for development of IBD.[35]

The localised inflammation is often associated with visceral pain, intestinal motility disturbances, and nutritional malabsorption.[46] Many patients present with low-grade elevations in systemic inflammatory markers such as C-reactive protein and white blood cell count.[47] These markers are non-specific and further differential diagnosis is necessary, which often makes IBD a diagnosis of exclusion. However, the composition of the microbiome appears to be predictive of a patient's intestinal inflammatory status. In intestinal germ-free animal models, the introduction of specific bacteria, such as *Actinobacteria*, *Campylobacter*, or *Enterobacteria*, increased the risk for the development of IBD if abundant enough to cause dysbiosis.[48,49] Similarly, both ulcerative colitis and Crohn's disease patients have been noted to have reduced levels of Firmicutes bacteria compared to healthy controls, and Crohn's disease patients were noted to have lower levels of the anti-inflammatory *Roseburia*, *Phascolarctobacterium*, and *Faecalibacterium prausnitzii* strains.[50,51] In addition to dysbiosis of the intestinal bacteria, higher levels of fungi such as *Aspergillus clavatus*, *Candida albicans*, and *Cryptococcus neoformans* have also been detected and associated with the development of Crohn's disease.[52]

By further differentiating the generated metabolites of the intestinal bacteria, recent publications indicate that a differential diagnosis is possible.[53] A majority of stool samples found an increase in tryptophan and other amino acids and a decrease in SCFA (especially propionic acid). Stool samples were more accurate in differentiating between Crohn's disease and ulcerative colitis than serum blood or urine samples. SCFA play a crucial role in maintaining intestinal immune barrier function through multiple pathways. They support B-cell development,[54] promoting the differentiation and expansion of regulatory T cells through effects on dendritic cells and macrophages,[55] maintaining mucosal integrity through inflammasome activation and IL-18 activation,[56] and exerting antiproliferative activity through inhibition of histone deacetylase.[57] The main receptors for SCFA on enterocytes are GPR43 and GPR109A, and are often dysfunctional or significantly reduced in patients with IBD compared to controls.[58] The lower expression of either of these receptors has been proposed as a potential target for treatment of acute inflammation in IBD, but agonists of GPR43 have not been successful to date. In contrast, supplementing SCFA in patients with IBD has shown benefits in reducing acute IBD symptoms.[59]

Bile acid metabolism is also intricately linked to the intestinal microbiome. Bile acids are deconjugated after aiding in lipid absorption by the enzyme bile acid hydrolase. Bile acids that are not deconjugated are metabolised by bacteria in the colon to secondary bile acids via desulfation that bind to the TGR5 (Figure 1).[60] Dysbiosis of intestinal microbiota can lead to a loss of bile acid hydrolase activity thus resulting in less absorption of deconjugated bile acids and more bile acids available in the colon for bacterial metabolism. Disturbed generation of secondary bile acids then influences the signalling cascade mediated through TGR5 leading to increased inflammation and overall lipid malabsorption.[61]

NOVEL TREATMENT APPROACHES FOR INFLAMMATORY BOWEL DISEASE

The current treatment approaches for IBD emphasise symptomatic relief of pain and the causative inflammation without addressing the underlying pathophysiology. As such, the mainstays of treatment are corticosteroids, anti-TNF-α therapy, and immunosuppressants that are given to suppress the inflammation.[62] However, standard therapy options and approaches do not provide adequate relief of symptoms and may often lead to progression in severity of the disease, requiring more aggressive treatment with potential for adverse effects and surgical intervention. Given the complex nature of IBD and the intricate connection between host and microbiome, different approaches have been developed that can be supplemented to alleviate IBD symptoms.

While genetic factors play a role in the development of IBD, they are not utilised in therapy but are primarily used in diagnosis and risk assessment. Environmental factors, however, can be addressed to reduce the risk of development of an IBD. These include diet, smoking, stress, and medications. Diets rich in sugar and long-chain fatty acids accelerate intestinal inflammation and are a known risk factor for the development of Crohn's disease.[46] Smoking increases the risk of developing Crohn's disease due to its effect on

humoral and cellular immune responses, but appears to lower the risk of ulcerative colitis due to the proposed promotion of colonic mucus production. The impact of environmental factors on the progression of IBD is still under research, including the potential of animal proteins for promotion of proinflammatory macrophages leading to colitis.

Microbiome-Focussed Therapies

To alter the intestinal microbiota, two therapeutic approaches are possible. The first approach aims to reduce pathogenic bacteria by using antibiotic therapy, while the second strengthens beneficial bacteria to re-establish symbiosis. Antibiotic therapy has been utilised since the 1970s and since then a range of antibiotic drug classes in combination with immunosuppressants have been used to treat IBD. The greatest rate in remission is observed with the use of rifaximin, a broad-spectrum antibiotic with local intestinal action and low oral bioavailability.[63] Metronidazole, either alone or in combination with amoxicillin and tetracycline, has also shown clinical improvement but is not as favourable a therapy due to the possible development of bacterial resistance. Local adverse effects and dysbiosis affecting the overall gut microbiome are the main issues with this approach.

The use of probiotic formulations over the past two decades has gained attention and in recent years specific microbial strains have been developed that lower remission rates in both Crohn's disease and ulcerative colitis, if taken for a ≥8-week period for ulcerative colitis, and between 10 weeks to 1 year for Crohn's disease.[63] The proprietary probiotic mixture VSL#3 and various *Bifidobacteria*, as well as the yeast *Saccharomyces boulardii*, was of benefit to patients with IBD in a majority of clinical studies. Studies not demonstrating a benefit for patients with IBD may be attributable to heterogeneity in doses or protocols. All of the current clinical trial protocols and investigational treatment approaches involving probiotic formulations or faecal transplants are not widely established in clinical practice, or have guidelines for their use, other than the use of *Escherichia coli* Nissle for maintenance of remission in ulcerative colitis in 5-aminosalicylic acid-intolerant patients.[64] An important note on the use of probiotics is the sufficient administration of colony-forming units (CFU) that can reach the intestinal lumen, so need to be formulated accordingly to withstand stomach acid. A minimum of 10^9 CFU (1,000,000,000 CFU) is recommended per day in order to achieve a therapeutic benefit, which may have to be increased in patients who are concomitantly on antibiotics.

Prebiotics are food items that serve as energy sources for intestinal bacteria and assist in the generation of SCFA, which are found to be low in IBD patients. Supplementation with prebiotics such as psyllium, wheat bran, and oligofructose-enriched inulin resulted in improved clinical outcomes and quality of life but was not always correlated with lower inflammation or remission rates.[63] Prebiotics in combination with probiotics (synbiotics) provided a better outcome in regards to remission rate.

The transplantation of faecal microbiota from a healthy donor to a patient with IBD has become a viable possibility with the approval of regulatory agencies to establish live biotherapeutic products and provide guidance on proper safety and handling.[63] Faecal microbiota transplantation has gained traction over the past 7 years for patients with severe forms of IBD that do not achieve sufficient symptom relief by any other means. Prior treatment with immunosuppressive and antibiotic drugs is important for successful faecal microbiota transplantation, to avoid native microbiota causing a local inflammatory response. Rectal engraftment in multiple small transfers is generally better tolerated than nasogastric or single-transfer administration. Promising results in maintenance of remission in ulcerative colitis have been found with the use of capsulated freeze-dried donor faecal transplant microbiota that can be taken orally for extended periods of time. In a small open-label study of 30 patients with ulcerative colitis in remission, administration of encapsulated faecal microbiota transplant for 6 weeks did provide adequate therapy without escalation of existing concomitant pharmacotherapy.[65] This microbiome-focussed therapy field is still developing, with more controlled clinical trials needed.

CONCLUSION

IBD are complex chronic disorders that remain challenging to diagnose and treat. By gaining a better understanding of the intricate interaction between the host and the intestinal microbiome and its dysregulation in IBD, differential diagnoses and treatment approaches can be better applied in clinical practice. New diagnostic criteria, such as SCFA variations in stool and expressed levels of specific intestinal bacteria, can assist practitioners in confirming a diagnosis and optimising therapy. Such therapies can be tailored to address dysregulation by reducing pathogenic bacteria or increasing beneficial bacteria. Advancing treatment approaches and availability is essential to benefit healthcare providers and patients alike.

References

1. Bienenstock J et al. Microbiota and the gut-brain axis. Nutr Rev. 2015;73(Suppl 1):28-31.
2. Dethlefsen L et al. Assembly of the human intestinal microbiota. Trends Ecol Evol. 2006;21(9):517-23.
3. Liu CJ et al. Is the delivery mode a critical factor for the microbial communities in the meconium? EBioMedicine. 2019;49:354-63.
4. Fettweis JM et al. The vaginal microbiome and preterm birth. Nat Med. 2019;25(6):1012-21.
5. Shao Y et al. Stunted microbiota and opportunistic pathogen colonization in caesarean-section birth. Nature. 2019;574(7776):117-21.
6. Eckburg PB et al. Diversity of the human intestinal microbial flora. Science. 2005;308(5728):1635-8.
7. Backhed F et al. Host-bacterial mutualism in the human intestine. Science. 2005;307(5717):1915-20.
8. Vallianou NG et al. Microbiome and diabetes: where are we now? Diabetes Res Clin Pract. 2018;146:111-8.
9. Maruvada P et al. The human microbiome and obesity: moving beyond associations. Cell Host Microbe. 2017;22(5):589-99.
10. Nishida A et al. Gut Microbiota in the Pathogenesis of Inflammatory Bowel Disease. J Clin Gastroenterol. 2018;11(1):1-10.
11. Tilg H et al. Gut microbiome and liver diseases. Gut. 2016;65(12).
12. Peng J et al. Interaction between gut microbiome and cardiovascular disease. Life Sciences. 2018;214:153-7.
13. Clemente JC et al. The role of the gut microbiome in systemic inflammatory disease. BMJ. 2018;360:j5145.
14. Radjabzadeh D et al. Diversity, compositional and functional differences between gut microbiota of children and adults. Scientific Reports. 2020;10(1).
15. Noto D, Miyake S. Gut dysbiosis and multiple sclerosis. Clin Immunol. 2020;108380.
16. Jiao Y et al. Crosstalk between gut microbiota and innate immunity and its implication in autoimmune diseases. Front Immunol. 2020;11: doi. org/10.3389/fimmu.2020.00282.
17. Graf D et al. Contribution of diet to the composition of the human gut microbiota. Microb Ecol Health Dis. 2015;26:26164.
18. Arumugam M et al. Enterotypes of the human gut microbiome. Nature. 2011;473(7346):174-80.
19. Long S et al. Metaproteomics characterizes human gut microbiome function in colorectal cancer. NPJ Biofilms Microbiomes. 2020;6(14).
20. Zhi C et al. Connection between gut microbiome and the development of obesity. Eur J Clin Microbiol Infect Dis. 2019;38(11):1987-98.
21. Salguero MV et al. Dysbiosis of Gram-negative gut microbiota and the associated serum lipopolysaccharide exacerbates inflammation in Type 2 diabetic patients with chronic kidney disease. Exp Ther Med. 2019;18(5):3461-9.
22. Fasano A. Zonulin and its regulation of intestinal barrier function: the biological door to inflammation, autoimmunity, and cancer. Physiologic Reviews. 2011;91(1).
23. Gecse KB. Differential diagnosis of inflammatory bowel disease: imitations and complications. Lancet. 2018;3(9):644-53.
24. de Graaf AA et al. Profiling human gut bacterial metabolism and its kinetics using [U-13C]glucose and NMR. NMR Biomed. 2010;23(1):2-12.
25. Shi N. Interaction between the gut microbiome and mucosal immune system. military medical research. 2017:4(14).
26. Sharma R et al. Molecular modulation of intestinal epithelial barrier: contribution of microbiota. J Biomed Biotechnol. 2010;2010:305879.
27. Neish AS. Microbes in gastrointestinal health and disease. Gastroenterology. 2009;136(1):65-80.
28. Umesaki Y et al. Segmented filamentous bacteria are indigenous intestinal bacteria that activate intraepithelial lymphocytes and induce MHC class II molecules and fucosyl asialo GM1 glycolipids on the small intestinal epithelial cells in the ex-germ-free mouse. Microbiol Immunol. 1995;39(8):555-62.
29. Lee J et al. Maintenance of colonic homeostasis by distinctive apical TLR9 signalling in intestinal epithelial cells. Nat Cell Biol. 2006;8(12):1327-36.
30. Umesaki Y et al. Expansion of alpha beta T-cell receptor-bearing intestinal intraepithelial lymphocytes after microbial colonization in germ-free mice and its independence from thymus. Immunology. 1993;79(1):32-7.
31. Mai V et al. Colonic bacterial flora: changing understandings in the molecular age. J Nutr. 2004;134(2):459-64.
32. Forchielli ML, Walker WA. The role of gut-associated lymphoid tissues and mucosal defence. Br J Nutr. 2005;93(Suppl 1):S41-8.
33. Maslowski KM et al. Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43. Nature. 2009;461(7268):1282-6.
34. Rhee SH et al. Principles and clinical implications of the brain-gut-enteric microbiota axis. Nat Rev Gastroenterol Hepatol. 2009;6(5):306-14.
35. Okumura R, Takeda K. Maintenance of intestinal homeostasis by mucosal barriers. BMC Inflamm Regen. 2018;38(5).
36. Johansson ME et al. The inner of the two Muc2 mucin-dependent mucus layers in colon is devoid of bacteria. Proc Natl Acad Sci USA. 2008;105(39):15064-9.
37. Salzman NH et al. Enteric defensins are essential regulators of intestinal microbial ecology. Nat Immunol. 2010;11(1):76-83.
38. Zimmermann M et al. Mapping human microbiome drug metabolism by gut bacteria and their genes. Nature.

2019;570(7762):462-7.

39. Murakami M, Tognini P. The circadian clock as an essential molecular link between host physiology and microorganisms. Front Cell Infect Microbiol. 2020;9: doi.org/10.3389/fcimb.2019.00469.
40. Willmann M et al. Distinct impact of antibiotics on the gut microbiome and resistome: a longitudinal multicenter cohort study. BMC Biol. 2019;17(76).
41. El Hage R et al. Propionate-producing consortium restores antibiotic-induced dysbiosis in a dynamic *in vitro* model of the human intestinal microbial ecosystem. Front Microbiol. 2019;10: doi.org/10.3389/fmicb.2019.01206.
42. Arredondo-Hernandez R et al. *Clostridium difficile* infection: an immunological conundrum. Arch Med Res. 2018;49(6):359-64.
43. Seto CT et al. Prolonged use of a proton pump inhibitor reduces microbial diversity: implications for clostridium difficile susceptibility. Microbiome. 2014;2:42.
44. Jackson MA et al. Proton pump inhibitors alter the composition of the gut microbiota. Gut. 2016;65(5).
45. Elinav E et al. NLRP6 inflammasome regulates colonic microbial ecology and risk for colitis. Cell. 2011;145(5):745-57.
46. Guan Q. A comprehensive review and update on the pathogenesis of inflammatory bowel disease. J Immunol Res. 2019;2019:7247238.
47. Chang S et al. Disease monitoring in inflammatory bowel disease. World J Gastroenterol. 2015;21(40):11246-59.
48. Younis N et al. Inflammatory bowel disease: between genetics and microbiota. Mol Biol Rep. 2020: doi.org/10.1007/s11033-020-05318-5.
49. Walker AW et al. High-throughput clone library analysis of the mucosa-associated microbiota reveals dysbiosis and differences between inflamed and non-inflamed regions of the intestine in inflammatory bowel disease. BMC Microbiol. 2011;11(7).
50. Knights D et al. Advances in inflammatory bowel disease pathogenesis: linking host genetics and the microbiome. Gut. 2013;62(10).
51. Sokol H et al. *Faecalibacterium prausnitzii* is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of crohn disease patients. Proc Natl Acad Sci USA. 2008;105(43):16731-6.
52. Li Q et al. Dysbiosis of gut fungal microbiota is associated with mucosal inflammation in crohn's disease. J Clin Gastroenterol. 2014;48(6):513-23.
53. Lavelle A, Sokol H. Gut microbiota-derived metabolites as key actors in inflammatory bowel disease. Nat Rev Gastroenterol Hepatol. 2020: doi: 10.1038/s41575-019-0258-z. [Epub ahead of print].
54. Kim M et al. Gut microbial metabolites fuel host antibody responses. Cell Host Microbe. 2016;20(2):202-14.
55. Singh N et al. Activation of GPR109A, receptor for niacin and the commensal metabolite butyrate, suppresses colonic inflammation and carcinogenesis. Immunity. 2014;40(1):128-39.
56. Macia L et al. Metabolite-sensing receptors GPR43 and GPR109A facilitate dietary fibre-induced gut homeostasis through regulation of the inflammasome. Nature Communications. 2015;6.
57. Davie JR. Inhibition of histone deacetylase activity by butyrate. J Nutrit. 2003;133(7 Suppl):2485S-93S.
58. Ferrer-Picon E et al. Intestinal inflammation modulates the epithelial response to butyrate in patients with inflammatory bowel disease. Inflamm Bowel Dis. 2020;26(1):43-55.
59. D'Souza WN et al. Differing roles for short chain fatty acids and GPR43 agonism in the regulation of intestinal barrier function and immune responses. PloS one. 2017;12(7).
60. Devkota S et al. Dietary-fat-induced taurocholic acid promotes pathobiont expansion and colitis in Il10-/- mice. Nature. 2012;487(7405).
61. Duboc H et al. Connecting dysbiosis, bile-acid dysmetabolism and gut inflammation in inflammatory bowel diseases. Gut. 2013;62(4):531-9.
62. Borg-Bartolo SP et al. Precision medicine in inflammatory bowel disease: concept, progress and challenges. F1000Research. 2020;9.
63. Oka R, Sartor RB. Microbial-based and microbial-targeted therapies for inflammatory bowel diseases. Dig Dis Sci. 2020;65(3).
64. Scaldaferri F et al. Role and mechanisms of action of *Escherichia coli* Nissle 1917 in the maintenance of remission in ulcerative colitis patients: an update. World J Gastroenterol. 2016;22(24):5505-11.
65. Adler E et al. Capsule-delivered faecal microbiota transplant is safe and well tolerated in patients with ulcerative colitis. Dig Dis Sci. 2019;64(9):2452-4.

Indoor Microbiome and The Rising Asthma Prevalence

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Abstract

The prevalence of asthma has increased in the past few decades in most developed and developing countries. Large-scale, cross-sectional epidemiological studies have reported several factors associated with asthma prevalence and severity, including parental asthma, tobacco smoking, preterm delivery, virus infection, and air pollution. However, a puzzling problem is that the time trends in the prevalence of these risk factors cannot explain the rise in asthma. For example, the prevalence of smoking and clinical pneumonia have been decreasing globally in the past few decades. Recent progress in high-throughput sequencing technology has promoted the progress of microbiome research and established associations between human and indoor microbiomes, and many metabolic, cognitive, and immune diseases including asthma and allergies. In this review, the authors systematically summarise the current literature, standard practice, and analysis pipeline in the field of indoor microbiome and asthma. The strength and limitation of different analytical approaches are discussed, including the utilisation of relative and absolute abundance in the associated studies. The authors discuss new frameworks of integrated microbiome research from different ecological niches, functional profiling from multiomics data, and how these new insights can facilitate understanding of asthma mechanisms and even the development of new personalised treatment strategies for the rising asthma epidemic.

INTRODUCTION

In the past 50 years the prevalence of asthma, including doctor-diagnosed asthma and asthma symptoms, has been rising in most developing and developed countries and is now one of the most common chronic disorders, affecting >300 million patients worldwide.[1,2] The prevalence of clinician-diagnosed asthma is generally >10% in several developed countries, including the USA, Canada, the UK, and Australia.[2] In Aberdeen, UK, the proportion of children with asthma increased from 28% in 1964 and 49% in 1989 to 64% in 1999.[3] The rise can be partly explained by the increased awareness and reporting rate of the disease among the public population, but

many studies also confirm a surge in asthma symptoms in adults and children.[4] The rising asthma prevalence has led to huge economic and social costs. In the USA, the annual asthma economic cost has risen from $12 billion in 1994 to $56 billion in 2011, which includes not only direct costs such as hospitalisation and medication, but also indirect costs such as the inability to work or attend school.[5] There is a worldwide concern of the prevalence increasing further in the future, and there is a need to identify the risks and protective factors for the global asthma epidemic and propose potential prevention and even personalised treatment strategies for asthma.

Many factors have been reported to be associated with asthma prevalence and severity. Parental asthma, tobacco smoking, and preterm delivery are suggested to be strong risk factors for paediatric asthma, whereas obesity, birth by caesarean section, respiratory syncytial virus infection, exposure to mould or fungi, and air pollution are also mildly-to-moderately associated with the symptoms.[6] The prevalence and mechanism is still largely unclear for adult-onset asthma, but risk factors identified so far are generally similar to those of paediatric asthma. For example, a recent large-scale, cross-sectional study evaluating asthma prevalence and related risk factors from a large representative general adult population from China reported that cigarette smoking, allergic rhinitis, paediatric pneumonia or bronchitis, and parental respiratory disease were risk factors for adult asthma.[7] A perplexing problem is that although the association between these risk factors and the incidence of asthma are well-supported by many epidemiology studies, the time trends in exposure to these risk factors cannot explain the rise in asthma prevalence. For example, parental or family history of asthma indicates genetic predisposition, and has been identified as one of the most important risk factors for asthma development.[6] Genome-wide association studies (GWAS) have identified the chromosomal *17q21* region containing *ORMDL3* as being strongly associated with both paediatric and adult asthma.[8,9] However, because the proportion of associated single nucleotide polymorphisms in the human population does not drastically change over a few decades, it is generally accepted that environmental rather than genetic factors are causing the rising prevalence of asthma. Smoking is a clear risk factor for asthma, but the prevalence of smoking decreased globally from 1980 to 2012: from 41.2% to 31.1% in men, and 10.6% to 6.2% in women.[10] Similarly, the incidence of clinical pneumonia in young children also decreased by 22.0% globally over the past two decades.[11] Most evidence regarding air pollution and allergens from other studies has also failed to explain the elevated asthma prevalence.[1,12-14] Therefore, there must be other underlying factors related to modern lifestyle contributing to the rising asthma epidemic in the past decades.

INDOOR MICROBIOME AND ASTHMA

New developments and lower costs in new sequencing technology, as well as their application in culture-free microbial research, have promoted the progress of microbiome research in the past 10 years. Large-scale international collaboration projects, such as the Human Microbiome Project and Earth Microbiome Project, have brought new dimensions to understanding the causes of some prevailing diseases. Numerous other small-scale microbiome studies of specific environmental niches, including those inside or on the surface of humans, animals, and plants, as well as indoor and outdoor environments, have also supplemented our knowledge.[15-18] New research has identified associations between the human or indoor microbiome and many metabolic, immune, and cognitive diseases, which, similar to asthma, have drastically increased since World War II, including obesity, diabetes, allergies, inflammatory bowel disease, and autism.[19] These findings shape new concepts for further research. Traditional concepts of public health mainly focus on identifying and understanding the spread of microbes causing infectious diseases and exploring approaches to prevent and treat these diseases. In contrast, the recent microbiome revolution highlights that many microbes inside the human body and indoor environment, previously thought to be of little importance, may be significant in maintaining human health and preventing disease. Instead of conferring risk from the microbes themselves, the loss of microbial diversity and protective microbes in gut and indoor environments, as a

result of changes in lifestyle, diet, and indoor environment linked to urbanisation, may be contributing to the increased prevalence of allergic, immune, and inflammatory diseases.[19]

The association between microbial composition or diversity in indoor environment and asthma symptoms has been explored in several studies. Population studies in different countries suggest that children who grow up on farms are protected against asthma and atopy.[20-22] The protection may be due to the wider range of bacterial or endotoxin exposure in the farm environment.[20,23,24] These findings are conceptually consistent with the hygiene hypothesis proposed by Strachan in 1989.[25] The protective effect is suggested to be associated with immunity training and maturation via T-helper cells.[20,23,24] Similarly, a higher fungal richness, represented by the number of fungal operational taxonomical units (OTU), was also protective against asthma development in a Latino birth cohort study in California, USA.[26] However, there are also studies suggesting that higher general microbial diversity does not protect against asthma development. Instead, the presence of specific microbes or the farm-like microbial composition have been suggested to be the key factors in reducing indoor-related asthma development.[27,28] Two cohort studies in Finland and Germany indicated the farm-like microbial composition, including a higher proportion of *Sphingobacteriia*, *Alphaproteobacteria*, and *Cyanobacteria*, is protective against asthma symptoms.[28] A birth cohort study from the USA found that a relative abundance of 373 bacterial taxa, but not the overall bacterial richness, was associated with childhood asthma.[27] Furthermore, there are several studies suggesting that high general microbial diversity could be a risk factor for impaired respiratory health. A birth cohort study in Connecticut, USA, and Massachusetts, USA, indicated high bacterial diversity was positively associated with asthma severity.[29] One study of moisture-damaged buildings also reported that fungal richness, especially for fungal classes *Dothideomycetes* and *Agaricomycetes*, was higher in the water-damaged buildings compared to undamaged buildings, suggesting that the high fungal richness could be a potential risk factor for occupants.[30] It remains an open question whether high microbial richness is a protective factor for development of asthma, or specific taxa or organism compositions may play an important role in asthma and respiratory health.

STANDARD PRACTICE AND TECHNICAL LIMITATION IN INDOOR MICROBIOME STUDY

Although techniques such as single-strand conformation polymorphism or denaturing gradient gel electrophoresis[20,31] are still used in microbiome studies of the indoor environment, the majority of current studies use next-generation sequencing techniques to characterise microbiome composition. These new techniques characterise composition by sequencing amplicon genes, including 16S ribosomal RNA for bacteria, and 18S ribosomal RNA gene or internal transcribed spacer region for fungi.[18] Several studies have begun to utilise shotgun metagenomics or even metatranscriptomics to characterise bacteria, fungi, viruses, and eukaryotes together.[32,33] The sequenced amplicon reads are assembled and compared to well-characterised and curated reference datasets such as Greengenes, Silva, or Unite for taxonomic classification,[34] with microbial richness then calculated based on the number of OTU identified in each sample. However, if the biomass is highly variable, or if the samples are enriched by one or more highly-abundant taxa, the standard analysing practice may skew the results describing community richness and composition.[35] If the sequencing depth is not high enough, the high-abundance taxa may swamp the rare taxa and lead to an underestimation of the overall microbial richness in the study sample. These technical limitations may be contributing to the inconsistent associations between microbial diversity and asthma symptoms previously discussed.

To identify potentially-protective or harmful microbes in the indoor environment, researchers have developed different analytical approaches. A standard approach involves screening associations between the relative or absolute abundance of microbes and asthma symptoms. This approach has been used in the majority of published microbiome studies, and has

identified protective and harmful microbes for asthma in the indoor environment.[27,29,31,36] The relative abundance is obtained directly from the high-throughput sequencing analysis, revealing the percentage of reads matched for each OTU in the total amount of reads produced. The absolute concentration takes total microbial biomass into consideration; the value is calculated by multiplying the relative abundance for each OTU with the total bacterial or fungal concentration derived from universal primer quantitative PCR.[36] It is generally accepted that the absolute abundance approach is superior to the relative abundance,[36] although the relative abundance approach is still used in the majority of microbial association studies. An increase in any one taxon will lead to a decrease in relative abundance of all other taxa, leading to a covariant effect. Thus, the relative approach may fail to link correct microbes to phenotypes and quantitative features, especially for samples with substantial microbial load variation.[37] One common problem of the relative approach is an overestimation of associated microbes. A methodology paper showed that the number of associated taxa with PM10 mass identified by the relative abundance approach was seven times higher than that identified by absolute abundance.[36] Another problem is misidentification. One study of the human gut showed that five out of eight taxa identified by the relative abundance approach were false-positive results.[37] The inconsistencies between the two approaches are generally more severe for samples with drastic biomass variation, but for samples with similar total microbial biomass the relative abundance approach can be comparable with the absolute abundance approach.

A recently-proposed novel analysis framework suggests applying an anchor-based approach to identify beneficial and harmful microbes.[28] Because the indoor microbiome in the farm environment confers a protective effect for asthma, it can be used as a reference to train a model to predict the protective effect of indoor microbiome in the non-farm environment. This approach has been verified in the Finnish (LUKAS) and German (GABRIELA) birth cohorts.[28] However, the farm microbiome composition can vary across geographic locations,[28,38,39] thus the model needs to use the local farm microbiome to predict the protective effect of the urban microbiome. As an example, a LUKAS farm microbiome was used to predict the protective effect of LUKAS urban samples, and a GABRIELA farm microbiome was used to predict GABRIELA urban samples. Thus, future studies should aim to identify the pan-microbiome for the farm environment or a global standardised reference of protective microbiome composition to facilitate a global utilisation of this approach.

INTEGRATED MICROBIOME RESEARCH

This review is mainly focussed on the indoor microbiome and asthma. However, the microbiome compositions in other ecological niches, such as the human intestine or respiratory tract, may also play important roles in respiratory health. Previous reviews systematically summarised beneficial and harmful microbes in the respiratory tract from several cohort and case-control studies, and demonstrated that healthy microbiota can act as gatekeepers to resist pathogens and protect against development of asthma and other respiratory diseases.[40,41] Similarly, the composition of the human gut microbiome can not only influence gastrointestinal disease, but also have long-lasting impacts on autoimmune, allergic, and metabolic diseases.[42,43] The association between gut microbiome and asthma has been demonstrated by several birth cohort studies[44,45] and mouse experiments.[41] Feeding *Clostridium* strains to pathogen-free mice induces regulatory T cells and reduces systemic IgE production,[46] whilst a diet with high fibre content changes the ratio of Firmicutes to Bacteroidetes, increasing Th2 cell effector function, which can protect against allergic inflammation in the lung.[47] Besides direct immunomodulation, gut microbiota can affect asthma in indirect ways. Obesity is a risk factor for asthma, and previous mouse experiments and large-scale microbial genotype–phenotype association studies have linked the composition of human gut microbiota to obesity.[48-50] Thus, asthma occurrence could be reduced by maintaining a healthy gut microbiota. Furthermore, preterm birth and caesarean section are also risk factors for asthma occurrence. A previous study

reported that genital tract microbial infection can explain up to 40-50% cases of preterm births. A recent case-control study revealed that women delivered preterm exhibited lower levels of *Lactobacillus crispatus* and higher levels of *Sneathia amnii* and a group of *Prevotella* species, and these taxa were correlated with proinflammatory cytokines in vaginal fluid.[52] Previous meta-analysis showed that children born by caesarean section have a 20% increase in the subsequent risk of asthma.[53] Reduced vaginal microbial exposure by caesarean section delivery can decrease diversity of gut microbiota, delay Bacteroidetes colonisation, and reduce Th1 responses in the first 2 years of life, contributing to asthma development for the newborns.[54] Thus, future microbiome studies can integrate marker gene and metagenomics data from different ecological niches, such as the indoor environment and human respiratory tract, gut, and vagina, to facilitate an overall assessment of microbiome exposure and how microbes enter, stimulate, and modulate human immune system activity during asthma development.

References

1. Eder W et al. The asthma epidemic. N Engl J Med. 2006;355(21):2226-35.
2. Anandan C et al. Is the prevalence of asthma declining? Systematic review of epidemiological studies. Allergy. 2010;65(2):152-67.
3. Devenny A et al. Respiratory symptoms and atopy in children in Aberdeen: questionnaire studies of a defined school population repeated over 35 years. BMJ. 2004;329:489.
4. de Nijs S et al. Adult-onset asthma: is it really different? Eur Respir Rev. 2013;22(127):44-52.
5. Nunes C et al. Asthma costs and social impact. Asthma Res Pract. 2017;3:1.
6. Castro-Rodriguez JA et al. Risk and protective factors for childhood asthma: what is the evidence? J Allergy Clin Immunol Pract. 2016;4(6):1111-22.
7. Huang K et al. Prevalence, risk factors, and management of asthma in China: a national cross-sectional study. Lancet. 2019;394(10196):407-18.
8. Moffatt MF et al. A large-scale, consortium-based genomewide association study of asthma. N Engl J Med. 2010;363(13):1211-21.
9. Bouzigon E et al. Effect of 17q21 variants and smoking exposure in early-onset asthma. N Engl J Med. 2008;359:1985-94.
10. Ng M et al. Smoking prevalence and cigarette consumption in 187 countries, 1980-2012. JAMA. 2014;311(2):183-92.
11. McAllister DA et al. Global, regional, and national estimates of pneumonia morbidity and mortality in children younger than 5 years between 2000 and 2015: a systematic analysis. Lancet Glob Health. 2019;7(1):47-57.
12. Mutius E et al. Prevalence of asthma and atopy in two areas of West and East Germany. Am J Respir Crit Care Med. 1994;149(2):358-64.
13. Lau S et al. Early exposure to house-dust mite and cat allergens and development of childhood asthma: a cohort study. Lancet. 2000;356(9239):1392-7.
14. Cullinan P et al. Early allergen exposure, skin prick responses, and atopic wheeze at age 5 in English children: a cohort study. Thorax. 2004;59(10):855-61.
15. Turnbaugh PJ et al. The human microbiome project. Nature. 2007;449:804-10.
16. Thompson LR et al. A communal catalogue reveals Earth's multiscale microbial diversity. Nature. 2017;551(7681):457-63.
17. Oh J et al. Biogeography and individuality shape function in the human skin metagenome. Nature. 2014;514:59-64.
18. Adams RI et al. Ten questions concerning the microbiomes of buildings. Build Environ. 2016;109:224-34.
19. Bello M et al. Preserving microbial diversity. Science. 2018;362(6410):33-4.
20. Ege MJ et al. Exposure to environmental microorganisms and childhood asthma. N Engl J Med. 2011;364(8):701-9.
21. Ege MJ et al. Prenatal exposure to a farm environment modifies atopic sensitization at birth. J Allergy Clin Immunol. 2008;122(2):407-12.
22. von Mutius E, Vercelli D. Farm living: effects on childhood asthma and allergy. Nat Rev Immunol. 2010;10:861-8.
23. Eder W, Mutius E. Hygiene hypothesis and endotoxin: what is the evidence? Curr Opin Allergy Clin Immunol. 2004;4(2):113-7.
24. van Strien RT et al. Microbial exposure of rural school children, as assessed by levels of N-acetyl-muramic acid in mattress dust, and its association with respiratory health. J Allergy Clin Immunol. 2004;113(5):860-7.
25. Strachan DP. Hay fever, hygiene, and household size. BMJ. 1989;299(6710):1259-60.
26. Dannemiller KC et al. Next-generation DNA sequencing reveals that low fungal diversity in house dust is associated with childhood asthma development. Indoor air. 2014;24(3):236-47.
27. O'Connor GT et al. Early-life home environment and risk of asthma among inner-city children. J Allergy Clin Immunol. 2018;141(4):1468-75.
28. Kirjavainen PV et al. Farm-like indoor microbiota in non-farm homes protects children from asthma development. Nat Med. 2019;25(7):1089-95.
29. Dannemiller KC et al. Indoor microbial communities: influence on asthma severity in atopic and nonatopic children. J Allergy Clin Immunol. 2016;138(1):76-83.
30. Pitkäranta M et al. Molecular profiling of fungal communities in moisture damaged buildings before and after remediation-a comparison of culture-dependent and culture-independent methods. BMC microbiol. 2011;11:235.
31. Pekkanen J et al. Indoor bacteria and asthma in adults: a multicentre case–control study within ECRHS II. Eur Respir J. 2018;51(2):pii:1701241.
32. Hsu T et al. Urban transit system microbial communities differ by surface type and interaction with humans and the environment. Am Soc Microbiol. 2016;1(3):e00018-16.
33. Hegarty B et al. Gene expression of indoor fungal communities under damp building conditions: implications for human health. Indoor air. 2018;28(4):548-58.
34. Knight R et al. Best practices for analysing microbiomes. Nat Rev Microbiol. 2018;16(7): 410-22.

35. Adams RI et al. A unique signal distorts the perception of species richness and composition in high-throughput sequencing surveys of microbial communities: a case study of fungi in indoor dust. Microbial Ecology. 2013;66(4):735-41.
36. Dannemiller KC et al. Combining real-time PCR and next-generation DNA sequencing to provide quantitative comparisons of fungal aerosol populations. Atmos Environ. 2014;84:113-21.
37. Vandeputte D et al. Quantitative microbiome profiling links gut community variation to microbial load. Nature. 2017;551:507-11.
38. Amend AS et al. Indoor fungal composition is geographically patterned and more diverse in temperate zones than in the tropics. Proc Natl Acad Sci USA. 2010;107(31):13748-53.
39. Barberán A et al. Continental-scale distributions of dust-associated bacteria and fungi. PNAS. 2015;112(18):5756-61.
40. Man WH et al. The microbiota of the respiratory tract: gatekeeper to respiratory health. Nat Rev Microbiol. 2017;15(5):259-70.
41. Huang YJ, Boushey HA. The microbiome in asthma. J Allergy Clin Immunol. 2015;135(1):25-30.
42. Rautava S et al. Microbial contact during pregnancy, intestinal colonization and human disease. Nat Rev Gastroenterol Hepatol. 2012;9(10):565-76.
43. Olszak T et al. Microbial exposure during early life has persistent effects on natural killer T cell function. Science. 2012;336(6080):489-93.
44. Stokholm J et al. Maturation of the gut microbiome and risk of asthma in childhood. Nat Commun. 2018;9:141.
45. Frati F et al. The role of the microbiome in asthma: the gut–lung axis. Int J Mol Sci. 2018;20(1):123.
46. Atarashi K et al. Induction of colonic regulatory T cells by indigenous Clostridium species. Science. 2011;331(6015):337-41.
47. Trompette A et al. Gut microbiota metabolism of dietary fiber influences allergic airway disease and hematopoiesis. Nat Med. 2014;20(2):159-66.
48. Turnbaugh PJ et al. A core gut microbiome in obese and lean twins. Nature. 2009;457: 480-4.
49. Turnbaugh PJ et al. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature. 2006;444(7122):1027-31.
50. Rothschild D et al. Environment dominates over host genetics in shaping human gut microbiota. Nature. 2018;555:210-5.
51. Lamont RF. Infection in the prediction and antibiotics in the prevention of spontaneous preterm labour and preterm birth. BJOG. 2003;110(s20):71-5.
52. Fettweis JM et al. The vaginal microbiome and preterm birth. Nat Med. 2019;25:1012-21.
53. Thavagnanam S et al. A meta-analysis of the association between caesarean section and childhood asthma. Clin Exp Allergy. 2008;38(4):629-33.
54. Jakobsson HE et al. Decreased gut microbiota diversity, delayed Bacteroidetes colonisation and reduced Th1 responses in infants delivered by caesarean section. Gut. 2014;63(4):559-66.
55. Huan T et al. Systems biology guided by XCMS Online metabolomics. Nat Methods. 2017;14:461-2.

Urinary Tract Infection in Children: A Review of the Established Practice Guidelines

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Abstract

Urinary tract infection (UTI) is a significant cause of morbidity in children. Delayed treatment is associated with complications that may result in chronic kidney disease and, subsequently, end-stage kidney disease. Over the years, clinical practice guidelines have advanced to ensure the best global practices in treating the infection and preventing its progression to chronic kidney disease. The established practice guidelines address five main questions: 1) which children should have their urine tested; 2) how the sample should be obtained; 3) which radiological tests are recommended after a diagnosis of UTI; 4) how the infection should be treated; 5) and how affected children should be followed up. There is a substantial overlap in the recommendations of the American Academy of Pediatrics (AAP) guidelines and the UK's National Institute for Health and Clinical Excellence (NICE) guidelines. Subtle differences, however, exist between the two established guidelines. An evidence-based paradigm shift of some traditional concepts about UTI in children has contributed to the revision and update of these guidelines. Further research is needed to clarify the role of host and genetic factors in renal scarring, as well as the diagnostic criteria for UTI. This narrative review aims to discuss the current recommendations of these established practice guidelines with an emphasis on the diagnosis, radiological investigation, treatment, and follow-up of UTI in children.

INTRODUCTION

Urinary tract infection (UTI) is a significant cause of morbidity in children.[1,2] Prevalence rates of a first-time symptomatic UTI are highest in both male and female infants during the first 12 months of life, with a marked reduction after this period.[1] UTI is broadly categorised into upper UTI (pyelonephritis) and lower UTI (cystitis). The usual trajectory of UTI spread involves the ascension of periurethral organisms through the urethra into the bladder (cystitis). These uropathogens then migrate upwards through the ureters to the renal parenchyma (pyelonephritis) and may be followed by haematogenous spread (bacteraemia). Pyelonephritis is associated with renal parenchymal scarring in approximately 10–30% of paediatric patients with febrile UTI.[3] Renal scars can result in hypertension, chronic kidney disease (CKD), and subsequently end-stage kidney disease.[4] Over the years, clinical practice guidelines have advanced to ensure the best global practices in managing UTI and preventing progression to CKD.

During childhood, 30–50% of children who have previously had an episode of UTI will have at least one recurrence.[5] Predisposition to recurrence of UTI has recently been linked to genetic factors; the identified genes were *HSPA 1B*, *CXCR1*, *CXCR2*, *TLR2*, *TLR4*, and *TGF-β1*.[6] However, nongenetic factors are also associated with infection risk and recurrence. These factors include constipation, severe acute malnutrition, obstructive uropathy, urolithiasis, absent circumcision in boys (particularly within the first year of life), and female sex after infancy.[7,8] Vesicoureteral reflux (VUR) also contributes to recurrence of UTI in children, which in turn promotes upper urinary tract involvement and renal scarring. Although postinfection renal scarring has traditionally been associated with subsequent CKD, its causal relationship with UTI-associated VUR has recently been challenged by some authors.[9,10] A high incidence of scar formation in post-UTI patients without VUR was noted in one of the studies.[9] The other report identified virulence of uropathogens, host defense factors, and genetic predisposition as risk factors for renal scarring.[10] Many of the sequelae from scarring are now attributed to pre-existing intrinsic renal disease.[11] Specifically, VUR-related renal damage is now linked to congenital dysplastic kidneys[12] and is regarded as part of the congenital anomaly of the kidney and urinary tract syndrome.[13]

These findings have led to a shift in clinical practice guidelines which until now recommended routine voiding cystourethrography (VCUG) or micturating cystourethrography (MCUG) after a first febrile UTI episode. The 2011 American Academy of Pediatrics (AAP) guideline now recommends a VCUG (or MCUG) after the second episode of UTI, with deference to parent preferences.[14] Similarly, the UK's National Institute of Health and Clinical Excellence (NICE) guideline dissuades clinicians from performing unnecessary invasive imaging tests and advocates a targeted investigative approach in high-risk children.[15] However, because the prevention of renal scarring is the objective of all therapeutic approaches for childhood UTI, it has been suggested that children at high risk of post-UTI renal scarring should still be treated and investigated pending the accumulation of more evidence to support any paradigm shift.[16] Clinical practice guidelines address five main questions: 1) which children should have their urine tested; 2) how the sample should be obtained; 3) which radiological tests are recommended after a diagnosis of UTI; 4) how the infection should be treated; 5) and how affected children should be followed up. This narrative review aims to discuss the current recommendations of the established practice guidelines with emphasis on the diagnosis, radiological investigation, treatment, and follow-up of UTI in children.

DIAGNOSIS OF URINARY TRACT INFECTION

Accurate diagnosis of UTI is critical for instituting appropriate treatment with antibiotics and preventing upper tract involvement and long-term renal disease. Proper interpretation of urinalysis and urine culture results form the basis for the diagnosis of UTI in children. Biomarkers of inflammation (such as pyuria and the presence of leukocyte esterase) and bacterial growth from urine cultures are usually required to establish a diagnosis. For the latter, which remains the gold standard, the reference standard for UTI diagnosis is a single uropathogen cultured from a specimen obtained at specific concentrations: $>10^3$ or 1,000 colony-forming units (CFU)/mL for a specimen from suprapubic aspiration (SPA); $>10^4$ or 10,000 CFU/mL for a catheter specimen; or $\geq 10^5$ or 10,000 CFU/mL for a 'clean-catch', midstream specimen.[17] Although some authors advocate the use of lower colony counts in symptomatic patients,[18] this proposal has not been incorporated in the established practice guidelines. However, a recent study observed that delay in initiating antibiotic therapy in children with a febrile UTI was associated with the development of renal scarring.[19] Specifically, the authors reported that a delay of ≥48 hours increased the odds of new renal scarring by approximately 47%.[19] This observation may emphasise the importance of rapid pretreatment diagnosis of UTI, or support the commencement of antibiotic therapy in children with febrile UTI without a positive urine culture result.

Identifying the most useful diagnostic test is critical for prompt treatment of UTI. However, there is no common agreement on the most

reliable single test. Some authors reported that urinalysis had excellent negative predictive value that was not enhanced by urine Gram stain in children with UTI.[20] Another study observed that point-of-care Gram stain was a useful rapid diagnostic tool for suspected UTI in young children; [21] it was concluded that pathogen-targeted therapy based on the point-of-care Gram stain would result in better antibiotic selection compared with empirical treatment.

In the revised AAP guideline, clinicians are encouraged to first consider the likelihood of a child having a UTI before starting their evaluation, given the fact that some host risk factors increase the likelihood of infection.[22] The AAP guideline recommendation for the diagnosis of UTI in children aged 2–24 months requires microscopic urinalysis results suggestive of infection (pyuria with or without bacteriuria) and the presence of ≥5x10^4 or 50,000 CFU/mL of a single uropathogen, cultured from a catheter or SPA urine specimen. This investigation can be undertaken once a clinician determines that the pre-evaluation likelihood of UTI merits obtaining a urine culture. The recommendation differs from the previous AAP guideline, which recommended urine testing for all children aged 2–24 months with unexplained febrile illness.[23] The revised AAP guideline recommends selective urine testing based on the probability of UTI. A set of risk factors help to determine 'low risk' of infection. These factors for females include Caucasian race, age <12 months, temperature ≥39° C, fever ≥2 days, and absence of another source of infection. For males, these factors include Caucasian race, temperature ≥39° C, fever >24 hours, absence of another source of infection. For both sexes, ≤2 factors are associated with ≤1% risk of UTI, and ≤3 is associated with ≤2% risk of UTI.[22] Caution is advised, however, in the application of this rule because the threshold probability for urine testing is not yet well established. Hence, evaluating each case individually is recommended, rather than urine testing based on certain identified risk factors alone.[14]

The NICE guideline, by comparison, recommends urine microscopy and culture for children aged 3–36 months with specific urinary symptoms, or with nonspecific symptoms who are at high risk of serious illness.[15] Dipstick urinalysis is recommended in children aged ≥3 years as the initial diagnostic step for UTI. For urine culture results, diagnosis of UTI should be based on a colony count of any Gram-negative bacilli, >10^3 or 1,000 CFU/mL of a Gram-positive cocci from a SPA specimen, >10^5 or 10,000 CFU of a single uropathogen per mL from a catheter urine specimen, or ≥10^5 or 10,000 CFU of a single uropathogen per mL from a clean-catch or midstream urine specimen.[15]

A comparative analysis of the two established guidelines indicates that the revised AAP guideline reduces emphasis from the role of dipstick urinalysis in the diagnosis of UTI. In contrast, the NICE guideline underscores its importance in the initial diagnosis of the infection in older children (≥3 years) (Table 1). The NICE guideline provides caveats which relate to the interpretation and implication of dipstick urinalysis results for leucocyte esterase and nitrate testing.[15] For instance, the sensitivity of nitrite testing is low and has been noted to be 53%, while its specificity is reportedly as high as 98%. Urine should be in the bladder for at least 1 hour for conversion of nitrate to nitrite to occur. In infants, who have short bladder transition time, nitrite testing may be falsely negative, reflecting its low sensitivity in this age group. The NICE guidelines suggest the use of dipstick testing as the initial diagnostic test for infants and children <3 years with a suspected UTI, and then if it is positive, the urine should be sent for culture.[15]

Secondly, disparities exist between the two guidelines regarding reference age ranges of pre-toilet-trained children (2–24 months versus 3–36 months), and the bacterial colony counts required for the diagnosis of UTI from the different sources of urine specimen.

The revised AAP guideline recommends the use of microscopic urinalysis findings, such as bacteriuria and pyuria, in conjunction with comparatively lower bacterial colony counts (5x10^4 or 50,000 CFU/mL, compared to >10^5 or 100,000 CFU/mL recommended by the NICE guideline). Furthermore, the AAP guideline suggests that the absence of pyuria in a true UTI is usually attributable to either a faulty method or insensitive laboratory definition of pyuria.[22] It maintains that the possibility of pyuria from other febrile illnesses does not preclude the fact that it is rarely absent in a true UTI.

Table 1: Comparative analysis of recommendations in the revised American Academy of Pediatrics (AAP)[14] and National Institute for Health and Clinical Excellence (NICE)[15] guidelines for urinary tract infection in children.

Parameters	AAP guidelines (2011)	NICE guidelines (2007)
Diagnosis of UTI		
- Using dipstick urinalysis		- Positive leucocyte esterase or nitrite test*
- Using microscopic urinalysis and urine culture	- Bacteriuria with or without pyuria - ≥$5x10^4$ CFU/mL (from SPA and catheter urine specimens)	- Colony count of any Gram negative bacilli or >10^3 CFU/mL of a Gram-positive coccus (from SPA urine specimen) - >10^5 CFU/mL (from catheter urine specimen) - ≥10^5 CFU/mL (from 'clean-catch' or midstream urine specimen)
Radiological investigation of UTI†		
- RBUS	- Recommended in febrile infants with first UTI	- Recommended in atypical or recurrent UTI in children aged <6 months
- DMSA renal scan	- Not recommended as routine investigation for first febrile UTI	- Recommended in children aged 6 months to 3 years with atypical or recurrent UTI‡
- VCUG (or MCUG)	- Not recommended as routine investigation for first febrile UTI	- Not recommended in children aged 6 months to 3 years with atypical or recurrent UTI‡‡
Treatment and follow-up of UTI		
- Route of antibiotics/duration	- Parenteral route for 48 hours (for critically ill patients) and switch to oral route if clinical improvement occurs. 7–10 days as the total duration of therapy	- For children aged <3 months: parenteral route for 2–3 days before a switch to oral route if clinical improvement occurs. 10 days as the total duration of therapy - For children aged >3 months with upper UTI: oral route using antibiotics with low-resistance pattern. 7–14-day duration of therapy§ - For children aged >3 months with lower UTI: oral route for 3 days
- Follow-up routine urine culture	- Not recommended**	- Not recommended
- Follow-up antibiotic prophylaxis	- Not recommended	- Not recommended

* Initial diagnostic step for UTI in children aged ≥3 years.

† Top-down approach recommends RBUS and DMSA renal scan as initial radiological investigations of UTI. Bottom-up approach recommends MCUG (VCUG) as the initial radiological investigation.

‡ Performed 4–6 months after UTI.

‡‡ If there is ureteral dilatation on RBUS or poor urine flow or non-*Escherichia coli* UTI, or family history of VUR.

§ Parenteral route for 2–4 days if the patient is vomiting, then switch to oral route for a total duration of 10 days.

** The previous guideline recommended follow-up urine culture after 48 hours of no clinical improvement.

AAP: American Academy of Pediatrics; CFU: colony-forming units; DMSA: dimercaptosuccinic acid; MCUG: mictuirating cystourethrography; NICE: National Institute for Health and Clinical Excellence; RBUS: renal and bladder ultrasonography; SPA: suprapubic aspiration; UTI: urinary tract infection; VCUG: voiding cystourethrography; VUR: vesico-ureteric reflux.

In contrast, the NICE guideline suggests that the absence of bacteriuria or pyuria does not completely exclude the diagnosis of UTI. This position on pyuria appears to be evidence-based considering the findings of two studies conducted in children with febrile neutropenia and UTI.[24,25] In these reports, the sensitivity of pyuria as a diagnostic parameter for UTI was found to be very low. These findings highlight the importance of bacteriuria and urine nitrite testing in this population of children with UTI, as neither of these diagnostic parameters would be affected by the absence of pyuria.[26] As a result, it seems reasonable to include urine nitrite and leucocyte esterase tests as part of the initial diagnosis of UTI in older children, as recommended by the NICE guideline.

Finally, the recommended techniques for collecting urine samples vary slightly between the two guidelines. The revised AAP guideline emphasises urine sample collection through catheterisation or SPA, while the NICE guideline adds absorbent urine-collection pads to the other options of clean-catch, catheterisation, or SPA samples. In the former, the urine collection technique is restricted to children aged 2–24 months. Recent evidence, however, suggests that the use of catheterisation for infants, and midstream or clean-catch urine (without cleansing the external genitalia for older children), constitute the most reliable methods for obtaining a good urine specimen for culture.[26] Catheterisation may be contraindicated in certain circumstances: gross infection of the external genitalia, labial adhesions in female children, or failure to visualise the urethral meatus in uncircumcised male children. Similarly, gross contamination of the external genitalia requires proper cleansing in school-aged children before any clean-catch urine collection.[27]

RADIOLOGICAL INVESTIGATION OF CHILDREN WITH URINARY TRACT INFECTION

Considerations addressed by the established clinical practice guidelines include radiological testing after a diagnosis of UTI, and how affected children should be followed up. Previous recommendations were predicated on evidence that VUR developed from recurrent UTI and subsequently led to renal scarring; therefore, the established guidelines had previously advocated comprehensive radiological investigations to detect possible VUR and renal scarring.[23,28]

The use of radiological investigations for evaluating a child with UTI remain controversial due to their invasive nature and radiation burden, as well as the current movement away from the concept of 'UTI–VUR–renal scar' progression. Two approaches to post-UTI imaging ('top-down' and 'bottom-up' approaches) have arisen.[29] In the top-down approach, the involvement of renal involvement during UTI helps predict the presence or absence of acute pyelonephritis, renal dysplasia, or acquired renal scarring.[5] Advocates of the top-down approach recommend initial investigation with renal and bladder ultrasonography (RBUS) and dimercaptosuccinic acid (DMSA) renal scan; VCUG (or MCUG) is performed only if renal involvement is observed. The bottom-up approach focusses on bladder involvement during UTI to detect VUR; this makes VCUG (or MCUG) an initial investigation. Although the top-down approach shows a high sensitivity in detecting VUR and renal scarring after a first febrile UTI, it is associated with the highest financial and radiation exposure costs. This drawback casts a doubt on its benefit and highlights the need for the clinician to apply the appropriate protocol that best suits the individual patient under investigation.

Further consideration is needed of the mismatch between VUR grading with VCUG, and ultrasound detection of abnormal morphology. Evidence supports the finding that when sonographic diagnosis of reflux is based solely on morphological criteria and degree of dilatation, correlation with VCUG findings appears poor. Additionally, evidence supports that a positive DMSA scan successfully identifies significant VUR in most instances, which strengthens a possible positive correlation between VCUG reflux grading and DMSA findings. This may support the possibility of using DMSA scan alone as the primary evaluation for children after a UTI episode and reduce use of VCUG in evaluating these children.

The NICE and revised AAP guidelines do not support routine radiological investigations for

children with first UTI.[15,22] In the NICE guidelines, radiological investigations are recommended depending on different factors: therapeutic response within 48 hours, evidence of atypical UTI, evidence of recurrent UTI, and the age of the child.[15] The guideline recommends RBUS in cases of atypical UTI, recurrent UTI, or children <6 months of age. DMSA renal scan is recommended only in children <3 years of age with atypical or recurrent UTI, and it is performed 4–6 months after UTI. By comparison, the revised AAP guideline recommends the performance of RBUS in febrile children aged 2–24 months with first UTI, to detect anatomic or structural abnormalities that may require further evaluation.[22] The guideline also discourages the use of VCUG (or MCUG) and DMSA as routine investigations after first febrile UTI.

The current practice of a restrictive approach in radiological investigations is supported by evidence from several studies that assessed the clinical importance of renal imaging.[30-33] The top-down approach in renal imaging, which forms the basis for the recommendations in the NICE and revised AAP guidelines, is supported by the findings of other studies.[34-36] Although UTI in children usually resolves with no sequelae, the fact that some children are predisposed to recurrence may partly be a reflection of an underlying congenital anomaly of the kidney and urinary tract and urinary tract obstruction, justifying the recommendation for RBUS as the initial imaging study following a first UTI.[5]

The American College of Radiology (ACR) Appropriateness Criteria® rates the following radiological investigations highly in the evaluation of select children with UTI: RBUS, radionuclide cystography, VCUG (or MCUG), and DMSA scan.[37] RBUS is considered useful for evaluating structural integrity of the kidneys and urinary tract, but is unreliable for detecting VUR.[38] VCUG (or MCUG) is sufficient for screening and grading VUR, whereas DMSA scan is adequate for evaluating renal scarring.[38] Thus, radiological investigations that seek to evaluate predisposing factors (structural anomalies) and complicating factors (VUR/renal scarring) of UTI would seem appropriate, despite the emerging evidence against the UTI–VUR–renal scar progression pathway.[11-13] This ACR rating appears to be the guiding principle in the recommendations of AAP and NICE established guidelines.

TREATMENT AND FOLLOW-UP OF URINARY TRACT INFECTION IN CHILDREN

The causative bacterial pattern determines the empirical antibiotic treatment of UTI in children. However, with rising antibiotic resistance of common uropathogens, clinicians should stay up-to-date with local bacterial resistance patterns that could influence antibiotic choices. The following factors should guide the choice and route of administration of empirical antibiotics: age of the child, severity of the clinical presentation, location of the infection (upper or lower tract), presence of complications, and prevalence and pattern of local antibiotic resistance.[39] One study recommended the following antibiotics for empirical therapy in pyelonephritis, according to age group: a combination of aminoglycoside/ampicillin or ceftazidime/ampicillin in early infancy; and an oral third-generation cephalosporin later in infancy and childhood.[39] However, the variable multiregional prevalence rates of extended-spectrum β-lactamase-producing bacteria have been increasing in acute care settings, and have brought the issue of multiantibiotic resistance to the fore. This trend thus negates the current choice of antibiotics used for empirical therapy.

Since the goals of treating acute UTI are to eliminate the infection, prevent complications, and reduce the likelihood of renal damage, the AAP guideline recommends that clinicians base the choice of antibiotics on local antimicrobial sensitivity patterns (if available) and adjust this choice according to sensitivity testing of the isolated uropathogen.[22] Moreover, the choice of route of antibiotics should be based on practical considerations as initiating treatment orally or parenterally is equally efficacious. The guideline also advocates for a 7–14-day duration of antimicrobial therapy. A Cochrane review analysed short-duration (2–4 days) versus standard-duration (7–14 days) oral antibiotics in 652 children with lower UTI.[40] The authors reported no significant difference in positive urine cultures between the two therapies immediately after treatment; [40] furthermore, there was no significant difference between short and standard duration therapies in the development of resistant organisms after the course of treatment. These findings support

that a 2–4-day course of oral antibiotics is as effective as a 7–14-day course in children with lower UTI. However, meta-analysis studies suggest that a single-dose or single-day course may be less effective than more prolonged courses of oral antibiotics, and are therefore strongly discouraged.[41,42]

While there is enough evidence to show that most children with UTI can be treated with oral antibiotics,[43-45] the revised AAP guideline recommends that "patients whom clinicians judge to be 'toxic' or who are unable to retain oral intake (including medications) should receive an antimicrobial agent parenterally until they exhibit clinical improvement (generally within 24–48 hours) and can retain orally administered fluids and medications".[22] However, oral antibiotics are as effective as parenteral therapy in children.[44] A randomised clinical trial demonstrated no difference in the prevalence of post-UTI renal scarring between children who were treated with oral antibiotics alone and those treated with both parenteral and oral antibiotics.[46] It is therefore not surprising that current guidelines have generally tilted towards oral antibiotic therapy for UTI in children.

The NICE guideline recommends parenteral antibiotics (precise duration not stated) for UTI in children aged <3 months, with 2–3-day duration recommended before switching to oral antibiotics, if there is clinical improvement.[15] For children aged >3 months with upper UTI, oral antibiotics with low resistance patterns are recommended for 7–14 days. If the patient is vomiting, the guideline recommends parenteral antibiotics for 2–4 days and a switch to oral antibiotics for a total duration of 10 days. For children aged >3 months with lower UTI, oral antibiotics for 3 days is advised.

Advice for the follow-up of children treated for UTI concerning repeat urine cultures differs between sources. Although several earlier studies support the necessity for routine urine cultures following the commencement of therapy,[47-53] a more recent study suggests that follow-up urine cultures were not useful in children hospitalised for UTI, including those with fever lasting beyond 48 hours or those with an underlying urological disease.[54] The previous AAP guideline recommended a repeat urine culture if the expected clinical response (i.e., resolution of fever) was not achieved within the first 48 hours of therapy.[21] This recommendation implies that fever beyond 48 hours is abnormal and should warrant investigation. However, one report had observed that fever persisted at 48 hours among young children hospitalised for UTI, [55] suggesting fever beyond 48 hours may not be an appropriate criterion for repeat urine cultures. The current AAP guideline does not advocate routine follow-up urine cultures. The NICE guideline similarly recommends that routine follow-up urine cultures in children who are well are unnecessary. Additionally, follow-up culture is not required in children who do not undergo radiological investigation.

Antibiotic prophylaxis aims to prevent the recurrence of UTI. Recurrent UTI, with or without VUR, is currently the most common reason for long-term antibiotic prophylaxis in infants and children. Other indications include febrile UTI in neonates and infants, and UTI with obstructive lesions. Low-dose nitrofurantoin (1–2 mg/kg once per day), and trimethoprim/sulfamethoxazole (2 mg/kg/night or 5 mg/kg twice weekly) are the antibiotics most commonly used in the prevention of UTI in children. Antibiotic prophylaxis can prevent recurrence of UTI, renal scarring, or both in young children following a UTI, with or without VUR.[5] However, emerging evidence has challenged the practice of follow-up prophylactic antibiotics in affected children. A recent study showed that patients with congenital pelvic–ureteric junction obstruction who were not administered prophylactic antibiotics had developed neither UTI or renal scarring on follow-up.[53] The efficacy of antibiotic prophylaxis has been judged low in several other studies.[57-62] The Swedish infant high-grade reflux trial compared the efficacy of continuous antibiotic prophylaxis and endoscopic injection treatment in the management of high-grade VUR in infants.[63] Similarly, it found that the resolution rate of high-grade VUR with injection treatment was higher than that of continuous antibiotic prophylaxis. This finding further suggests that antibiotic prophylaxis appears less effective in preventing UTI recurrence in children with underlying predisposing factors. Perhaps the current burden of proof against antibiotic prophylaxis has influenced the recommendations of the current clinical

practice guidelines. The NICE guideline does not recommend routine antibiotic prophylaxis in infants and children after first UTI, [15] and the recent AAP guideline also discourages the practice after first UTI in children aged 2–24 months.[22]

CONCLUSION

There is substantial overlap in the recommendations of both AAP and NICE guidelines for the diagnosis, radiological investigation, antibiotic treatment, and follow-up of UTI in childhood. Subtle differences, however, exist between the two guidelines. An evidence-based paradigm shift on some traditional concepts about UTI in children has influenced the revision and update of these guidelines. Regarding the controversial issue of 'UTI–VUR–renal scar' trajectory, further research is required to establish host and genetic factors that may predispose to renal scarring. In future clinical practice guidelines, these factors should be considered in order to reduce the need for invasive radiological investigations. Finally, a revision of the current recommendations for UTI diagnosis may be necessary. Specifically, it may be reasonable to reduce the diagnostic threshold to $\geq 10^4$ or 10,000 CFU/mL against the revised AAP recommendation of $\geq 5 \times 10^4$ or 50,000 CFU/mL, and augment diagnostic testing with a reliable detector of significant pyuria such as the leukocyte esterase test. The leukocyte esterase test would help strengthen the clinical significance of urine culture because of its utility in differentiating true UTI from an inflammatory response to urine contamination or asymptomatic bacteriuria.

References

1. Shaikh N et al. Prevalence of urinary tract infection in childhood: a meta-analysis. Pediatr Infect Dis J. 2008;27(4):302-8.
2. Chang SL, Shortliffe LD. Pediatric urinary tract infections. Pediatr Clin North Am. 2006;53(3):379-400.
3. Shaikh N et al. Identification of children and adolescents at risk of renal scarring after a first urinary tract infection: a meta-analysis with individual patient data. JAMA Pediatr. 2014;168(10):893-900.
4. Lin KY et al. Acute pyelonephritis and sequelae of renal scar in pediatric first febrile urinary tract infection. Pediatr Nephrol. 2003; 18(4):362-5.
5. Paintsil E. Update on recent guidelines for the management of urinary tract infections in children: the shifting paradigm. Curr Opin Pediatr. 2013;25(1):88-94.
6. Zaffanello M et al. Genetic risk for recurrent urinary tract infections in humans: a systematic review. J Biomed Biotechnol. 2010; 2010:321082.
7. Uwaezuoke SN et al. The prevalence and risk of urinary tract infection in malnourished children: a systematic review and meta-analysis. BMC Pediatr. 2019; 19:261.
8. Schoen EJ et al. New-born circumcision decreases the incidence and costs of urinary tract infections during the first year of life. Pediatrics. 2000;105(4 Pt 1):789-93.
9. Garin EH et al. Primary vesicoureteral reflux in childhood. Adv Pediatr. 2002; 49:341-57.
10. Lee YJ et al. Risk factors for renal scar formation in infants with a first episode of acute pyelonephritis: a prospective clinical study. J Urol. 2012;187(3):1032-6.
11. Montini G et al. Febrile urinary tract infections in children. N Engl J Med. 2011; 365:239-50.
12. Williams G et al. Vesicoureteral reflux. J Am Soc Nephrol. 2008;19(5):847-62.
13. Ichikawa I et al. Paradigm shift from classic anatomic theories to contemporary cell biological views of CAKUT. Kidney Int. 2002;61(3):889-98.
14. Newman TB. The new American Academy of Pediatrics urinary tract infection guideline. Pediatrics. 2011;128(3):572-5.
15. National Institute for Health and Clinical Excellence (NICE). Urinary tract infection in children (CG54). Available at: http://www.nice.org.uk/CG054. Last accessed: 30 November 2019.
16. Park YS. Renal scar formation after urinary tract infection in children. Korean J Pediatr. 2012;55(10):367-70.
17. Rushton HG. Urinary tract infections in children: epidemiology, evaluation, and management. Pediatr Clin North Am. 1997;44(5):1133-69.
18. Heidrich FJ et al. UTI: diagnosis and evaluation in symptomatic pediatric patients. Clin Pediatr (Phila). 2000;39(8):461-72.
19. Shaikh N et al. Early antibiotic treatment for pediatric febrile urinary tract infection and renal scarring. JAMA Pediatr. 2016;170(9):848-54.
20. Cantey JB et al. Lack of clinical utility of urine Gram stain for suspected urinary tract infection in pediatric patients. J Clin Microbiol. 2015; 53:1282-5.
21. Yodoshi T et al. Utility of point-of-care Gram stain by physicians for urinary tract infection in children ≤36 months. Medicine (Baltimore). 2019; 98(14):e15101.
22. Roberts KB. Urinary tract infection: clinical practice guideline for the diagnosis and management of the initial UTI in febrile infants and children 2 to 24 months. Pediatrics. 2011;128(3):595-610.
23. American Academy of Pediatrics, Committee on Quality Improvement, Subcommittee on Urinary Tract Infection. Practice parameter: the diagnosis, treatment, and evaluation of the initial urinary tract infection in febrile infants and young children. Pediatrics. 1999;103(4 Pt 1):843-52.
24. Sandoval C et al. Urinary tract infections in pediatric oncology patients with fever and neutropenia. Pediatr Hematol Oncol. 2012;29(1):68-72.
25. Klaassen IL et al. 2011. Pyuria is absent during urinary tract infections in neutropenic patients. Pediatr Blood Cancer. 2011; 56:868-70.

26. Doern CD, Richardson SE. Diagnosis of urinary tract infections in children. J Clin Microbiol. 2016; 54:2233-42.

27. Lohr JA et al. Hospital-acquired urinary tract infection. Pediatrics. 1989; 83:193-9.

28. Smellie JM et al. Medical versus surgical treatment in children with severe bilateral vesicoureteric reflux and bilateral nephropathy: a randomised trial. Lancet. 2001;357(9265):1329-33.

29. Routh JC et al. Vesicoureteral reflux: current trends in diagnosis, screening, and treatment. Eur Urol. 2012;61(4):773-82.

30. Schroeder AR et al. Impact of a more restrictive approach to urinary tract imaging after febrile urinary tract infection. Arch Pediatr Adolesc Med. 2011; 165:1027-32.

31. Hoberman A et al. Imaging studies after a first febrile urinary tract infection in young children. N Engl J Med. 2003;348(3):195-202.

32. Jahnukainen T et al. Ultrasonography after the first febrile urinary tract infection in children. Eur J Pediatr. 2006;165(8):556-9.

33. Pennesi M et al. Managing children under 36 months of age with febrile urinary tract infection: a new approach. Pediatr Nephrol. 2012; 27:611-5.

34. Lee JH et al. Is a routine voiding cystourethrogram necessary in children after the first febrile urinary tract infection? Acta Paediatr. 2012; 101:e105-9.

35. Hannula A et al. Imaging the urinary tract in children with urinary tract infection. Acta Paediatr. 2011; 100(12):e253-9.

36. Tsai JD et al. Screening high-grade vesicoureteral reflux in young infants with a febrile urinary tract infection. Pediatr Nephrol. 2012; 27:955-63.

37. American College of Radiology. ACR Appropriateness Criteria: urinary tract infection-child. Available at: https://acsearch.acr.org/docs/69444/Narrative/. Last accessed: 30 November 2019.

38. White B. Diagnosis and treatment of urinary tract infections in children. Am Fam Physician. 2011;83(4):409-15.

39. Beetz R, Westenfelder M. Antimicrobial therapy of urinary tract infections in children. Int J Antimicrob Agents. 2011; 38:42-50.

40. Michael M et al. Short versus standard duration oral antibiotic therapy for acute urinary tract infection in children. Cochrane Database Syst Rev. 2003;(1):CD003966.

41. Tran D et al. Short-course versus conventional length antimicrobial therapy for uncomplicated lower urinary tract infections in children: a meta-analysis of 1279 patients. J Pediatr. 2001;139(1):93-9.

42. Keren R, Chan E. A meta-analysis of randomized, controlled trials comparing short- and long-course antibiotic therapy for urinary tract infections in children. Pediatrics. 2002; 109(5):E70.

43. Hoberman A et al. Oral versus initial intravenous therapy for urinary tract infections in young febrile children. Pediatrics. 1999;104(1 Pt 1):79-86.

44. Hodson EM et al. Antibiotics for acute pyelonephritis in children. Cochrane Database Syst Rev. 2007;(4):CD003772.

45. Bloomfield P et al. Antibiotics for acute pyelonephritis in children. Cochrane Database Syst Rev. 2005;(1):CD003772

46. Bocquet N et al. Randomized trial of oral versus sequential IV/oral antibiotic for acute pyelonephritis in children. Pediatrics. 2012; 129:e269-75.

47. Ansari BM et al. Urinary tract infection in children, part 1: epidemiology, natural history, diagnosis, and management. J Infect. 1995;30(1):3-6.

48. Winberg J et al. Epidemiology of symptomatic urinary tract infection in childhood. Acta Paediatr Scand Suppl. 1974; 252:1-20.

49. Johnson CE. New advances in childhood urinary tract infections. Pediatr Rev. 1999;20(10):335-43.

50. Shapiro ED. Infections of the urinary tract. Pediatr Infect Dis J. 1992;11(2):165-8.

51. Watson AR. Urinary tract infection in early childhood. J Antimicrob Chemother. 1994;34(Suppl A):53-60.

52. Feld LG et al. Urinary tract infections in infants and children. Pediatr Rev. 1989; 11:71-7.

53. Lerner GR. Urinary tract infections in children. Pediatr Ann. 1994;23(9):466-73.

54. Currie ML et al. Follow-up urine cultures and fever in children with urinary tract infection. Arch Pediatr Adolesc Med. 2003; 157:1237-40.

55. Bachur R. Nonresponders: prolonged fever among infants with urinary tract infections. Pediatrics. 2000; 105(5):e59.

56. Islek A et al. Probability of urinary tract infection in infants with ureteropelvic junction obstruction: is antibacterial prophylaxis really needed? Pediatr Nephrol. 2011; 26:1837-41.

57. Montini G et al. Prophylaxis after first febrile urinary tract infection in children? A multicenter, randomized, controlled, non-inferiority trial. Pediatrics. 2008;122(5):1064-71.

58. Song SH, Kim KS. Antibiotic prophylaxis in pediatric urology. Indian J Urol. 2008;24(2):145-9.

59. Le Saux N et al. Evaluating the benefits of antimicrobial prophylaxis to prevent urinary tract infections in children: a systematic review. CMAJ. 2000;163(5):523-9.

60. Faust WC, Pohl HG. Role of prophylaxis in vesicoureteral reflux. Curr Opin Urol. 2007;17(4):252-6.

61. Hewitt IK et al. Antibiotic prophylaxis for urinary tract infection-related renal scarring: a systematic review. Pediatrics. 2017; 139(5):e20163145.

62. Pennesi M et al. Is antibiotic prophylaxis in children with vesicoureteral reflux effective in preventing pyelonephritis and renal scars? A randomized, controlled trial. Pediatrics. 2008; 121(6):e1489-94.

63. Nordenström J et al. The Swedish infant high-grade reflux trial: Study presentation and vesicoureteral reflux outcome. J Pediatr Urol. 2017;13(2):130-8.

Molecular Identification and Antifungal Susceptibility Profiles of Non-*albicans Candida* Species Clinical Isolates

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Abstract

Background: There is an increasing incidence of life-threatening systemic mycoses, specifically fulminant infections by the *Candida* species in hospitalised patients and in those who are immunocompromised. Management of the limited number of antifungal drugs currently available requires the identification of infections containing drug-resistant isolates.

Objectives: The aim of this study was to identify the non-*albicans Candida* species as azole-resistant fungi, isolated from sputum and bronchoalveolar lavage specimens of hospitalised cases.

Methods: The subjects included hospital-acquired infection (HAI) cases, with a primary diagnosis using a direct microscopic examination, performed for the detection of probable fungi. The molecular tests of PCR-restriction fragment length polymorphism (RFLP) and real-time PCR were performed to confirm the identity and molecular typing of the *Candida* isolates. Antifungal susceptibility testing (AFST), by the Clinical and Laboratory Standards Institute (CLSI) broth microdilution (BMD) minimum inhibitory concentration (MIC) (M27-A2) method, was performed on the hospital-isolated *Candida* species.

Results: During 24 months, from August 2014 to September 2016, a total of 198 samples were obtained from cases with proven HAI. The results of experimental studies on the specimens showed 93 (47%) positive cases for a fungal or bacterial infection, of which 54 (58%) had a fungal infection. It was hypothesised that all of the isolated organisms were causative agents of the HAI.

Conclusions: The results showed that the medium CHROMagar™ *Candida* is an accessible and easy-to-use method for the identification of infection, but not as accurate and reliable as PCR-RFLP and real-time PCR methods. Results also showed decreasing susceptibility to azoles (itraconazole in this study) of the *Candida* species.

BACKGROUND

The *Candida* species is the cause of several infections, including bloodstream infections and disseminated candidiasis. Despite recent advancements in the diagnosis and treatment of candidiasis, *Candida* is the fourth leading cause of bloodstream infection pathogens in the USA and seventh in Europe.[1] Life-threatening systemic fungal infections have increased amongst immunocompromised patients, particularly fulminant infections caused by *Candida* spp. Patients immunocompromised as a result of HIV infection, allogenic haematologic stem cell transplantation, chemotherapy for acute leukaemia, or infant prematurity, are at risk of *Candida* infections.

Although *Candida albicans* is known as the most common cause of invasive candidiasis, there has been an increase in non-*albicans* infections, such as *C. krusei, C. glabrata, C. lusitaniae, C. tropicalis,* and *C. parapsilosis*. Some non-*albicans Candida* species, including *C. auris, C. tropicalis, C. krusei, C. parapsilosis,* and *C. lusitaniae*, are classified as notable pathogenic fungi that cause invasive candidiasis, mainly because of their innate resistance to antifungal drugs.[2-6] Treating invasive fungal infections has been difficult because of the limited availability of antifungals, as well as the relative toxicity, drug interaction, and drug-resistance challenges.[7] Systemic and invasive candidiasis can be treated with the limited number of available antifungal medications, such as polyenes, allylamines, azoles, and the newly produced echinocandin class of molecules.[1] Adverse side effects, toxicity, and drug resistance in *Candida* species have been observed worldwide. Studies on the prevalence of *Candida* infections and antifungal susceptibility testing (AFST) can help to make decisions on clinical strategies. Studying the incidence of *Candida* infections, as well as AFST, is helpful in terms of planning clinical measures. Rapid identification of *Candida* spp. is also beneficial as early management of antifungal treatment can take place.[1] Additionally, management of the limited number of antifungals currently available requires the identification of infections containing drug-resistant isolates, and the discovery of factors that promote the evolution of drug resistance.[4]

Objectives

The aim of this study was to identify the non-*albicans Candida* species from azole-resistant fungi, isolated from the sputum and bronchoalveolar lavage specimens of hospitalised cases. The performance of three rapid methods of identification at the species level was evaluated, and two susceptibility tests for the *C. albicans* clinical isolates were used.

METHODS

Subjects

Respiratory tract specimens were taken from patients with hospital-acquired infection (HAI) and infection symptoms occurring in the first 48 hours after arriving at the UMS University educational hospitals, in Urmia, Iran. Additionally, other clinical samples including pressure ulcers, skin abscess, vaginal discharge, and nail scrape were obtained from patients during hospitalisation.

Culture and Identifications

The collected specimens were transported to the Medical Mycology Center, School of Medicine, UMS University. The primary diagnosis included direct microscopic examination (potassium hydroxide preparation [KOH]: 10%; Giemsa: 20% stained slides), which was performed for the detection of the possible fungi causing the HAI. This was followed by growing the cultures on sabouraud dextrose agar ([SDA]: 4%), Czapek yeast extract agar (CYA), corn meal agar, and CHROMagar™ *Candida*, for the identification of yeast isolates.[8]

Molecular Identification of *Candida* Species

First, DNA extraction was performed: molecular tests (PCR-restriction fragment length polymorphism [RFLP] and real-time PCR) were performed to confirm the identity and molecular typing of *Candida* isolates. DNA extraction of *Candida* cells was performed using phenol-chloroform and the glass beads manual method (making a lysis solution with 10 mM ethylenediaminetetraacetic acid, 1% sodium dodecyl sulfate, 100 mM sodium chloride, 100 mM Tris-HCl, and 1% TritonX-100 [Sigma-Aldrich, St Louis, Missouri, USA]).[9]

There are two important non-coding regions, internal transcribed spacers (ITS) 1 and 2, containing a variable subregion inside and a conservative subregion outside. The ITS fragments are located between the 18s and 28s rRNA genes of the *Candida* species. The variability of the ITS regions have proved useful in phylogenic studies of the *Candida* species. The target gene rRNA was amplified using ITS primers (ITS1: 5'-TCC GTA GGT GAA CCT GCG G-3'; ITS2: 5'-TCC TCC GCT TAT TGA TAT GC-3'). The PCR profile included: 5 µl of the DNA template; PCR buffer (20 mM Tris-HCl; pH 8.0); 50 mM potassium chloride; 0.1 mM each of the forward and reverse primers; and 1.5 U of Taq DNA polymerase in a total reaction volume of 50 µl. The reactions were performed in a Thermocycler model XL (Bioer Technology, Hangzhou, China). The PCR-RFLP, using the restriction enzyme MspI, was performed to create a differential pattern to identify the *Candida* species (Table 1; Figure 1).[10] A real-time PCR for the confirmation of each RFLP-identified Candida isolate was carried out at the Molecular Research Center, EUMS, Esfahan, Iran.

Susceptibility Test

AFST, by the Clinical and Laboratory Standards Institute (CLSI) broth microdilution (BMD) minimum inhibitory concentration (MIC) (M27-A2) method, was performed on hospital-isolated *Candida* species. The medium for testing antifungal agent constituted synthetic complete medium and RPMI-1640 (Sigma-Aldrich), supplemented with glutamine but without bicarbonate, as recommended. Supplementing medium to a final concentration of 20 g/L (2%) glucose has been shown to result in better growth of yeast isolates, without markedly altering the MIC of antifungal agents.[10] Preparation of the stock drug solution included the antifungal drug itraconazole (Taj Pharmaceuticals Ltd., Birmingham, UK), prepared by weighing 16 mg on an analytical balance calibrated to 2 decimal places, as it was recommended that >16 mg (32 mg) of powder was used. Antifungal drug stock solution was prepared to a concentration 100 times the highest concentration to be tested (320 mg/mL).

Table 1: Clinical specimens collected from patients with *Candida* colonisation.

Clinical specimen	Number	Percentage (%)
Bronchoalveolar lavage	12	60
Vaginal discharge	5	25
Nail scrape	2	10
Sputum	1	5
Total	20	100

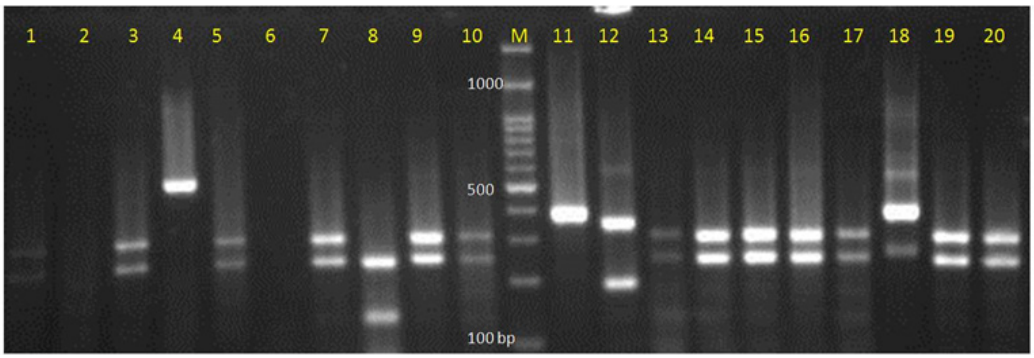

Figure 1: Electrophoretic picture of digestion with restriction enzyme MspI in the PCR-restriction fragment length polymorphism method.

Lanes: 1, 3, 5, 7, 9, 10, 13, 14, 15, 16, 19, 20 show *C. albicans*; lane 11: *C. parapsilosis*; lane 12: *C. glabrata*; lane 8: *C. tropicalis*; lane 4: undigested PCR product; and lane M: 100 base pair DNA marker.

Dimethyl sulfoxide (DMSO) was suitable for dissolving itraconazole, as well as other antifungal drugs (ketoconazole and flucytosine). The drug's stock solutions were used, and the DMSO was prepared and sterilised beforehand.

For the yeast cell suspension, a 24-hour culture was prepared on 4% SDA and adjusted by a spectrophotometry system (wavelength: 530 nm; optical density: 75–77), ensuring that the yeast cell suspensions had equal concentration to the 0.5 McFarland standard.

Treatment

MIC testing of itraconazole was performed as per the procedure in the CLSI guidelines M27-A2,[9-20] using RPMI-1640 broth, an inoculum of 0.5–2.5X10^3 colony-forming units (cfu)/mL, incubation at 35°C, and MIC endpoint criteria of prominent inhibition of growth.[10] A serial dilution of the drug solution was made adding 100 µl of free bicarbonate RPMI-1640 into the first well (each lane of a 96-well plate), followed by a reduction in concentration (16.0000 to 0.0313 µg/mL). For the treatment, 100 µl of 1,000 times diluted yeast cells suspension was added to 10 wells, plus a negative control (without fungal cells) and a positive control (fungal cells only). After incubating for 24–48 hours at 35°C, turbidities of fungal growth were detected on a light screen. The MIC for the azole family were described as the lowest concentration of the drug that could reduce 50% of fungal growth. MIC of itraconazole for *Candida* cells were obtained by observing the first well without any growth.

RESULTS

Throughout the 24 months between August 2014 and September 2016, 198 clinical specimens were tested for fungal isolation. Bronchoalveolar lavage and sputum were the most frequent specimens with *Candida* isolation (as pathogens or colonising) (Table 1). *Candida* species, dominantly *C. albicans*, were obtained from 20/198 clinical specimens used in this study. Clinical cases were in the age range of 1–78 years. The identification results showed 93 (47.0%) patients with fungal or bacterial colonisation, and 25 (26.9%) had a fungal infection. It is thought that all of the isolated organisms were causing agents of infection in the tested cases. Among all isolated *Candida* species, *C. albicans, C. dubliniensis, C. glabrata,* and *C. tropicalis* were confirmed. These findings are based on the PCR-RFLP results confirmed by real-time PCR (Table 2). With the exception of *C. dubliniensis*, the results of molecular identifications were approximately similar in the two molecular methods. *C. dubliniensis* isolates in this study were identified by CHROMagar *Candida* and confirmed by real-time PCR. Additionally, a drug-resistant *Candida* isolate was identified as *C. parapsilosis* by PCR-RFLP and real-time PCR. Susceptibility testing (MIC) of *Candida* isolates results showed that 48% were resistant and 52% were sensitive to itraconazole. MIC results showed that tested *C. parapsilosis* were resistant against itraconazole (MIC: 0.125–0.250 µg/mL). Moreover, of the 23 tested samples of *C. albicans/C. dubliniensis*, 11 (47.8%) were resistant (MIC range: 0.125–0.250 µg/mL). Only one case of *C. glabrata* was sensitive to itraconazole with an MIC range of ≤0.125 µg/mL.

DISCUSSION

The prevalence of systemic *Candida* infections, for which hospital care is needed, has recently elevated significantly for several reasons. Patients at risk of systemic fungal infections (including candidiasis), such as those with a serious underlying disease, immunosuppression caused by cytotoxic/anti-rejection chemotherapy, corticosteroid therapy, and long-term antibiotic therapy, are on the increase.[11] Furthermore, fungal infections are recognised as a serious concern amongst the elderly population, as age is a risk factor because of its effect on mortality rates.[12,13] Presence of *Candida* colonisation on the skin surface and mucosa is a potential source of systemic infection, and the hands of medical personnel have been evidenced to play an important role in nosocomial infections with transmission from healthcare workers to patients in hospitals.[14] Moreover, transmission from common sources such as contaminated intravenous fluids, hospital food, and medical equipment has been shown.[14,15]

In the present study, most of the subjects were admitted to specialist medical wards, including nephrology and pulmonology. A significant number of the patients hospitalised were immunosuppressed after a kidney transplant, an important risk factor for *Candida* colonisation and infection.

Table 2: Molecular identifications and minimum inhibitory concentration of itraconazole on clinical *Candida* isolates.**

Number	Strain number	Age/sex	Source	CHROM agar *Candida*	PCR-RFLP	Real-time	MIC 24	MIC 48
1	KDY1072	35/F	BAL	*C. glabata*	*C. albicans*	*C. albicans*	S	S
2	KDY1155	48/M	BAL	*C. albicans*	*C. glabrata*	*C. glabrata*	S	S
3	KDY1237	36/M	BAL	*C. dubliniens*	*C. albicans*	*C. dubliniens*	4	R
4	KDY1271	43/F	Nail scrape	*C. albicans*	*C. albicans*	*C. albicans*	S	S
5	KDY1244	60/F	Vaginal discharge	*C. albicans*	*C. albicans*	*C. albicans*	S	S
6	KDY1108	55/M	BAL	*C. albicans*	*C. albicans*	*C. albicans*	8	R
7	KDY1239	28/F	Vaginal discharge	*C. albicans*	*C. albicans*	*C. albicans*	R	R
8	KDY1253	26/M	BAL	*C. albicans*	*C. albicans*	*C. dubliniens*	R	R
9	KDY1210	59/M	BAL	*C. albicans*	*C. albicans*	*C. albicans*	S	S
10	KDY1201	66/F	BAL	*C. albicans*	*C. tropicalis*	*C. tropicalis*	8	R
11	KDY1110	73/M	BAL	*C. albicans*	*C. albicans*	*C. albicans*	S	S
12	KDY1147	78/F	BAL	*C. albicans*	*C. albicans*	*C. albicans*	8	R
13	KDY1130	11/F	BAL	*C. albicans*	*C. albicans*	*C. albicans*	R	R
14	KDY935	34/M	Vaginal discharge	*Candida* spp.	*	*C. parapsilosis*	R	R
15	KDY1203	1/M	Sputum	*C. albicans*	*C. albicans*	*C. albicans*	8	R
16	KDY1159	56/M	BAL	*C. albicans*	*C. albicans*	*C. albicans*	8	R
17	KDY1208	57/F	Nail scrape	*C. albicans*	*C. albicans*	*C. albicans*	S	S
18	KDY1061	50/F	Vaginal discharge	*C. albicans*	*C. albicans*	*C. albicans*	8	R
19	KDY1162	17/F	Vaginal discharge	*C. albicans*	*C. albicans*	*C. albicans*	S	S
20	KDY1253	26/M	BAL	*C. dubliniens*	*C. albicans*	*C. dubliniens*	S	S
21	KDY1227	9/M	Urine	*C. albicans*	*C. albicans*	*C. albicans*	S	S
22	KDY1116	42/M	BAL	*C. dubliniens*	*C. albicans*	*C. albicans*	S	S
23	KDY1658	-		*C. albicans*	*C. albicans*	*C. albicans*	S	S
24	KDY953	34/F	Sputum	*C. albicans*	*C. albicans*	*C. albicans*	S	S
25	KDY618	80/F	BAL	*Candida* spp.	*C. parapsilosis*	*C. parapsilosis*	R	R

*Undigested DNA in RFLP profile
**MIC range: 0.063–0.25µg/ml

BAL: bronchoalveolar lavage; F: female; M: male; MIC: minimum inhibitory concentration; R: resistant; RFLP: restriction fragment length polymorphism; S: sensitive.

Two studies from the USA have shown a medieval prevalence of *Candida* yeasts at nephrology wards.[16,17] This study showed that there was no difference in sex preponderance with regards to either colonisation or infection by *Candida* yeasts; however, the elderly patients (>50 years old) were more at risk of infection. Similar to other studies, kidney transplant, immune suppression, and haemodialysis were shown to be the main risk factors in this study. Several other studies have indicated that immunosuppression, broad spectrum antibiotic therapy, and diabetes are important predisposing factors of candidiasis.[15,17] Another recent study classified antibiotic treatment 3 months after kidney transplant, cytomegalovirus infection, and diabetes as independent risk factors associated with invasive fungal infections.

Amongst all collected specimens in this study, most of the *Candida* cells were isolated from bronchoalveolar lavage, which indicated *Candida* colonisation of the mucosal layer of the oropharynx and the lower respiratory tract. Post-transplant immunosuppression may have predisposed the patients to mucosal colonisation by *Candida*. Several researchers have also reported a high prevalence of *Candida* species causing pulmonary colonisation infection. This study's findings of Candida identification showed that *C. albicans* was the most common yeast isolate. This result is consistent with that of several other studies.[17] Another study from the USA reported *C. albicans* as the most common fungal isolate from 28 kidney transplant, 17 liver transplant, six lung transplant, and four heart transplant patients.[9] Nonetheless, non-*albicans Candida* species, such as *C. glabrata* and *C. dubliniensis,* have been identified as leading isolates in other studies. The importance of real-time PCR in this research was highlighted by its ability to differentiate *C. dubliniensis*, which could not be differentiated by PCR-RFLP and a restriction enzyme. Using real-time PCR for the molecular identification of *Candida* yeasts has been reported by several researchers. A recent study from China reported the identification of four *Candida* species, including *C. albicans*, *C.tropicalis*, *C. glabrata*, and *C. dubliniensis*. Another study from Germany used real-time PCR assays for identifying *C. albicans, C. parapsilosis, C. glabrata, C. tropicalis,* and *C. dubliniensis*.[10] There were no coincidences amongst the three methods used in the present study, which involved at least seven *Candida* isolates at the species level. For example, *C. tropicalis* identified by the CHROMagar™

differential medium was not confirmed by PCR-RFLP and real-time PCR. However, one case of *C. albicans* identified by real-time PCR was identified as *C. glabrata* by CHROMagar™

and PCR-RFLP. These results of AFST (MIC) indicated that approximately half of the clinical isolates of *C. albicans* exhibited itraconazole resistance. Two thirds of cases of *C. dubliniensis* and both isolates of *C. parapsilosis* were also resistant. A study from Turkey reported 11/23 isolates of *C. albicans* and 3/5 isolates of *C. glabrata* were resistant to itraconazole (MIC: ≥16 µg/mL).[12]

CONCLUSION

In conclusion, the authors found that immunosuppressions and/or iatrogenic interventions predispose patients hospitalised at medical wards to yeast colonisation/infection. *C. albicans* is the predominant species amongst the isolates that colonises in the upper respiratory tract. It was also found that *C. albicans* had a decreasing susceptibility to itraconazole (an azole antifungal drug). Molecular identification of *Candida* species and their resistance patterns is essential to optimising patient management and reducing unnecessary drug use.

References

1. Bhattacharjee P. Epidemiology and antifungal susceptibility of *Candida* species in a tertiary care hospital, Kolkata, India. Curr Med Mycol. 2016;2(2):20-7.
2. Kaur R et al. Identification and antifungal susceptibility testing of *Candida* species: a comparison of Vitek-2 system with conventional and molecular methods. J Glob Infect Dis. 2016;8(4):139-46.
3. Wiederhold N et al. The fungal Cyp51 specific inhibitor VT-1598 demonstrates *in vitro* and *in vivo* activity against *Candida auris*. Antimicrobial agents and chemotherapy. Antimicrob Agents Chemother. 2019;63(3).

4. Demers E et al. Evolution of drug resistance in an antifungal-naive chronic *Candida lusitaniae* infection. Proc Nat Acad Sci U S A. 2018;115(47):12040-5.
5. Chew KL et al. Delay in effective therapy in anidulafungin-resistant *Candida tropicalis* fungaemia: potential for rapid prediction of antifungal resistance with whole-genome sequencing. J Glob Antimicrob Resist. 2019;16:105-7.
6. Thomaz D et al. An azole-resistant *Candida parapsilosis* outbreak: clonal persistence in the intensive care unit of a Brazilian teaching hospital. Front Microbiol. 2018;9:2997.
7. Nami S et al. Current antifungal drugs and immunotherapeutic approaches as promising strategies to treatment of fungal diseases. Biomed Pharmacother. 2019;110:857-68.
8. Naji S et al. Interspecies differences of *Candida* species causing recurrent vulvovaginal candidiasis in response to fluconazole treatment. Tehran Univ Med J. 2017;75(4):280-7.
9. Shoham S, Marr K. Invasive fungal infections in solid organ transplant recipients. Future Microbiol. 2012;7(5):639-55.
10. Zhang J et al. Development of *Candida*-specific real-time PCR assays for the detection and identification of eight medically important *Candida* species. Microbiol Insights. 2016;9:21-8.
11. Vincent J et al. Epidemiology, diagnosis and treatment of systemic Candida infection in surgical patients under intensive care. Intensive Care Med. 1998;24(3):206-16.
12. Kucukates E et al. Identification of *Candida* species and susceptibility testing with Sensititre YeastOne microdilution panel to 9 antifungal agents. Saudi Med J. 2016;37(7):750-7.
13. Guimarães T et al. Epidemiology and predictors of a poor outcome in elderly patients with candidemia. Int J Infect Dis. 2012;16(6):e442-7.
14. León C et al. What's new in the clinical and diagnostic management of invasive candidiasis in critically ill patients. Intensive Care Med. 2014;40(6):808-19.
15. Ahmed A et al. Risk prediction for invasive candidiasis. Indian J Crit Care Med. 2014;18(10):682-8.
16. Khan A et al. Fungal infections in renal transplant patients. J Clin Med Res. 2015;7(6):371-8.
17. Chakrabarti A et al. Incidence, characteristics and outcome of ICU-acquired candidemia in India. Intensive Care Med. 2015;41(2):285-95.
18. Sahin SZ et al. Invasive fungal infections in renal transplant recipients: epidemiology and risk factors. Mycopathologia. 2015;180(1-2):43-50.
19. Aboutalebian S et al. Molecular epidemiology of otomycosis in Isfahan revealed a large diversity in causative agents. J Med Microbiol. 2019;68(6):918-23.
20. Mirhendi H et al. Identification of pathogenic *Aspergillus* species by a PCR-restriction enzyme method. J Med Microbiol. 2007;56(11):1568-70.