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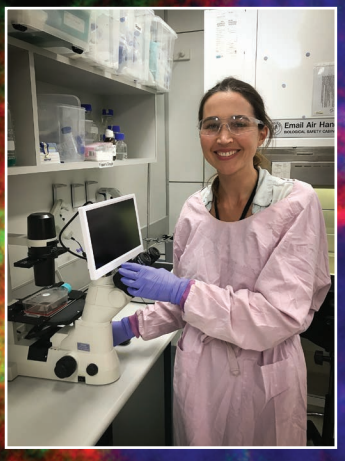
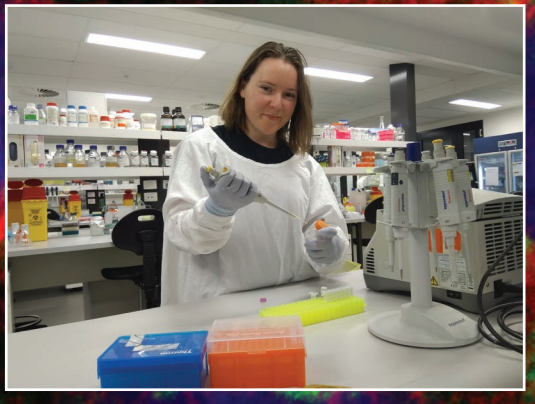
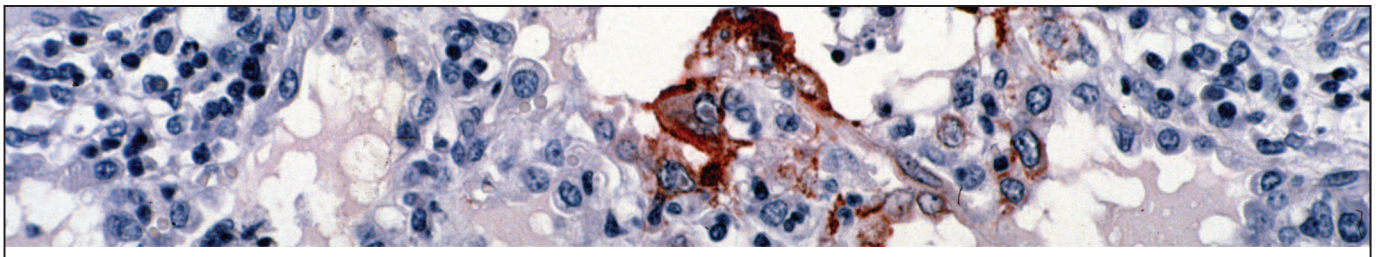







Research communications by early career scientists



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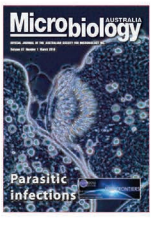

Contact:
Michelle Harris-Spencer
ASM National Office, 9/397 Smith Street, Fitzroy, Vic. 3065
Tel: 1300 656 423
Email: admin@theasm.com.au

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Contents

Cover image: Background image is confocal microscopy of IBV-infected A459 cells with calnexin staining for the endoplasmic reticulum in red, DAPI staining for nuclear DNA in blue and TGN46 staining for the Golgi apparatus in green (image provided by Marios Koutsakos). Inset images are of our seven early career scientists whose articles feature in this issue.

The Australian Society for Microbiology Inc.

9/397 Smith Street
Fitzroy, Vic. 3065
Tel: 1300 656 423
Fax: 03 9329 1777
Email: admin@theasm.com.au
www.theasm.org.au
ABN 24 065 463 274

For *Microbiology Australia* correspondence, see address below.

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Editorial correspondence
Prof. Ian Macreadie
Tel: 0402 564 308 (Ian)
Email: ian.macreadie@gmail.com

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Publishing enquiries
Jenny Foster
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Research communications of early career scientists

This issue of *Microbiology Australia* comprises research communications by early career scientists (names in bold below), all members of The Australian Society for Microbiology (ASM). These peer-reviewed articles showcase some of the exciting and diverse research going on in laboratories of our ASM members.

A number of the articles address medically important microbes and their treatment, in particular, urinary tract and respiratory infections. **Rhys White** addresses the problem of *E. coli*'s resistance to 3rd generation cephalosporins and fluoroquinolones in Australia and New Zealand. The treatment of nontypeable *Haemophilus influenzae* by haem restriction is discussed by **Brianna Atto**, David Gell and Stephen Tristram, while **Maria Koutsakos** and Stephen Kent discuss an overlooked and underestimated cause of respiratory illness, influenza B viruses.

Disease prevention is also a focus of several articles. The use of live probiotics to disrupt invasive interactions by the fungus *Candida albicans* with the human gut is discussed by **Bronwyn Smit**, Anna Kuballa, Samantha Coulson and Mohammad Katouli. Prevention of water-borne diseases through improved methods of monitoring water treatments is discussed by **Chris Owens**, Peter Cox, Paul Byleveld, Nicholas Osborne and Md. Bayzidur Rahman.

Sudip Dhakal discusses the awesome power of yeast, a model eukaryote, in providing greater understanding of Alzheimer's disease and in researching prevention strategies.

The last research article, from **Emma Harding**, Grace Yan and Peter White, analyses viral fossils in marsupial genomes, discussing how sequences encoded within them could be guardians for their hosts.

COVID-19 has meant a tough 2 years for students, researchers, front-line microbiologists and for the ASM. Ian Macreadie provides a reflective article on how it affected him personally and as a university academic, But life went on. Karena Waller provides a report on ASM's National conference, which was once again affected by lockdowns. Fortunately, a contingency plan was ready and the conference went ahead very successfully. On another positive note for microbiologists providing solutions, Cheryl Power reviews the book, *Vaxxers. The Inside Story behind the AstraZeneca Oxford Vaccine and the Race against the Virus*, while Barbara Porter reviews Peter Doherty's new book on the scientific response to the COVID-19 pandemic, *An Insider's Plague Year*.

In addition to Early Career Researchers, ASM encourages student members to undertake research assisted through Summer Student Research Awards. Priscilla Johanesen reports on our 17 ASM student members who were fortunate in being able to do summer research placements supported by 2021 Summer Student Research Awards. Abstracts of the outcomes of their research projects are presented.

Microbiology Australia continues to be freely available worldwide and is highly utilised by members and other interested parties around the globe. CSIRO Publishing has now made metrics on individual articles in *Microbiology Australia* available for viewing in real time. In the next issue readers will see some further format changes to further improve accessibility and use of articles. Also, we now offer our ASM members an opportunity to contribute stand-alone (non-themed) articles without invitation. Like other *Microbiology Australia* articles, these will be subject to rigorous peer review. Please contact the editor or editorial board members for further information.

The Editorial Board of *Microbiology Australia* comprises Ipek Kurtböke (Chair), Dena Lyras (ASM President), Rebecca LeBard (Vice President Communications), Ian Macreadie (Editor-in-Chief), Ross Barnard, Mary Barton, Linda Blackall, Prue Bramwell, Gary Lum, Sam Manna, Wieland Meyer, Chris Owens, Cheryl Power, William Rawlinson, Tom Ross, Paul Selleck, David Smith and Helen Smith. We have overseen the production of this issue, which encourages our talented early career researchers and informs others of their import works.

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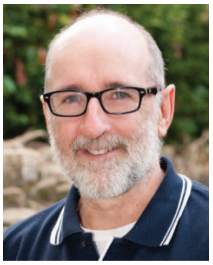

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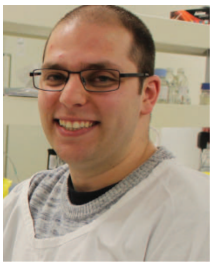
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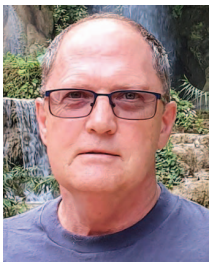
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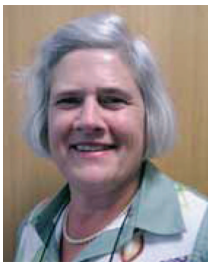
Cheryl Power


William Rawlinson


Tom Ross


Helen Smith

Living with uncertainty: pandemics and the long and short of COVID-19

To view the public lecture from the ASM 2021 meeting presented by Laureate Professor Peter Doherty go to: https://www.theasm.org.au/

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Escherichia coli: placing resistance to third-generation cephalosporins and fluoroquinolones in Australia and New Zealand into perspective

Rhys T White

School of Chemistry and Molecular Biosciences and Australian Infectious Disease Research Centre, The University of Queensland, Brisbane, Qld 4072, Australia; *and* Australian Centre for Ecogenomics, The University of Queensland, Brisbane, Qld 4072, Australia. Tel.: +61 7 3365 8549; Email: rhystwhite@gmail.com

Abstract. At least 300 million urinary tract infections (UTIs) occur annually worldwide. Uropathogenic *Escherichia coli* (UPEC) are the leading cause of UTIs. The discovery of antibiotics has revolutionised modern medicine. Yet, overusing antibiotics has accelerated the emergence of antimicrobial resistance (AMR), with UPEC driving the dissemination of AMR globally. Resistance to broad-spectrum antibiotics like third-generation cephalosporins (3GCs) and fluoroquinolones threatens public health. Extended-spectrum β-lactamase (ESBL)-producing *E. coli* precipitate resistance, particularly when these antibiotics are used as empirical therapies against UPEC. In response, the Centers for Disease Control and Prevention in the United States have listed ESBL-producing Enterobacterales, such as *E. coli* as a severe threat. Additionally, the World Health Organization have classified 3GCs and fluoroquinolones as the highest priority (critically important antimicrobials), where these therapies are only recommended following susceptibility testing. The present report demonstrates the distributions of *E. coli* cases with resistance to 3GC and fluoroquinolones in Australia and New Zealand and contextualises trends with European reports. This investigation emphasises the value of epidemiology and the justification of evidence-based interventions using data as an essential resource for reducing resistance to our 'first-line' antibiotics.

Received 25 March 2021, accepted 21 July 2021, published online 8 September 2021

The burden of antimicrobial resistance

Antimicrobial resistance (AMR) is a well established global priority due to the increasing impacts on public health. Across the United States (US), at least 2.8 million antimicrobial-resistant infections occur each year[1]. Globally, antimicrobial-resistant infections cause more than 700 000 deaths annually, with 10 million deaths expected by 2050[2]. The increasing incidence and global dissemination of AMR are: (1) limiting treatment options; (2) resulting in prolonged illnesses; (3) increasing morbidity and mortality; and (4) increasing healthcare-related costs on a global scale[3,4]. In Australia, an estimated 200 000 healthcare-associated infections occur annually, including multidrug-resistant infections[5]. The same healthcare-associated infections are estimated to generate healthcare costs between A$2–3 billion (US$1 = A$1.11[6]) and contribute to 7000 deaths annually[5].

Increasing AMR rates affect current antimicrobial therapeutic guidelines, particularly as third-generation cephalosporins (3GCs) are recommended as 'first-line' treatments to avoid prescribing 'last-line' antibiotics such as carbapenems and colistin. In response, the World Health Organization has devised a list of highest priority, critically important antibiotics, which includes 3GCs and fluoroquinolones[7]. Extended spectrum β-lactamase (ESBL)-producing organisms inactivate broad spectrum 3GC antibiotics. Additionally, the US Centers for Disease Control and Prevention (CDC) listed ESBL-producing Enterobacterales (ESBL-E) as a serious threat. In 2017, the CDC predicted that ESBL-E accounted for the deaths of 9100 individuals in the US, with 197 400 confirmed cases, and an attributable healthcare cost of US$1.2 billion[1] (US$1 = A$1.31[6]). In New Zealand, ESBL-producing *Escherichia coli* incidence rates increased from 3.7 per 100 000 in the early to mid-2000s[8,9] to 113.8 per 100 000 in the early to mid-2010s[10]. Furthermore, the Australian Commission on Safety and Quality in Health Care (ACSQHC) have reported a 55.5% increase in fluoroquinolone-resistant *E. coli* between 2015 (n = 11 094/149 916, 7.4%) and 2018 (n = 17 253/169 145, 10.2%)[11]. These data from the US, New Zealand, and Australia represents a serious concern, as resistance to fluoroquinolones may indicate resistance to one of the last available oral treatment options. This is particularly concerning for low socio-economic, disadvantaged, and under-resourced communities across regional, rural, and remote regions who rely on these antimicrobials. These communities are at a higher risk of infections

for which quinolones are indicated. For example, non-ototoxic ciprofloxacin ear drops are the mainstays of treatment for chronic suppurative otitis media, which affects 9 in 10 Aboriginal and Torres Strait Islander peoples younger than 3 years of age in remote Northern Territory (Australia) communities[12].

Antimicrobial therapy and incidence epidemiology of urinary tract infections

With increasing AMR rates impacting public health, common infections are becoming more persistent and harder to treat. These include urinary tract infections (UTIs), which are typically self-limiting and are one of the most frequently occurring bacterial infections. The Institute for Health Metrics and Evaluation estimates 391.3 million UTIs occurred worldwide in 2017, mostly reported among females (Figure 1*a*)[13]. UTI-associated healthcare costs are at least GB£4 billion[14] (US$1 = GB£0.65[6]) annually. If undiagnosed or untreated, UTIs can progress to systemic bacteraemia infections, which can trigger sepsis and septic shock. Gram-negative bacteria are a common cause of UTIs[15]. In 2018 for example, *E. coli* was the predominant (n = 2948/8797, 33.5%) cause of UTIs and bacteraemia across Australia[15]. Most of these *E. coli* cases were defined as community-onset (n = 2425/2948, 82.3%; culture collected ≤48 h after admission) rather than hospital-onset (n = 523/2948, 17.7%; >48 h after admission)[15]. Acute UTIs are treated empirically within the first 48 h of symptoms using a 3–14-day course of oral antibiotics (i.e. trimethoprim/sulfamethoxazole, cephalexin, nitrofurantoin, ciprofloxacin, or amoxicillin with clavulanate)[16]. Hospitalisation

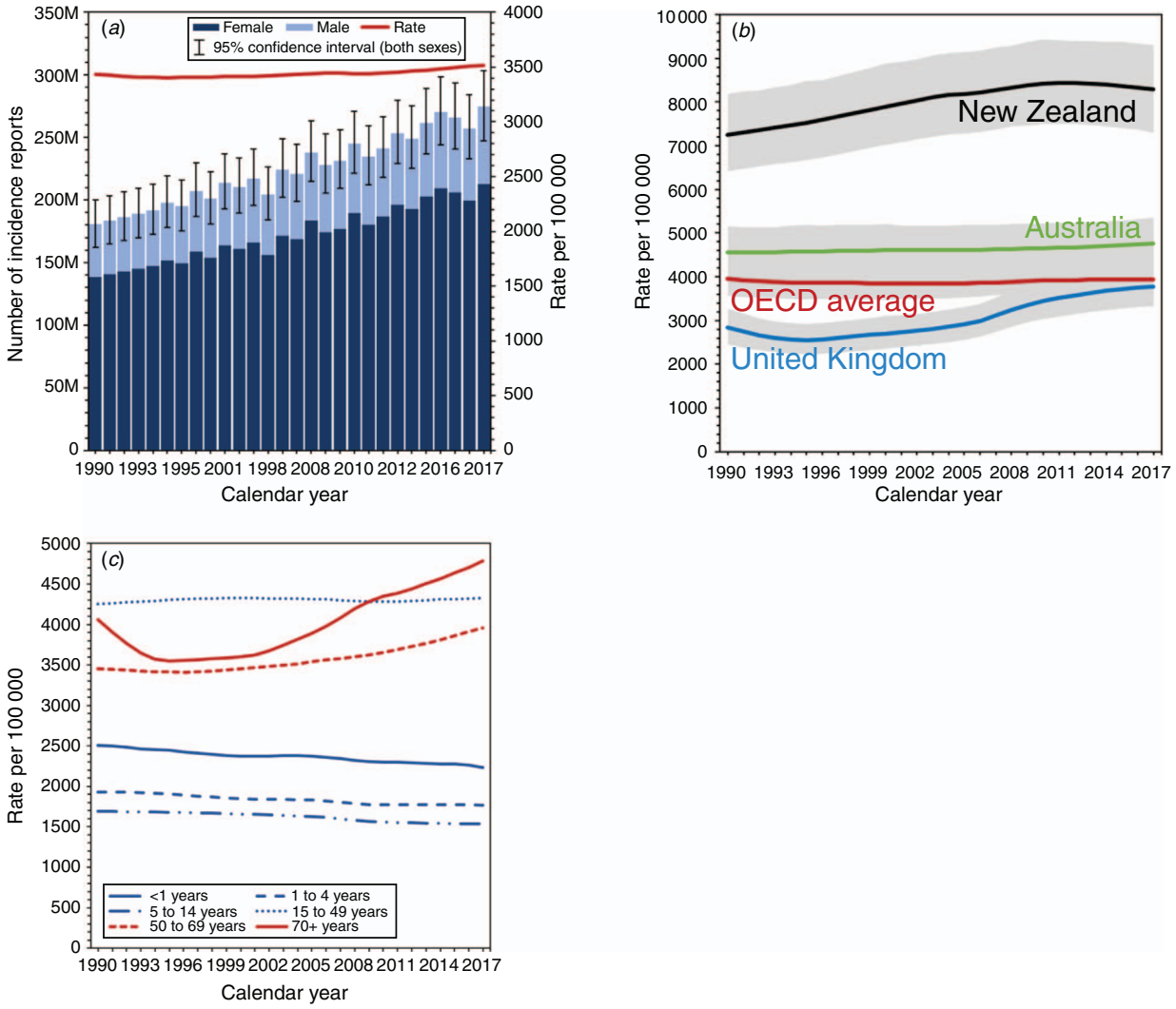

Figure 1. Global burden of urinary tract infections. (*a*) Global incidences reported, males (light blue) and females (navy) all ages, 1990 to 2017. Error bars represent the 95% confidence interval for total counts of infections reported in both sexes. The incidence rate per 100 000 is represented by the red line. (*b*) Age-standardised incidence rate in males and females from 1990 to 2017. Colour lines represents the point estimates for the incidence rate per 100 000 people for: the average from Organisation for Economic Co-operation and Development (OECD) member countries (red), Australia (green), New Zealand (black), and the United Kingdom (blue). Grey shading represents the 95% confidence intervals. (*c*) Global number of incidences by age group, males and females all ages, 1990 to 2017. Age groups are represented by corresponding line patterns with age groups 50–69 and 70+ highlighted in red (legend). Data retrieved from the Global Burden of Disease Collaborative Network[13].

for UTI can often result in the inappropriate use of broad-spectrum antimicrobials[17]. The increasing empiric use of broad-spectrum antibiotics may drive multidrug-resistant infections, particularly in recurrent cases of UTIs[18].

While changes among the annualised global age-standardised incidence rate over the past 27 years are negligible[13], increases have been observed in countries where mechanisms are in place for reporting UTIs, such as the United Kingdom, Australia, and New Zealand (Figure 1*b*). In 2017, the annualised incidence was substantially higher in New Zealand (8290 per 100 000) when compared with Australia (4759 per 100 000) and the average from 36 Organisation for Economic Co-operation and Development (OECD) member countries (3928 per 100 000). Meanwhile, incidence across all OECD member countries has remained stable, with approximately 1 case per 25 persons. Notably, the epidemiology of UTIs has changed, with increases in incidence in persons aged 50+ years (Figure 1*c*), raising concerns as the proportion of persons aged 65+ years across Australia and New Zealand has increased over the past two decades. While older persons are at higher risk of UTIs, over-diagnosis has been reported in these age groups[19]. Historical statistics in New Zealand show that one in every nine persons were aged 65+ years in 1996 compared with one in seven persons in 2017[20]. Similarly, in Australia, one in every eight persons were aged 65+ years in 1997 compared with one in six persons in 2017[21]. These groups are more susceptible to age-related decline in immune system function and other age-related health co-morbidities. This susceptibility leads to greater morbidity and poorer, undesirable health outcomes such as urosepsis and mortality in as little as 12 h from the onset of illness.

Coordinating national data on antimicrobial resistance across Australia and New Zealand

In 2014, the ACSQHC established the Antimicrobial Use and Resistance in Australia (AURA) Surveillance System to coordinate local and national data collection and analyses on AMR across Australia. AURA encompasses a national collaboration of clinicians and microbiologists known as the Australian Group on Antimicrobial Resistance (AGAR) (www.agargroup.org). AGAR is historically responsible for using standardised methodologies to undertake ongoing targeted surveillance of AMR within clinically relevant pathogens. Recently, AGAR has focused on the Gram-negative Sepsis Outcome Program, which involves collections of AMR and demographic data on isolates cultivated from patient episodes of bacteraemia. Similarly, in New Zealand, the Institute of Environmental Science and Research Ltd (ESR), contributes to the national public health surveillance of AMR among human pathogens (https://surv.esr.cri.nz/index.php). The ESR are responsible for the antimicrobial susceptibility testing and epidemiological typing of clinically relevant pathogens, including ESBL-E. The national public health surveillance into ESBL-E across New Zealand is monitored through an established network of hospitals and pathology laboratories, which conduct periodic point-prevalence surveys of isolates from throughout the country which commenced in 1996. Both the AURA and ESBL-E schemes across Australia and New Zealand, respectively, review resistance in pathogens found in blood cultures. This allows for a direct comparison with European countries that regularly release comparable data from the European Antimicrobial Resistance Surveillance Network (EARSNet) scheme[22].

Resistance to 3GC and fluoroquinolones in *E. coli* is lower in Australia and New Zealand compared with European countries

A standardised definition to measure antimicrobial usage (AU) across jurisdictions is called the Defined Daily Dose (DDD). This is essentially the total units of antibiotics that have been used, divided by a DDD correction factor that is reviewed by the World Health Organization every 3 years (https://www.whocc.no/atc_ddd_index/). With the DDD definition, comparisons between AU in Australia and Europe can be made from data collected from AURA and EARSNet. Although Australia has an overall downward trend in AU since 2016, Australia remains in the top seven countries for AU when compared with Europe[11]. For example, at least two in five hospital patients across Australia received antibiotic treatment on any given day in 2014; where 24.3% of prescriptions were noncompliant with guidelines and 23.0% were inappropriate[23]. These undesirable prescribing habits may have arisen because of the lack of timely susceptibility testing, which has been described as 'too slow to guide logical choice of antibiotic therapy in critically ill patients'[5]. While some bacterial infections are still susceptible to various antibiotics (or combinations of antibiotics), this extensive use has to some degree contributed to the acquisition of AMR in most bacterial pathogens, whether multidrug-resistant or not[24,25]. Most European countries observed an increase in the rates of resistance to fluoroquinolones and 3GC in *E. coli* between 2016 and 2018. In comparison, the rates across Australia and New Zealand remain relatively low (Figure 2). The prevalence of fluoroquinolone resistance in *E. coli* across Australia increased between the start of 2016 (14.0%) and end of 2017 (14.4%). In contrast, resistance to 3GCs across Australia decreased between the same period of 2016 (11.8%) and 2017 (11.5%). Conversely, fluoroquinolone-resistant *E. coli* across New Zealand increased 6-fold over the same period (2.0% in 2016 compared with 12.0% in 2017). Additionally, in New

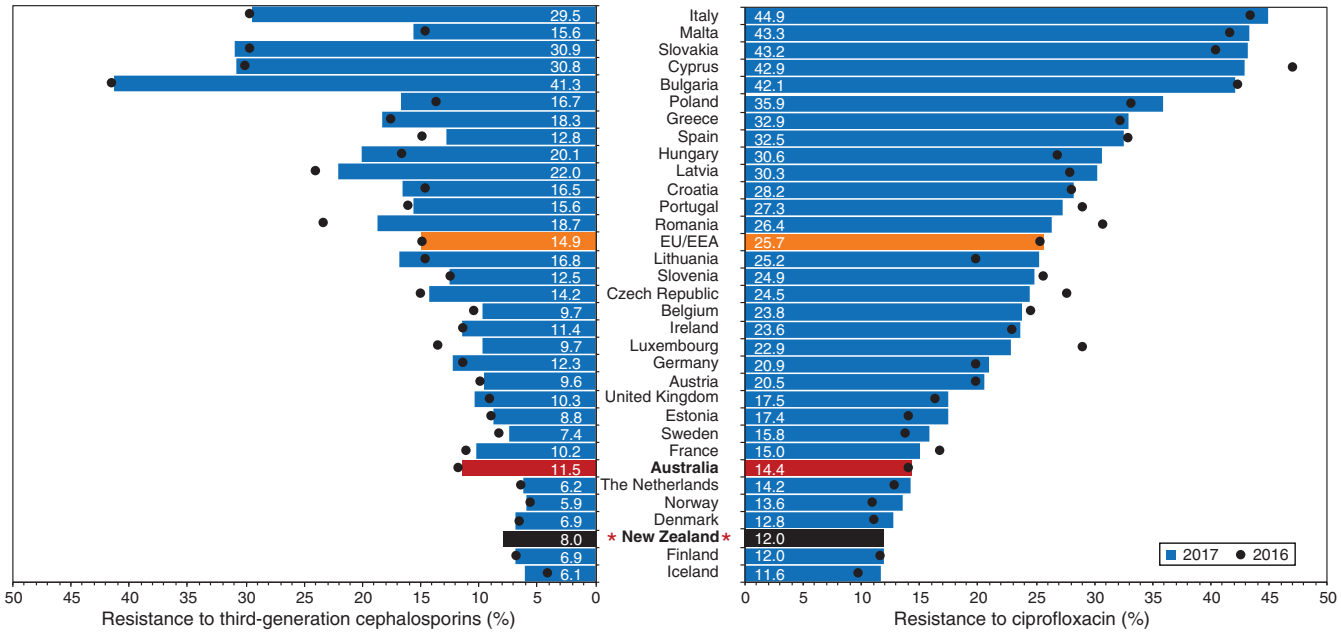

Figure 2. Prevalence of *Escherichia coli* resistant to third-generation cephalosporins (left) and ciprofloxacin (right) in Australia, New Zealand, and European countries, 2016 and 2017. European Union (EU) and European Economic Area (EEA) countries' population-weighted mean percentages are highlighted in orange, Australia in red, and New Zealand in black. For New Zealand, data represents isolates from 2015 only. Adapted from 'AURA 2019: third Australian report on antimicrobial use and resistance in human health' by the Australian Commission on Safety and Quality in Health Care (ACSQHC). Sydney, Australia: ACSQHC (2019).

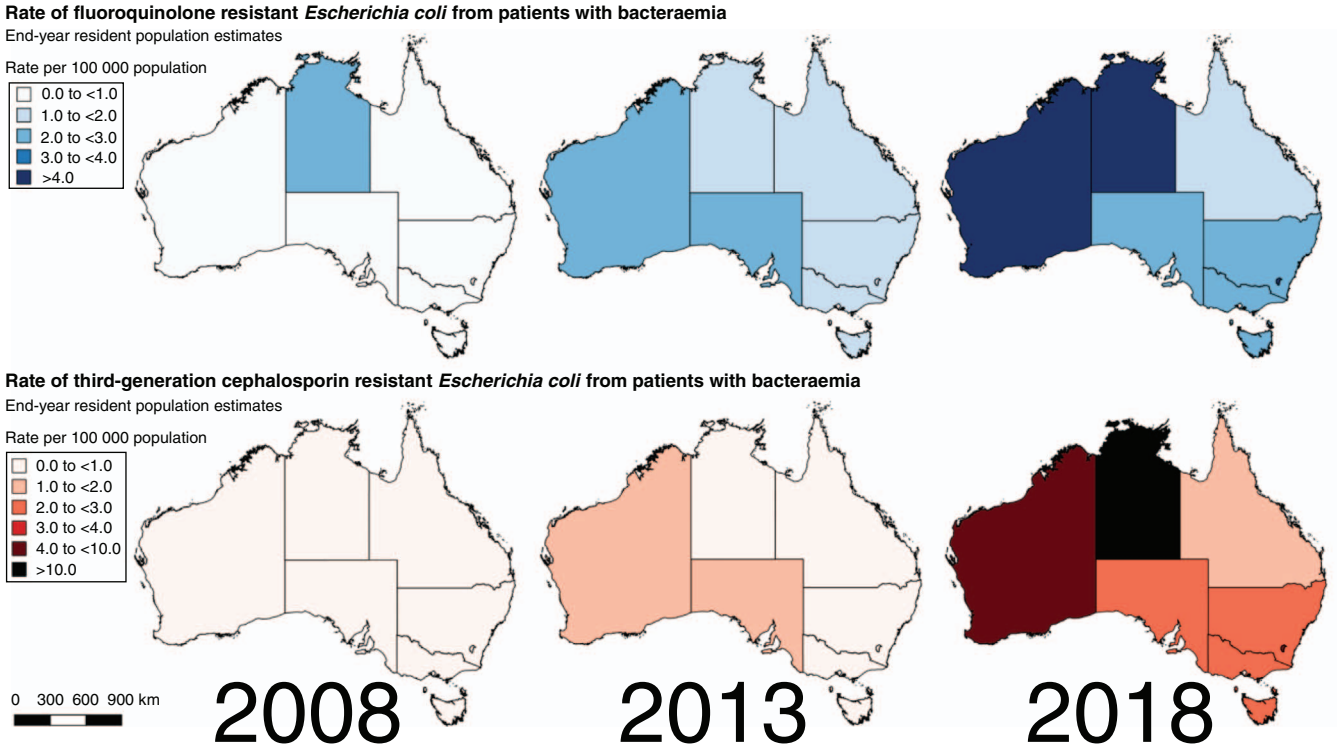

Figure 3. Annualised incident rates of resistance to fluoroquinolones (blue) and third-generation cephalosporins (red) amongst *Escherichia coli* from bacteraemia cases across Australia, 2008 to 2018. Incidence is expressed as rate per 100 000 based on the estimated resident population, States and Territories, from the Australian Bureau of statistics (Copyright © 2018 by the Commonwealth of Australia). Adapted from the 'Gram-negative Survey 2008 Antimicrobial Susceptibility Report' by the Australian Group on Antimicrobial Resistance (AGAR). Canberra, Australia: AGAR (2011); 'Gram-negative Survey 2013 Antimicrobial Susceptibility Report' by AGAR. Canberra, Australia: AGAR (2014); and 'Gram-negative Sepsis Outcome Programs 2018 report' by AGAR. Canberra, Australia: AGAR (2019).

Zealand there was a 4-fold increase in the incidence of 3GCs resistant infections (2.0% in 2016 compared with 8.0% in 2017). Nevertheless, the prevalence of resistance to fluoroquinolones and 3GCs across both Australia and New Zealand remained below the population-weighted averages (25.7% and 14.9%, respectively) of the European Union and European Economic Area countries' (Figure 2).

Comparisons of AMR in *E. coli* across Australia and New Zealand

ACSQHC uses reports like the AURA 2019 to survey the volume of AU within hospitals, the community, and aged care homes[11]. Notably, the total AU rate has increased from 22 DDDs per 1000 population in 2000 to 24 in 2009[26]. However, generally AU rates in Australia have been on a downward trend since 2010[11]. While AU is overall declining, *E. coli* with resistance to critical antibiotics like ciprofloxacin (an oral fluoroquinolone) and ceftriaxone (an injectable 3GC) are increasing (Figure 3). In contrast, metrics collected between 2016 and 2018 demonstrate a decline in the number of community dispensing rates under the government-subsidised medications Pharmaceutical Benefits Scheme. While 41.5% (n = 10 215 109) of the Australian population received at least one prescription for antimicrobials in 2017, the age-standardised rates of antimicrobial prescriptions dispensed per 1000 inhabitants decreased by 4.7% from 1120 in 2016 to 1067 in 2017[11]. Similarly, there was a decline in the number of residents in Australian aged care homes who were prescribed at least one antimicrobial between 2016 and 2018. While 8.8% (n = 1087/12 307) of residents in aged care homes received at least one prescription for antimicrobials in 2017, the rates of antimicrobial prescriptions dispensed per 1000 aged care home residents decreased from 98.6 in 2016 to 88.3 in 2017[11].

In New Zealand, surveillance conducted by the ESR reveals that the total-hospital AU rate has increased from 17 in DDDs per 1000 population per day in 2006 to 26 in 2012, before stabilising between 2012 and 2015[27]. This is also reflected by the longitudinal trend of increasing ESBL-producing clinical *E. coli* between 2006 (n = 56/87, 64.4%)[28] and 2016 (n = 386/521, 74.0%)[29]. The annualised rate of ESBL-producing *E. coli* circulating New Zealand is has also increased nation-wide, particularly between 2008 and 2013 (Figure 4). However, the data show reductions in major metropolitan areas in contrast to the increase observed in rural regions.

Conclusion

Since the discovery of penicillin, antibiotic treatments have revolutionised modern medicine and will forever impact global public health. Today, antimicrobials are extensively used against bacterial infections worldwide. The rapid emergence of resistance to 'first-line' and readily accessible antimicrobials like fluoroquinolones and third-generation cephalosporins requires urgent action. It is essential to place the prevalence of antimicrobial resistance into perspective and identify key indicators of their burden. From a clinical perspective, the appropriateness of antimicrobial use must be improved by supporting 'antibiotic stewardship' through initiatives at the local, national, and international level. This will reduce undesirable prescribing habits. While resistance to fluoroquinolones and third-generation cephalosporins is lower in Australia and New Zealand compared with European countries, *E. coli* with resistance ciprofloxacin (an oral fluoroquinolone) and ceftriaxone (an injectable

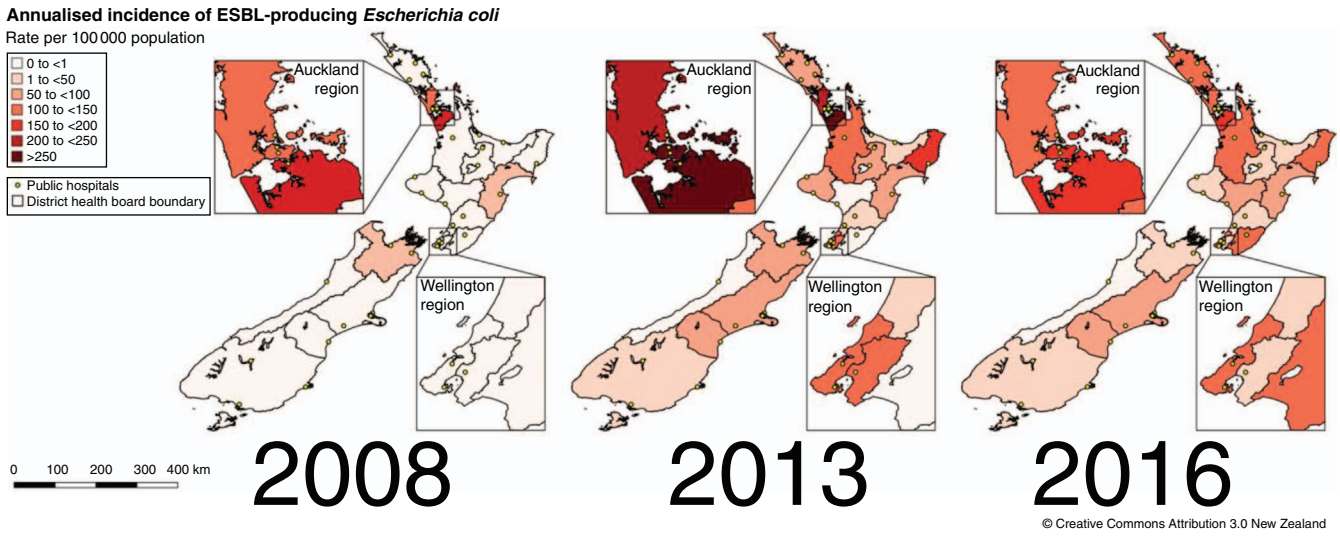

Figure 4. Extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* annualised incidence rates by district health board, New Zealand, 2008 to 2016. Incidence is expressed as rate per 100 000 based on the estimated resident population, District Health Boards, from Statistics New Zealand's data which are licensed for re-use under the Creative Commons Attribution 4.0 International licence. Incidence rates for (i) Capital & Coast and Hutt; and (ii) Canterbury and South Canterbury District Health Boards are combined. Adapted from the 'Annual survey of extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae, 2008' by the Institute of Environmental Science and Research Ltd. (ESR). Wellington, New Zealand: ESR (2009); 'Annual survey of extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae, 2013' by the ESR. Wellington, New Zealand: ESR (2014); and '2016 survey of extended-spectrum β-lactamase-producing Enterobacteriaceae' by the ESR. Wellington, New Zealand: ESR (2018).

third-generation cephalosporin) are increasing. It is important to maintain the decline in antimicrobial usage rates across hospitals, the community, and aged care homes as reported in Australia. Here, I have emphasised current trends in antimicrobial resistance and shown that work is still required to reduce the incidence of resistance to fluoroquinolones and third-generation cephalosporins.

Conflicts of interest

The author declares no conflicts of interest.

Declaration of funding

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Biography

Rhys White is working towards completing his PhD in Genomics and Bioinformatics at The University of Queensland, Australia. Rhys graduated with a BSc (Hons) from Cardiff University (United Kingdom) in 2016. Before commencing his PhD, Rhys started his career with Public Health Wales where he refined his analytical skills alongside clinicians. Rhys' research interests are in microbial genomics where he uses comparative genomic approaches to better understand the evolution, emergence, and dissemination of global Enterobacterales.

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Influenza B viruses: underestimated and overlooked

Marios Koutsakos[A,D] *and Stephen J Kent*[A,B,C]

[A]Department of Microbiology and Immunology, University of Melbourne, at the Peter Doherty Institute for Infection and Immunity, Melbourne, Vic. 3000, Australia
[B]Melbourne Sexual Health Centre and Department of Infectious Diseases, Alfred Hospital and Central Clinical School, Monash University, Melbourne, Vic. 3004, Australia
[C]ARC Centre for Excellence in Convergent Bio-Nano Science and Technology, University of Melbourne, Parkville, Vic. 3010, Australia
[D]Tel.: +61 3 9035 4179; Email: marios.koutsakos@unimelb.edu.au

Abstract. Influenza B viruses circulate globally every year causing respiratory disease with significant clinical and socio-economic impacts. IBV are considered exclusive human pathogens with no established animal reservoirs, which suggests with concerted effort it may be possible to eradicate this virus from human circulation. However, this requires a deeper understanding of IBV virology and immunology and the design of vaccines that induce universal immunity to antigenic variants of IBV.

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Introduction

Influenza A and B viruses (IAV and IBV) circulate annually causing seasonal epidemics around the world. Influenza viruses are single-stranded negative sense RNA viruses with segmented genomes belonging to the family of *Orthomyxoviridae*[1]. They replicate is the respiratory tract and cause influenza disease which can vary from asymptomatic and mild upper respiratory tract disease to severe lower respiratory tract disease and in some cases fatal disease[1]. Although IAV exists in a wide range of animal hosts, IBV does not have an established animal reservoir[2]. The potential of antigenically novel IAV viruses to 'jump' from animals into humans and cause severe disease, and is some instances global pandemics, has placed IAV in the spotlight. The lack of an established animal reservoir, and therefore lack of pandemic potential, for IBV has left this type of influenza virus considerably underestimated and overlooked. However, IBV has substantial health and socio-economic impacts annually. Additionally, the lack of an animal reservoir means that it may be possible to eradicate this virus from human circulation with highly effective, broadly protective vaccines and broad population coverage. To achieve that, a thorough understanding of IBV virology and immunology is needed.

The underestimated impact of IBV infections

Seasonal epidemics caused by IAV and IBV result in 3–5 million cases of severe disease and 290 000–650 000 deaths annually[1]. IBV accounts for on average 23% of the annual influenza burden[3] but can comprise up to ~80% of infections in some countries in selected years[4]. It is estimated that IBV infections result in 7.9 million lower respiratory tract infections and 1.4 million hospitalisations annually[5]. Although the clinical severity of IBV was initially thought to be lower than that of IAV, recent studies have contested this notion, with hospitalisation and mortality rates in adults being similar for IAV and IBV[6,7]. Importantly, IBV incidence is higher in children, in which IBV can cause severe systemic complications and frequent hospitalisation and death, with up to 52% of influenza-related paediatric deaths being attributed to IBV[8–10]. Additionally, in children under the age of 16 years, IBV can have higher mortality rates than IAV and a significant rate of ICU admission[11]. Fatal infections of IBV in children are associated with secondary bacterial pneumonia as well as cardiac injury[12]. Lastly, IBV infections account on average for 37% of influenza-associated healthcare costs, with projected costs in the US of US$0.96–2.6 billion annually[13]. Overall, IBV has significant clinical and socioeconomic impacts. This impact could be minimised with highly effective vaccines and intervention strategies.

Known and unknowns of the IBV life cycle

IBV replicates in epithelial cells of the respiratory tract. The virus uses its surface glycoprotein haemagglutinin (HA) for attachment to sialic acid receptors on the cell surface and subsequent membrane fusion in endosomes. This results in the release of eight viral ribonucleoprotein (vRNP) complexes, which replicate in the nucleus of the cell. Viral RNA replication combined with protein expression are followed by assembly and budding of newly formed virions from the cell surface. The viral surface glycoprotein neuraminidase (NA) releases virions from attached sialic acid receptors on the cell surface[14]. During IBV infection, the viral non-structural 1 (NS1) protein of IBV has a critical role in counteracting immune recognition by innate receptors such as RIG-I as well as interferon-stimulated genes such as protein kinase R (PKR) and ISG-15, which are potent inhibitors of IBV[14,15]. Interestingly, the NS gene of IBV exhibits the highest rate of selection pressure among the genes of IBV[16]. Given its critical role in counteracting innate immune responses, understanding the evolution of the IBV NS1 protein in humans would be of great interest.

Although the life cycle of IAV and IBV is in many ways similar, it is pertinent to note that the two types of influenza viruses encode different sets of accessory proteins (Figure 1). Specifically, IBV lacks expression of immunomodulatory virulence factors PB1-F2 and PA-X found in IAV. Conversely, IBV encodes a unique open reading frame (ORF) called NB, that overlaps with the NA ORF[15]. NB is a small transmembrane protein that is heavily glycosylated and is incorporated in the IBV virion. Despite the high conservation of NB in IBV, NB expression is dispensable for virus viability and replication *in vitro*[17,18] and its role in viral replication is unclear[18]. Dissecting the role and

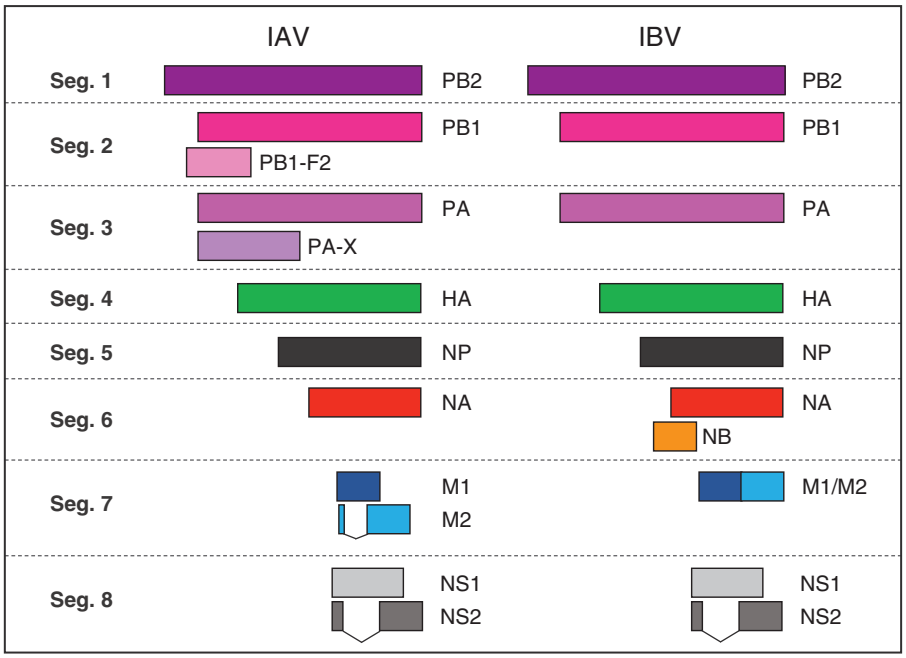

Figure 1. Comparison of genomes of IAV and IBV. Each segment is depicted with the encoded open reading frames for IAV and IBV. The 8 gene segments and known open reading frames (ORFs) of IAV and IBV are shown. Segment 2 and 3 of IAV encode overlapping ORFs PB1-F2 and PA-X respectively, which are not found in IBV. Conversely, IBV encodes an overlapping ORF on segment 6 (NB), not found in IAV. Segment 7 for both viruses encodes two ORFs, which in IAV are expressed by alternative splicing, while in IBV an alternative stop-start codon mechanism is utilised.

function of NB in the life cycle of IBV would assist the understanding of the IBV life cycle.

Host species tropism of IBV

Another important difference between IAV and IBV is host species tropism. Although IAV can be found in many animal species, IBV is considered exclusively a human pathogen. However, it is important to note that natural infections of animals with IBV have been reported for a variety of species (Table 1) and some have been recapitulated experimentally. However, most of these infections have occurred in animals in proximity with humans (domestic and farm animals or animals in zoos/research centres) and likely represent isolated reverse zoonosis events. A notable exception is the

Table 1. Infection of animals with IBV.

Animal[A]	Location, year	Detection methods	Frequency of animals positive (%)	Comments	Reference
Natural infections					
Harbor seals *(Phoca viulina)* and gray seals *(Halichoreus grypus)*	The Netherlands, 1995–1999	HAI and ELISA to HA, NA and NP	8/391 (2)	– 580 samples prior to 1995 were seronegative – 1 RT-PCR$^+$ throat swab in 1999 – B/Seal/The Netherlands/1/99 virus isolated	19
Harbor seals *(Phoca viulina)* and gray seals *(Halichoreus grypus)*	The Netherlands, 2002–2012	HAI	10/625 (1.6)	– Seropositive samples only detected in 2010 (9/21) and 2011 (1/150)	20
Caspian seals *(Phoca capsica)*	Caspian Sea, 1997–2000	ELISA with whole virus	5/77 (6)	3 of seropositive animals in 2000 were <1 year old, suggestive of recent introduction of IBV	21
South American fur seals *(Arctocephalus australis)*	Uruguay, Sep. 2004	HAI	25/37 (67.6)	– Seropositive cut-off set at HAI >80 for 1993 strain – Lower seropositivity rates for 1999 and 2001 strains	22
Horse	Japan, 1977	HAI	16/504 (3.2)	– Seronegative animals in the study: cattle (n = 812), dogs (n = 158), cats (n = 52), mink (n = 62), rats (n = 33), chickens (n = 389), ducks (n = 10), pigeons (n = 250), wild birds (n = 55)	23
Swine	Japan, 1968–1977	HAI	1/1030 (0.1)		
Pigs	USA, 2010–2012	HAI, verified by NT	41/560 (7.3)	– 3 RT-PCR$^+$ nasal swabs – Limited region of virus sequenced	24
Pigs	Great Britain, Oct. 1991–Feb. 1992	HAI, verified by NT and immunoblot	8/2000 (0.4)	Seropositive samples spread across England and Wales	25
Chimpanzees	The Netherlands, 1986, 1992, 1998, 2000	Magnetic bead-based assay, verified by immunoblot	80/305 (26.2)	Housed in biomedical research Centre	26
Gorillas	Not specified, reported in 2014		45/77 (58.4)	Zoo animals	
Orangutans	Indonesia, 1994–1998		135/179 (75.4)	House in animal rehabilitation center	
Dogs	Taiwan, June–July 1971	Virus isolation from nasal swabs	1/372 (0.3)	No virus isolated from cats (n = 28)	27
Dogs	Japan, Jan. 2009–Feb. 2010	NT, verified by immunoblot	6/366 (1.6)	Samples from indoor domestic dogs, no illness reported	28
Horses	Canada, 1960–1963	Complement fixation assay	Numbers not reported (30)	Animals from farms	29
Guinea pigs	Ecuador	ELISA with whole virus, recombinant HA and NP, verified by immunoblot	28/40 (70)	Animals raised as livestock	30
Birds	Not specified, reported in 1980	Not specified	Numbers not reported (4.1)	Full text study not available	31
Ruminants	Not specified, reported in 1984	Not specified	Not specified	Full text study not available	32
Animal	**Inoculation**	**Disease**	**Transmission**	**Comments**	**Reference**
Experimental infections[B]					
Pigs	Intranasal and intratracheal	ILI and lung lesions	Yes (contact)	– Limited transmission	24
Guinea pigs	Intranasal	Histopathological changes in nasal tissue	Yes (contact and aerosol/ droplet)	– Replication in upper respiratory tract – High efficiency of transmission	33
Cynomolgus macaques *(Macaca fascicularis)*	Intranasal and intratracheal	– Fever, loss of appetite/ weight loss – No sneezing or coughing – Lung lesions	Not assessed	– Replication in upper and lower respiratory tract – Inflammatory cytokine detected in upper respiratory tract	34

[A]Species indicated where available. [B]Other than mice and ferrets. HAI, hemagglutination inhibition assay; ELISA, enzyme-linked immunosorbent assay; HA, hemagglutinin; NA, neuraminidase; NP, nucleoprotein; NT, neutralisation assay.

presence of IBV in seals that has been detected across species of seals and geographical sites between 1995 and 2012[19–22]. The single virus isolated from a seal in 1999 had high homology to a human IBV isolate. However, it is not known whether the presence of IBV in seals represents a single introduction from humans and subsequent spread amongst seals between 1995 and 2012 or multiple distinct reverse zoonosis events in that period. Overall, while a variety of mammals are susceptible to natural IBV infection, there is no evidence of established animal reservoirs in any species.

Understanding the factors that contribute to the exclusivity of IBV in humans is of great importance. The host species restriction of avian IAV in birds, and the requirement for significant adaption for efficient replication and transmission in humans, occurs at many stages of the life cycle, including HA mediated attachment and entry as well the activity of the influenza virus replication machinery[35]. Interestingly, it was recently reported that the IBV HA exhibits optimal activity in the pH and temperature conditions of the human upper respiratory tract, more so than IAV strains tested in that study, indicating significant host adaptation in the human host environment[36]. Additionally, IBV can interact with mammalian (human and murine) but not avian homologues of host proteins required to support viral replication[37,38], providing a potential mechanistic basis for the lack of IBV in avian species. The IBV NS1 protein can counteract the effects of the antiviral protein ISG-15 in a species-specific manner, by interacting with human and non-human primate ISG-15 but not with canine or murine homologues[39]. Overall, these studies demonstrate considerable adaptation of IBV to mammalian and often specifically human hosts, which may restrict the ability of IBV to efficiently replicate in other species. It is important to note the recent discovery of IBV-like viruses in lower vertebrates[40]. These viruses show similar genome architecture to human IBVs[40] and encode functional homologues of HA and NA but are not recognised by human serum samples[41]. Understanding the virology and host-restriction of these viruses could provide novel insights into IBV evolution and host species tropism.

Antigenic diversity and immune responses to IBV

Two antigenically and genetically distinct lineages of IBV co-circulate globally. These lineages, named B/Yamagata/16/1988-like (or B/Yamagata) and B/Victoria/2/1987-like (or B/Victoria), are estimated to have diverged in the 1970s[42]. While B/Victoria viruses were dominant in the late 1980s in most countries, B/Yamagata viruses dominated in the 1990s, during which B/Victoria viruses were virtually absent globally, except for a 1996/1997 outbreak in Asia[42]. B/Victoria viruses re-emerged in 2001 and the two lineages have co-circulated since[42,43].

Both IBV lineages undergo gradual antigenic drift by accumulating escape mutations in the head domain of the HA protein – the major antigenic target of protective antibodies[14]. Mutations are primarily focused on sites surrounding the receptor binding site of the HA and overlap with sites of antibody recognition. Interestingly, since 2015 the B/Yamagata HA has not acquired any mutations in those sites. Instead, it has acquired 7 mutations on the NA protein[16], although the effects of these mutations in antigenic evolution and immune escape are unclear. In contrast, since 2015 the B/Victoria viruses have undergone significant diversification of their HA gene, including the recurrent but independent emergence of viruses with 2–3 amino acid deletions in one of the antigenic sites[16]. These deletions significantly alter the antigenicity of those domains and have necessitated the inclusion of these strains in the influenza vaccine[44]. Intriguingly, similar amino acid deletions have been previously detected in IBV strain from 1940–1988[16], an observation that warrants further investigation as it indicates this might be a common escape mechanism of IBV.

Although such mutations can escape antibody recognition, conserved domains of the HA protein can be recognised by broadly cross-reactive antibodies[45,46]. These can target highly conserved sites of the HA head as well as the HA stem domain and cross-react with both IBV lineages[46]. Cross-recognition of the two lineages can also occur by cytotoxic T cells, which can recognise and kill virally infected cells, providing an additional level of immune protection[47]. The repeated isolation of multiple broadly cross-reactive antibodies in different studies indicates that such antibody responses may not be uncommon, although their prevalence and abundance in serum samples is unknown. Nonetheless, their discovery indicates that universal immunity across both lineages of IBV is feasible. Antibodies to the IBV NA also show broad cross-reactivity across both lineages and can mediate protection from challenge[48]. Understanding how such broadly cross-reactive immune responses to HA and NA are generated through infections and vaccination during the human lifespan will assist in the design of broadly cross-protective vaccines.

Vaccination strategies against IBV

Influenza vaccines primarily comprise unadjuvanted inactivated split virions or recombinant proteins that induce antibodies towards the HA and vaccine composition needs to be updated annually to accommodate for the emergence of escape mutants. A live attenuated influenza vaccine (LAIV) is also approved in some countries.

Traditionally, a trivalent influenza vaccine (TIV) has been used that includes two IAV strains along with one IBV strain from the lineage predicted to dominate the upcoming influenza season. However, due to the frequent mismatch of the predicted and the circulating IBV lineage[8], in 2012 the WHO recommended where possible the use of a quadrivalent vaccine (QIV) that includes one IBV strain from each lineage. Despite this, the average vaccine effectiveness for IBV is only 54%[49] and the advantages of the QIV formulation remain contested[50]. An alternative to annual administration of a strain-specific vaccine would be the design of a universal vaccine that induces broadly cross-reactive immunity and does not require annual reformulation. This can be achieved by rationally designing vaccines that focus the immune response to highly conserved sites of the IBV HA and NA proteins, although such vaccines are only in pre-clinical development. Overall, despite the introduction of QIV, current vaccination strategies against IBV only provide modest and partial protection and further research is needed to improve vaccine effectiveness. The development of more effective IBV vaccines will assist efforts to eliminate IBV from human circulation.

Future directions

Despite the consistent seasonal circulation globally and the significant health and socio-economic impacts of IBV, initial misconceptions of relatively lower clinical severity have left IBV underestimated and overlooked. As a result, there is only limited focus on the control of IBV infections. Significant advances in the last decade have demonstrated the potential for universal immunity across both lineages of IBV. The lack of animal reservoir and subsequently pandemic potential, once a reason for neglecting IBV, is now considered its Achilles' heel and could allow for the high-level suppression or even elimination of this virus. However, this can only be achieved by global concerted efforts to understand the antigenic evolution of IBV, the generation of broadly cross-reactive immunity and the rational design of universal vaccines.

Conflicts of interest

The authors declare no conflicts of interest.

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Biographies

Dr Marios Koutsakos is an NHMRC Emerging Leadership Investigator and Research Fellow in Professor Kent's group in the Department of Microbiology and Immunology, The University of Melbourne at the Peter Doherty Institute for Infection and Immunity. His research focuses on understanding immune responses to IBV and the rational design of universal vaccines as well as IBV–host interactions.

Professor Stephen Kent is an infectious diseases physician and viral immunologist in the Department of Microbiology and Immunology, The University of Melbourne at the Peter Doherty Institute for Infection and Immunity. He heads a lab studying immunity to HIV, influenza, COVID-19 and the development of vaccines against such pathogens, from preclinical development to human clinical trials. Stephen remains active in infectious diseases clinical medicine at the Alfred Hospital and Melbourne Sexual Health Centre.

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Exploiting the struggle for haem: a novel therapeutic approach against *Haemophilus influenzae*

Brianna Atto[A,C], *David Gell*[B] *and Stephen Tristram*[A]

[A]School of Health Sciences, University of Tasmania, Newnham Drive, Launceston, Tas. 7248, Australia
[B]School of Medicine, University of Tasmania, 17 Liverpool Street, Hobart, Tas. 7000, Australia
[C]Email: brianna.atto@utas.edu.au

Abstract. Over the past decade, nontypeable *Haemophilus influenzae* (NTHi) has gained recognition as a major opportunistic pathogen of the respiratory tract that imposes a substantial global burden of disease, owing to a high rate of morbidity and ensuing complications. Further amplifying the global impact of NTHi infections is the increasing spectrum and prevalence of antibiotic resistance, leading to higher rates of treatment failure with first- and second-line antibiotics regimes. The threat of antibiotic resistance was recognised by the World Health Organization in 2017, listing NTHi as a priority pathogen for which new therapies are urgently needed. Despite significant efforts, there are currently no effective vaccine strategies available that can slow the growing burden of NTHi disease. Consequently, alternative preventative or therapeutic approaches that do not rely on antibiotic susceptibility or stable vaccine targets are becoming more attractive. The nutritional dependency for haem at all stages of NTHi pathogenesis exposes a vulnerability that may be exploited for the development of such therapies. This article will discuss the therapeutic potential of strategies that limit NTHi access to this vital nutrient, with particular focus on a novel bacteriotherapeutic approach under development.

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NTHi is a major respiratory pathogen for which new therapies are needed

Nontypeable *Haemophilus influenzae* (NTHi) is a common coloniser of the upper respiratory tract in healthy children (20–80%) and adults (20–30%), the prevalence of which varies considerably across geographical regions[1–4]. However, in susceptible individuals, NTHi represents a major cause of opportunistic infections in the respiratory tract, namely acute otitis media and sinusitis in children, and lower respiratory tract infections in elderly individuals or those with chronic obstructive pulmonary disease[5]. Collectively, these infections and subsequent long-term health complications, such as hearing loss or decline in lung function, impart a significant global disease burden[5,6]. Further amplifying the global impact of NTHi infections is the rapidly expanding spectrum and prevalence of antibiotic resistance, leading to treatment failure with first- and second-line antibiotics[5,7]. The high morbidity and long-term antibiotic prescription associated with NTHi infections, collectively expose a substantial proportion of the population to antimicrobial agents, driving resistance to a broad-spectrum of antibiotics in the community[8,9]. The threat of antibiotic resistance was recognised by the World Health Organization in 2017, listing NTHi as a priority pathogen for which new therapies are urgently needed[10]. Owing to the high genetic heterogeneity and phase-variable expression of conserved antigen targets, there are currently no effective vaccine strategies available that can slow the growing burden of NTHi disease[11]. Consequently, novel preventative or therapeutic approaches that do not rely on antibiotic susceptibility or stable vaccine targets are becoming more attractive.

Haem-iron acquisition is a major determinant of NTHi pathogenesis

The pathogenesis of NTHi is largely dictated by interactions with host airway epithelia. Although the exact mechanisms are poorly understood, NTHi adhesion and colonisation of the host pharyngeal epithelium, followed by migration to privileged anatomical sites, is required to elicit an infection[12]. Survival and persistence at the site of infection is mediated by host-cell internalisation, formation of biofilms, or modulation of the immune response that protects bacterial populations from immune or antibiotic clearance[13–15]. In addition to being an essential growth requirement, access to iron-containing haem plays an important role in the ability of NTHi to perform these interactions and as such, the ability to sequester host-derived sources of haem is a key determinant of pathogenesis[16,17]. The consequence of NTHi haem starvation, either by disruption of acquisition mechanisms or by environmental restriction, has been demonstrated to attenuate virulence in animal models of invasive disease and otitis media[18–21]. Strategies that interrupt NTHi acquisition or utilisation of host-derived sources of haem may therefore have a significant impact on the ability of NTHi to cause disease.

A new therapeutic approach: exploitive competition for haem-iron

Recently, we discovered strains of the closely related commensal *Haemophilus haemolyticus* (Hh) that also inhabit the pharyngeal niche and secrete a novel haemophore (since named haemophilin; Hpl) that elicits potent inhibitory activity against NTHi[22,23].

Functional and proteomic investigation demonstrated that Hpl is a previously unrecognised haem uptake mechanism of Hh, which inhibits NTHi growth through exploitative competition for haem. We have since conducted several investigations *in vitro* and *in vivo* to test the NTHi-inhibitory capacity of Hpl-producing strains of Hh (Hh-Hpl$^+$) and propose their therapeutic utility as a respiratory probiotic.

In vitro *investigations*

In a broth co-culture system, NTHi strains were outcompeted by Hh-Hpl$^+$ and suffered a complete loss of fitness over subsequent generations[24]. Similarly, in tissue culture models of nasopharyngeal (D562) and lung epithelia (A549), Hh strains with high levels of *hpl* expression protected cell monolayers against adhesion and invasion by NTHi[25] (Figure 1). Significant inhibition of NTHi adherence and invasion was maintained when Hh-Hpl$^+$ treatment doses were 10–100-fold lower than the NTHi challenge. In both *in vitro* models, NTHi-inhibitory activity correlated with levels of *hpl* expression and Hpl protein quantified from competition media. The absence of NTHi-inhibitory activity in a *hpl* knockout or native non-producing strains confirmed that the inhibitory phenotype was mediated by the ability to produce Hpl.

In vivo *investigations*

Considering the NTHi-inhibitory activity *in vitro* we hypothesised that natural pharyngeal carriage of Hh strains with the *hpl* open reading frame would be associated with a lower prevalence and/or density of NTHi colonisation in healthy individuals. Real-time PCR was used to quantitatively compare the oropharyngeal carriage load of NTHi and Hh populations with the Hh-*hpl*$^+$ or Hh-*hpl*$^-$ genotype from 257 healthy adults in Australia. Compared to carriage of Hh-*hpl*$^-$ strains, adult (18–65 years) and elderly (>65 years) participants that were colonised with Hh-*hpl*$^+$ were 2.43 (95% CI, 1.95–2.61; $P < 0.0001$), or 2.67 times (95% CI, 2.63–2.70; $P = 0.0036$) less likely to carry NTHi, respectively. Colonisation with high densities of Hh-*hpl*$^+$ correlated with low NTHi carriage load and a 2.63-times (95% CI, 2.56–2.70, $P = 0.0112$) lower likelihood of acquiring/maintaining NTHi colonisation status between visits[26] (Figure 2).

Potential translation as a respiratory probiotic to prevent NTHi infections

The presence of healthy carriers of NTHi indicates that a complete eradication of NTHi is not necessary to prevent infection. Furthermore, higher NTHi pharyngeal carriage loads are correlated with an increased susceptibility to otitis media *in vivo*[27–30] and an increased severity of airway inflammation, exacerbations, and daily symptoms in chronic obstructive pulmonary disease[31,32]. Thus, even small reductions in NTHi carriage might have beneficial clinical outcomes. Using a model designed to predict the risk of otitis media in children

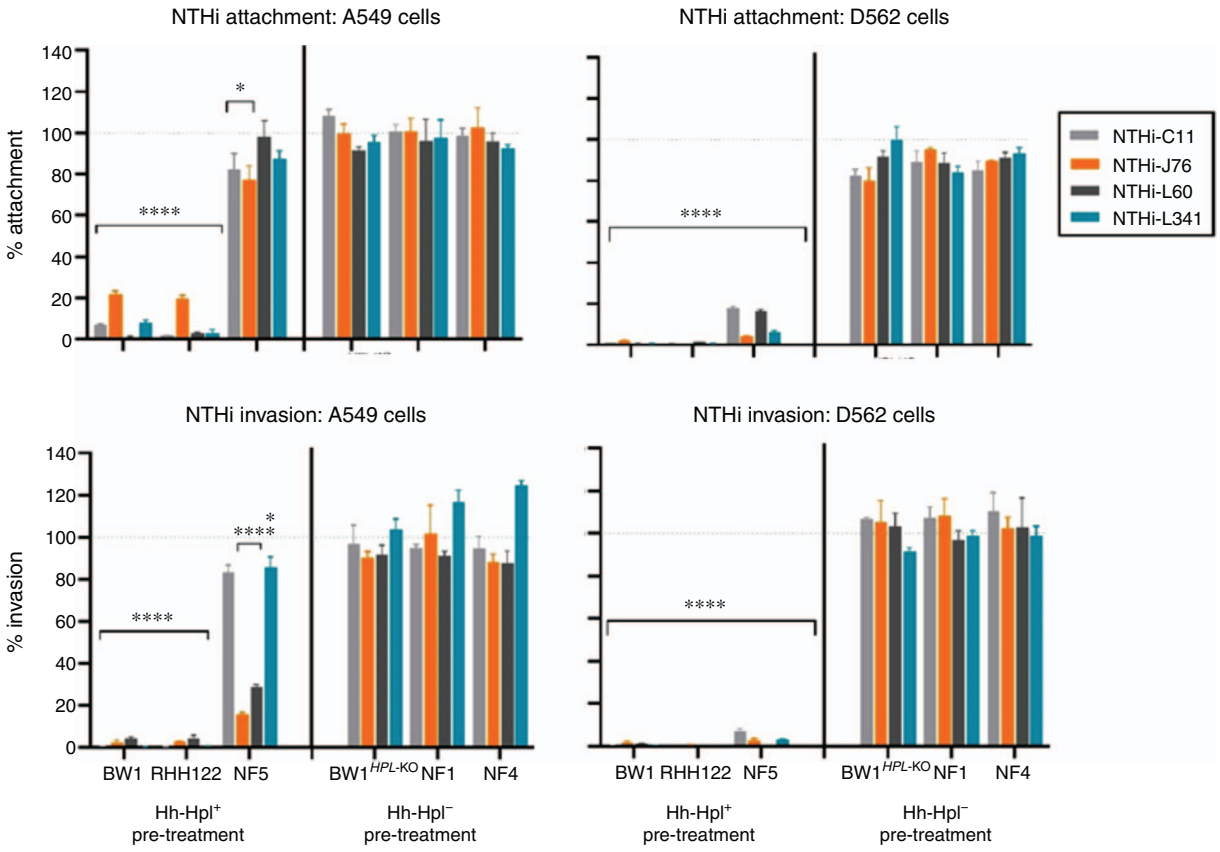

Figure 1. NTHi attachment and invasion of A549 and D652 cells post treatment with *Haemophilus haemolyticus* (Hh) strains (BW1, RHH122, NF5, NF5, NF1) or the *hpl* knockout (BW1$^{hpl\text{-}KO}$). The percent attachment of NTHi (compared to media control) to A549 (*a*) and D562 (*b*) cell monolayers post 4-h pre-treatment with Hpl-producing Hh (Hh-Hpl$^+$) or Hh strains that do not produce Hpl (Hh-Hpl$^-$). Percent of internalised NTHi (compared to media control) after exposure to A549 (*c*) and D562 (*d*) cell monolayers post 4-h pre-treatment with Hh-Hpl$^+$ or Hh-Hpl$^-$. Error bars represent the ±SEM (standard error of the mean) of three biological replicates, measured triplicate: *$P < 0.05$, ****$P < 0.0001$.

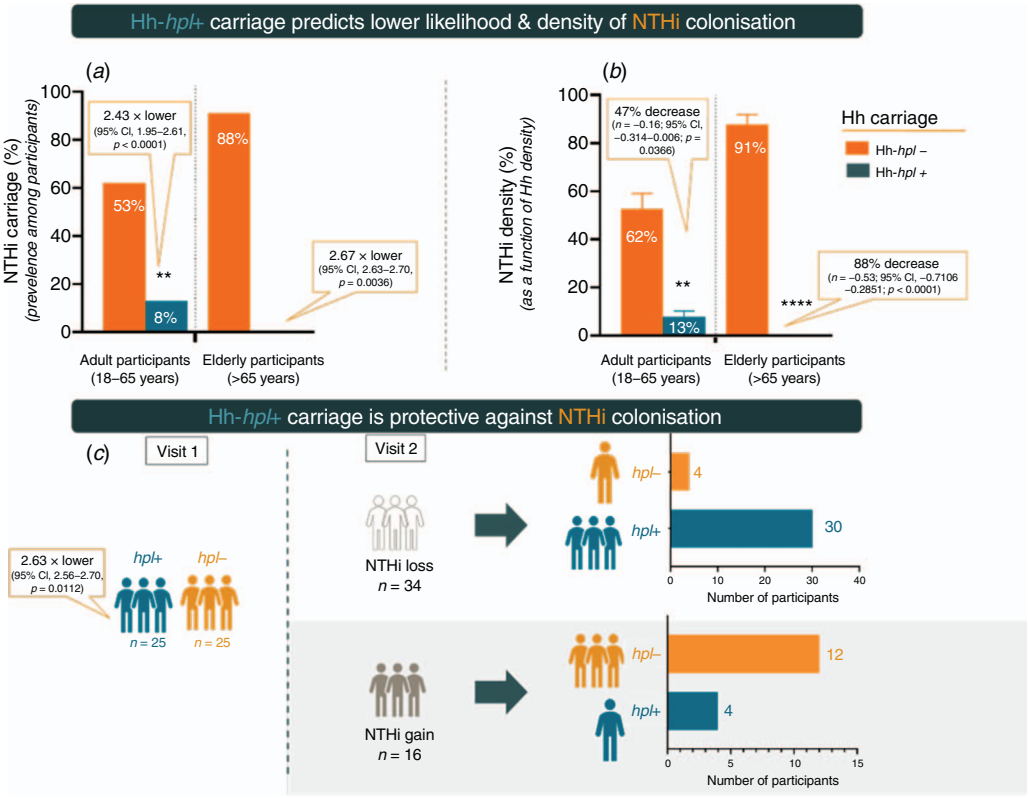

Figure 2. NTHi dominance in oropharyngeal swabs of healthy adult (18–65 years) or elderly (>65 years) participants co-colonised with Hh. NTHi oropharyngeal carriage prevalence (*a*) or proportion of NTHi (as a function of total Hh) (*b*) among participants concurrently carrying Hh strains that possess the *hpl* ORF (Hh-*hpl*$^+$) or do not possess the *hpl* ORF (Hh-*hpl*$^-$). Hh-*hpl*$^+$ (predominant) denotes instances where *hpl*$^+$ is the predominant Hh genotype (>0.5 of total Hh). NTHi colonisation status in participants carrying *hpl*$^+$ ($n = 25$) or *hpl*$^-$ ($n = 25$) strains of Hh on follow-up testing (visit 2) 2–6 months after their initial visit (visit 1). Error bars represent ±SEM (standard error of the mean); statistical significance was determined by simple logistic regression (*a*) or nonparametric Spearman correlation (*b*); $^{**}P < 0.005$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

based on NTHi pharyngeal carriage load[30], we could predict a ≈40% decrease in the risk of infection, provided the level of protection conferred by Hpl-producing Hh to model cell lines was preserved in the context of the respiratory tract. Hh also possesses favourable characteristics suited to probiotic applications; it has not been implicated as a causative agent of respiratory tract infection[33,34] and as a normal pharyngeal inhabitant, is able to thrive in the niche amongst other microbial inhabitants[35]. Additionally, probiotic-based therapies have a narrow spectrum of activity that do not damage host tissue, provoke collateral damage to the healthy microbiome or promote enrichment of resistant clones[36]; properties which make them an asset against the emergence of antibiotic resistance.

In conclusion, Hpl-producing Hh may be a promising respiratory probiotic candidate for the prevention of NTHi infections by inhibiting requisite pharyngeal colonisation.

Conflicts of interest

The authors declare no conflicts of interest.

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Biographies

Brianna Atto is a final year PhD candidate at the University of Tasmania. She is interested in the development of novel therapeutic strategies that overcome antibiotic resistance to treat and prevent infections. Her research is currently exploring the therapeutic value of upper respiratory commensals to tackle the growing disease burden of the major respiratory pathogen nontypeable *Haemophilus influenzae*.

David Gell is a protein chemist at the University of Tasmania with an interest in the role of haem-binding proteins at the interface of host-microbe interactions.

Stephen Tristram lectures in Medical Microbiology in the School of Health Sciences at the University of Tasmania and is an Associate Professor and Academic Lead of the Laboratory Medicine Programs. He has been researching antibiotic resistance and pathogenesis in *Haemophilus influenzae* for over 20 years.

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Interaction of *Candida albicans* with human gut epithelium in the presence of Live Biotherapeutic Products (LBPs)

Bronwyn Smit[A,D], *Anna Kuballa*[B], *Samantha Coulson*[C] *and Mohammad Katouli*[A]

[A]School of Science, Technology and Education, University of the Sunshine Coast, Maroochydore DC, Qld 4556, Australia
[B]School of Health and Behavioural Sciences, University of the Sunshine Coast, Maroochydore DC, Qld 4556, Australia
[C]Servatus Biopharmaceuticals, Coolum Beach, Qld 4573, Australia
[D]Email: bronwyn.smit@research.usc.edu.au

Abstract. *Candida albicans* is a semi-ubiquitous pathobiont that is known to significantly impact human health and wellbeing, causing a significant financial strain on the medical system. Due to increasing antifungal resistance, there is a growing need for novel fungal therapeutics to treat diseases caused by this fungus. The development and use of Live Biotherapeutic Products (LBPs) is an innovative and novel approach to potentially treating Candidiasis and other comorbidities associated with *C. albicans* infection. To evaluate their anti-pathogenic efficacy, it is necessary to understand the underlying mechanisms involved, via the use of biomimetic cell models. In this study, six LBPs were chosen to investigate their competitive inhibitory effect against *C. albicans* using a co-culture of Caco-2 cells and mucous-secreting HT29-MTX cells to mimic human gut epithelium. The LBP strains were supplied by Servatus Biopharmaceuticals and identified as SVT 01D1, SVT 04P1, SVT 05P2, SVT 06B1, SVT 07R1 and SVT 08Z1. Five out of the six LBPs showed a significant reduction in the adhesion of *C. albicans* and all six LBPs reduced *C. albicans* invasion in the co-culture cells to varying degrees. There was no significant difference between co-inoculation of *C. albicans* with the LBPs or pre-inoculation of LBPs before the addition of *C. albicans*. The potential of these LBPs as novel anti-fungal therapeutics for the treatment of *C. albicans* diseases can be further documented in clinical trials.

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Introduction

The gut microbiome is arguably one of the most mysterious ‘organs’ of the human body and is vital to all aspects of human health and our sense of wellbeing. Over the past few decades significant research has been conducted to understand the dynamics involved in microbe-to-microbe and microbe-to-gut interactions and how these impact on overall human health. One microbe that has been the subject of ongoing investigation is *Candida albicans,* an opportunistic pathogenic yeast found in about 70% of people[1]. As a polymorphic fungus, it is generally considered to inhabit the body as a commensal, kept under control by the host’s beneficial microbiota. However, in certain circumstances, such as in immunocompromised individuals[2], and during prolonged antibiotic therapy[3], *C. albicans* is able to overgrow within its local environment, that is, within the gastrointestinal (GI) tract or translocate across the gut epithelium leading to systemic Candidiasis. This disease has a high morbidity and mortality ranging from 20% to 49%[4–6]. Studies suggest that *C. albicans* adversely affects inflammatory bowel disease (IBD) exacerbating inflammation in the gut and delaying healing of ulcerative colitis, for example in humans and mice[7–9]. An increased abundance of *C. albicans* has been observed in patients with IBD compared with healthy subjects suggesting that fungi may play a role in its pathogenesis[10,11].

Current gaps and future direction

Scientific investigations have identified various fungal genes known to play a role in *C. albicans* pathogenicity. However, there are still gaps in current knowledge of atypical virulence mechanisms, particularly in understanding the ability of *C. albicans* to invade gut epithelial cells. The majority of studies have focused on the interaction of *C. albicans* with oral/vaginal epithelial cells as opposed to gastrointestinal (GI) epithelial cells[12]. While antibiotics are still the drug of choice to treat bacterial and fungal infections in clinic, Live Biotherapeutic Products (LBPs) have been suggested as an alternative for treating infections. LBPs are defined by the Food and Drug Administration (FDA) Centre for Biologic Evaluation and Research (CBER) as ‘a live biological product that: (1) contains live organisms, such as bacteria; (2) is applicable to the prevention, treatment

or cure of a disease or condition of human beings; and (3) is not a vaccine'[13]. They are further described as 'medicinal products containing live micro-organisms (bacteria or yeasts) for human use' by the European Pharmacopoeia (Ph. Eur.) (which excludes faecal microbiota transplants and gene therapy agents from this category)[14]. The investigation of LBPs in treating inflammatory, autoimmune and even malignant conditions is accelerating at an astonishing rate, being recognised as novel drug candidates that aim to change the medical paradigm in treating human illness[15]. In this study we investigated the competitive inhibitory effects of six LBPs on adhesion and invasion of *C. albicans* using a co-culture of Caco-2:HT29-MTX cells as a model of human gut epithelium to provide insight into the potential of these LBPs for managing Candidiasis.

Scope of this project

Current methods to investigate pathogenic interactions of microbes on gut epithelium rely on using cell lines that resemble biomimetic synthetic intestines, mainly Caco-2 or HT-29 cell lines[16–18]. Caco-2 cells are differentiated in culture medium to form a polarized cell monolayer with tight junctions and microvilli to resemble important characteristics of human intestinal mature enterocytes. The main drawback of this cell line is that it does not produce a sufficient mucus layer. HT29, with methotrexate (MTX) adaptation, differentiates in culture media to secret mucin[19], an essential component of the gut epithelium. We used a co-culture of Caco-2 and HT29-MTX cells to investigate the interaction of *C. albicans* ATCC 10231 with the gut epithelium. Six LBP candidates were selected and provided by Servatus Biopharmaceuticals: SVT 01D1, SVT 04P1, SVT 05P2, SVT 06B1, SVT 07R1 and SVT 08Z1.

Interaction of *C. albicans* ATCC 10231 at a final concentration of 10^6 CFU/mL with the co-culture Caco-2:HT29-MTX (9:1) alone and in the presence of each of the LBPs (10^6 CFU/mL) was assessed by measuring reduction in *C. albicans* colonisation when co-inoculated with LBP strains, and when pre-inoculated for 60 min with LBP strains prior to inoculation of *C. albicans*. The number of adhering

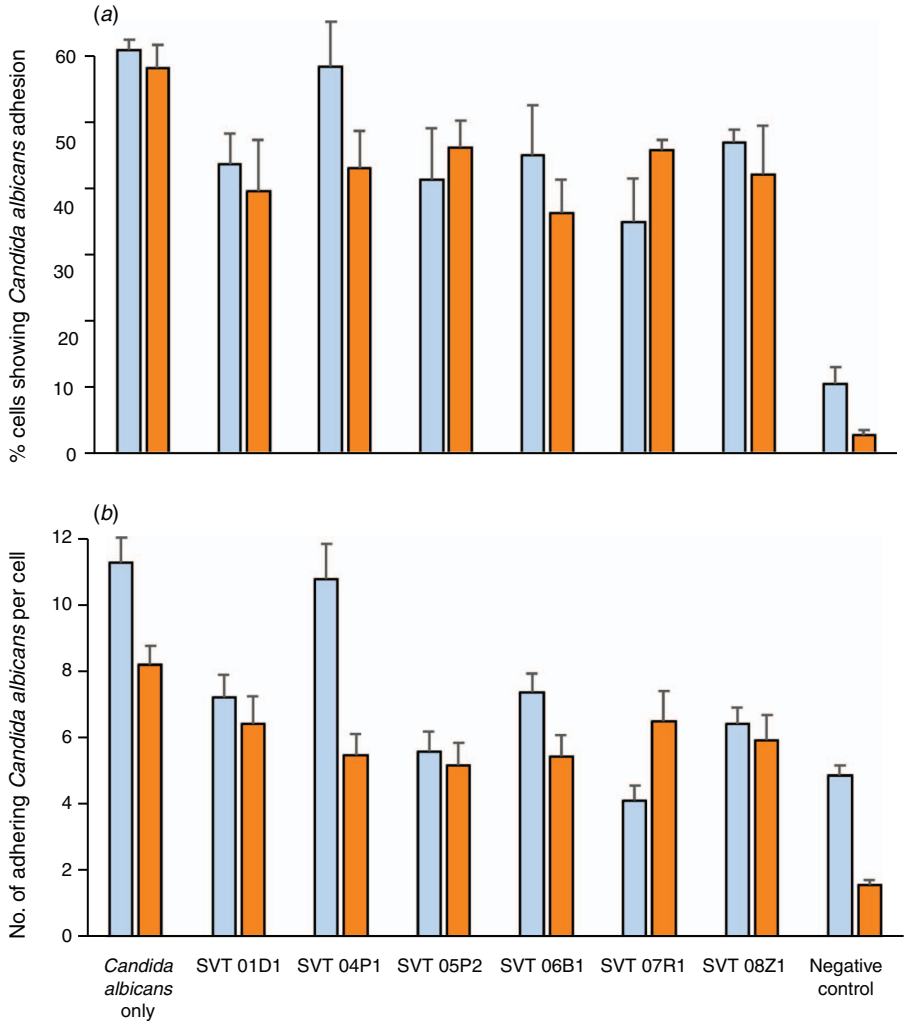

Figure 1. Percent colonisation on co-culture of Caco-2:HT29-MTX by *C. albicans* ATCC 10231 (*a*) and number of adhering *C. albicans* per cell (*b*) when co-inoculated with LBPs (blue) and following pre-inoculation with LBPs (orange). *E. coli* strain 46-4 was used as a negative control. Error bars represent SEM.

C. albicans per cell was recorded to identify the competitive ability of LBP strains to inhibit adherence of *C. albicans* per cell. The results indicated that the LBP strains (except SVT 04P1) reduced the colonisation of *C. albicans* on the co-culture cells by 21–43% (Figure 1*a*) and adhesion per cell by 21–64% (Figure 1*b*) both in co-inoculation and pre-inoculation assays.

All LBP strains (except SVT 04P1) demonstrated a significant reduction in colonisation and adhesion per cell of *C. albicans* ($P < 0.01$ in both co- and pre-inoculation). Overall, reduction in number of adhering *C. albicans* ATCC 10231 was seen for both co-inoculation and pre-inoculation, with SVT 07R1 showing the highest reduction ($P = 0.0005$).

In scanning electron microscopy (Figure 2) of the co-culture assay, *C. albicans* ATCC 10231 was shown to be highly invasive in that the *C. albicans* hypha was seen penetrating the epithelial cell monolayer. A similar procedure to the adhesion assay was used for co-inoculation and pre-inoculation in the invasion assay. A suspension of *C. albicans* was inoculated into 96-well plates at a final concentration of 10^7 CFU/well. After 90 min the wells were inoculated with nystatin (24 µg/mL) for 60 min to kill any extracellular *C. albicans*, followed by incubation with 0.1% Triton-X-10 (Sigma-Aldrich) for 15 min to lyse the monolayer releasing invading pathogens and enumerating them. The results showed a reduction in invasion of *C. albicans* in the presence of LBP strains that demonstrated variable efficacy (Figure 3) with SVT 01D1 showing the highest reduction overall.

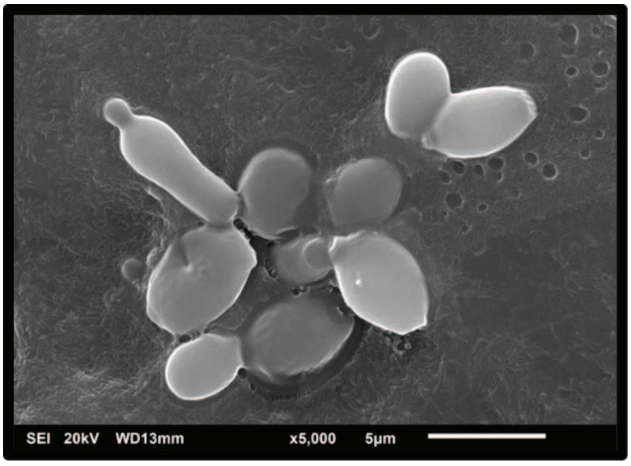

Figure 2. Scanning electron micrograph showing invasion of *C. albicans* strain ATCC 10231 into Caco-2/HT29-MTX cells after a 20-min incubation. Scale bar = 5 µm.

Discussion

The escalating need to develop alternative approaches for managing *C. albicans* infection has highlighted the potential for the use of LBPs as anti-fungal therapeutics. We showed that most LBPs used in this study showed a significant reduction in the adhesion and invasion of *C. albicans* in our human gut epithelium cell culture model, although these effects varied among the LBPs. The potential use of these LBPs as a therapeutic or as a prophylactic measure was also tested using co-inoculation and pre-inoculation models of the LBPs against the *C. albicans*. While there was a significant reduction in colonisation and invasion of the cells by *C. albicans* in the presence of LBPs, we did not observe a significant difference between co-inoculation and pre-inoculation of LBPs one hour before the addition of *C. albicans*. Poupet *et al*. studied the curative effect of LBP *L. rhamnosus* Lcr35® on *Caenorhabditis elegans* survival after *C. albicans* exposure, and found that the 2-h and the 4-h pre-inoculation periods were most protective against *C. albicans* infection[20]. Future studies to investigate the effect of longer incubation periods of LBPs used in our study can provide a better understanding

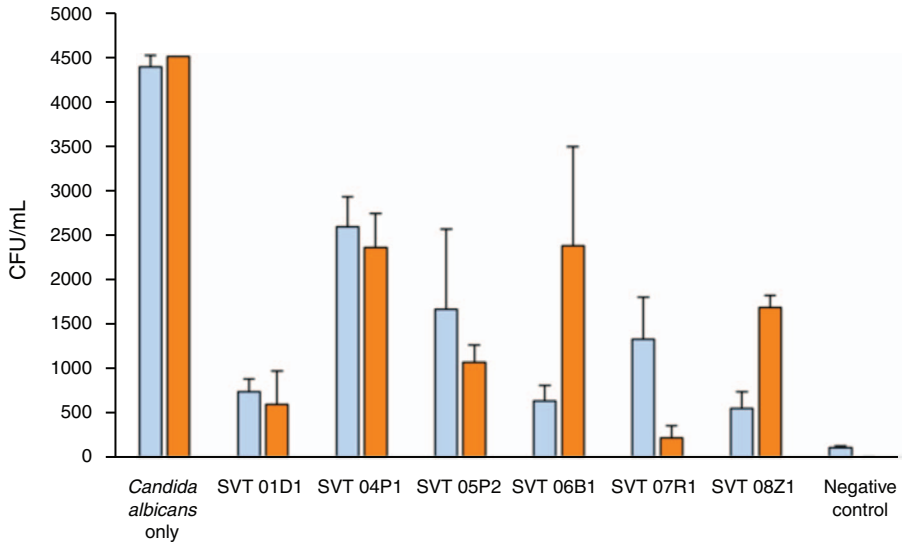

Figure 3. The number of invading *C. albicans* ATCC 10231 cells in a co-culture of Caco-2:HT29-MTX cells when co-inoculated with the LBPs (blue) and following pre-inoculation with LBPs (orange). Error bars represent SEM.

of the impact of pre-inoculation time in clinical studies aiming to assess the prophylactic effect of LBPs against *C. albicans*. Furthermore, it could prove insightful to explore the efficacy of various other LBP strains against *C. albicans*. Similarly, the use of further *Candida* strains in future studies for comparison would strengthen our findings.

Although the most reliable model to establish the impact of LBPs against *C. albicans* and other enteric pathogens is clinical trials in humans, *in vitro* studies utilising a co-culture of Caco-2 and HT29-MTX cells lines as used in this study, provide a suitable model to mimic the human gut epithelium. Caco-2 cells can be differentiated in the culture medium to form a polarized cell monolayer with tight junctions and microvilli that resemble important characteristics of human intestinal mature enterocytes. The other cell line, HT29, with methotrexate (MTX) adaptation, differentiates in culture media to secret mucin. In this study we used this co-culture model to investigate the efficacy of the LBPs against *C. albicans*, however, to achieve a far more reliable and robust gut epithelium model which resembles biomimetic molecular mechanisms in the intestinal niche, further improvements of this model such as the use of secretory IgAs and/or various other crucial antibodies/cytokines necessary for managing gut microbiome homeostasis are necessary[21].

This fascinating field of research has significant potential for determining the link in a chain of events involving interactions between *C. albicans* and the gut epithelium where LBPs are used to treat the invading pathogens.

Future studies

We are currently investigating the cellular response of the gut epithelial cells to *C. albicans* colonisation by comparing global gene expression (using RNA sequencing) with and without co-inoculation of LBPs. The RNAs will represent a snapshot of interaction/non-interaction of *C. albicans* with gut epithelium cells following the competitive adhesion of *C. albicans* with and without LBPs and identify genes that play a major role during these interactions. This will further our understanding of the mechanisms associated with using LBPs to treat invading pathogens.

Conflicts of interest

The authors declare no conflicts of interest.

Declaration of funding

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Biographies

Born in South Africa, **Bronwyn Smit** is a naturalised New Zealand citizen, currently residing in Queensland. She completed her undergraduate degree in Biomedical Science at the University of Auckland, followed by a master's degree in forensic science. Aside from briefly working in environmental science, Bronwyn explored career opportunities in the arts and hospitality industries before developing a fascination with the gut microbiome and its overwhelming impact on human health and disease. This led to her current PhD project in Microbiology at University of the Sunshine Coast, investigating the competitive inhibition of *Candida albicans* by live biotherapeutic products in the human gut.

Dr Anna Kuballa obtained a Bachelor of Biomedical Science degree (Hons) majoring in microbiology from the James Cook University. She graduated with a Doctor of Philosophy in the field of molecular biology from the University of Queensland, Brisbane in 2007. She continued her research as a post-doctoral research fellow at the University of the Sunshine Coast where she currently holds an academic research and teaching position. Dr Kuballa's published contributions centre around the molecular pathways involved in inflammation and microbial infection, with a special interest in the microbiome of inflammatory bowel disease and the breast milk microbiome.

Samantha Coulson joined Servatus in 2018 as Head of the Clinical Research Department and holds a PhD in Medicine from the University of Queensland. With over 15 years' experience, Samantha has a diverse background in both academia and industry with extensive knowledge of the human microbiome. She is adept in designing, initiating, leading and completing multidisciplinary research projects and also in managing product research, innovation and development programs. As Head of Clinical Research Samantha manages all aspects of Servatus' human clinical trial projects and preclinical studies, together with a small but highly experienced research team and global collaborators.

Associate Professor Mohammad Katouli obtained his PhD in 1980 from University of Ulster in UK. He then joined the Research and Development Department of DP Pharmaceuticals. In 1985, he took the position of the Head of Microbiology Department at the Pasteur Institute in Tehran. Between 1988 and 1998, he worked as a senior research fellow at the Microbiology and Tumor Biology Centre of the Karolinska Institute, Stockholm, Sweden. In 1998, he took a teaching and research position at University of the Sunshine Coast. His current interest is gut microbiota and the role of *E. coli* in pathogenesis of IBD.

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Indigenous microbial surrogates in wastewater used to understand public health risk expressed in the Disability-Adjusted Life Year (DALY) metric

Christopher EL Owens[A,B,C,H], *Peter T Cox*[B], *Paul M Byleveld*[D], *Nicholas J Osborne*[E,F,G] and *Md Bayzidur Rahman*[A]

[A]The Kirby Institute, Faculty of Medicine and Health, University of New South Wales, NSW, Australia
[B]Sydney Water Corporation, Parramatta, NSW, Australia
[C]Centre for Economic Impacts of Genomic Medicine, Macquarie Business School, Macquarie University, NSW, Australia
[D]NSW Health, Sydney, NSW, Australia
[E]School of Public Health, Faculty of Medicine, University of Queensland, Qld, Australia
[F]School of Population Health, Faculty of Medicine and Health, University of New South Wales, NSW, Australia
[G]European Centre for Environment and Human Health, University of Exeter, Royal Cornwall Hospital, Truro, UK
[H]Tel.: +61 403 854 950; Email: chris.owens@unswalumni.com

Abstract. In any wastewater recycling scheme, the protection of public health is of primary importance. In Australia, the public health requirements applying to the treatment of recycled water are stringent. They use the Disability-Adjusted Life Year (DALY) metric to set a level of negligible public health risk. The target maximum risk of 10^{-6} DALY per person per year has been adopted in Australian water recycling guidelines since 2006. A key benefit of the DALY approach is its ability to standardise the understanding of risk across disparate areas of public health. To address the key challenge of translating the results of monitoring of microorganisms in the recycled water into this quantitative public health metric, we have developed a novel method. This paper summarises an approach where microbial surrogate organisms indigenous to wastewater are used to measure the efficiency of water recycling treatment processes and estimate public health risk. An example of recent implementation in the Greater Sydney region of Australia is provided.

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Introduction

Large scale water recycling schemes are increasing in importance. Climate change and urbanisation are placing pressure on the continued ability to meet regional water demand through conventional water sources such as lakes and aquifers[1]. Similarly, the global challenges posed by inadequate water supply, sanitation, and hygiene highlight the need to increase safe water recycling practices[2].

Various forms of water recycling are taking place in Australia[3]. The largest scale applications involve domestic wastewater contributed by the public to the sewer. When treated to a high standard, recycled water can be safely used by industry and domestic users. There are many Australian schemes where recycled water is supplied through a network that is entirely separate to the drinking water supply (Figure 1), such as the Rouse Hill scheme in north-west Sydney[4]. Such schemes offset the use of the conventional water sources for non-drinking uses with relatively low human exposure, including garden watering and toilet flushing. There are also schemes that are used to safely augment a drinking water supply, such as the Beenyup scheme in Perth, which injects highly treated water into the water table[5,6]. Doing so replenishes the groundwater that is later treated for drinking.

Water safety planning and the health-based target for pathogen reduction

In the *Australian Guidelines for Water Recycling*, EPHC-NRMMC-AHMC[3] set out a framework for the effective and reliable management of safety of water recycling schemes. The approach is formalised in a 12-element recycled water safety plan. The preparation and implementation of a water safety plan is a key regulatory requirement of water supply authorities across Australia. Similar requirements apply in many international jurisdictions.

In formulating the water safety plan, much attention is paid to the risk posed by enteric pathogens in the untreated source water. This risk defines the types of treatment processes required and how well these processes must perform to protect public health. In Australia, the level of pathogens in recycled water deemed to pose a negligible risk is based on a specified public health measurement (referred to as a 'health-based target'). The target is an upper limit of 10^{-6} DALY

Figure 1. Recycled water used for non-drinking purposes in the Greater Sydney region of Australia.

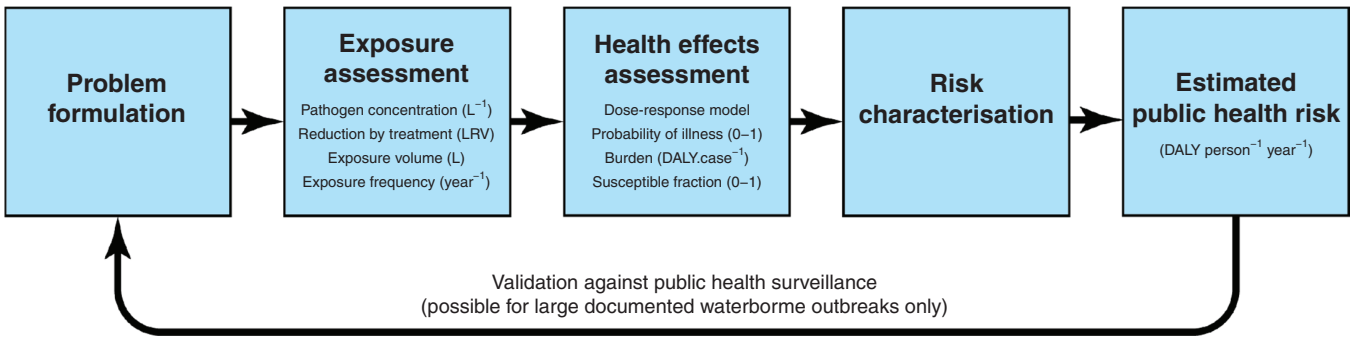

Figure 2. Quantitative microbial risk assessment process.

person per person per year, and it was adopted by the National Health and Medical Research Council over a decade ago[3].

Quantitative microbial risk assessment

When considering the application of the DALY metric to recycled water exposure scenarios, a method is required for translating pathogen levels into public health risk. Quantitative microbial risk assessment (QMRA) is the recognised process to do this[7,8]. It models the nature of exposure, associated health effects, and other relevant factors across a theoretical reference population (Figure 2). Results are then compared to a targeted tolerable (negligible) reference level.

QMRA has been formally embedded in water recycling guidelines in Australia since 2006[3]. It has a much longer history of being used to estimate health risks in drinking water supplies[9,10]. However, novel developments in the approach to implementation are not often presented in literature. The next section of this paper provides a case study of adaptive implementation of the guidelines in the Greater Sydney region of Australia. The approach represents the further development of the method discussed in *Microbiology Australia* by Cox *et al.*[4].

Implementation in the Greater Sydney region of Australia

The case study focuses on a water recycling scheme that supplies to commercial and local government entities. The water is used for purposes categorised as 'municipal irrigation', as defined by EPHC–NRMMC–AHMC[3]. The frequency and volume of public exposure associated with this use are reflected in the required level of pathogen reduction.

The level of pathogen reduction by the wastewater treatment processes was estimated based on monitoring indigenous surrogate organisms representing three major pathogen groups (Table 1) across the treatment process during typical operating

Table 1. Surrogate organisms monitored to understand the fate of each major pathogen group.

Monitored indigenous surrogate	Pathogen group represented	QMRA reference pathogen	Key reference
Escherichia coli	Bacteria	*Campylobacter jejuni*	[3]
Clostridium perfringens spores	Protozoa	*Cryptosporidium* spp.	[3]
Bacteriophage MS2	Viruses	Noroviruses (dose-response) Adenoviruses (occurrence)	[13]
As for protozoa	Helminths	Not used	[3]

conditions. The surrogates are understood to have similar removal characteristics to their represented pathogens but are generally of higher concentration in domestic wastewater[3,11,12]. The use of surrogates was warranted due to the levels of pathogens in wastewater being too low to fully measure the high level of reduction required. This reflects the stringency of public health requirements and the analytical limitations of detecting pathogens in low concentrations in treated water. The more common approach of challenge-testing the treatment processes was not feasible due to the potential to disrupt the co-located wastewater disposal processes. Similarly, testing during commissioning did not occur due to the original purpose of the plant being for environmental discharge rather than for water recycling.

At the studied water recycling plant, located in Greater Sydney, the microbial surrogates were monitored at three phases: before treatment, following the biological digestion process, and following the chlorination process. A total of 27 samples were analysed over two campaigns, from April 2015 to May 2016 (n = 14) and from April to June 2019 (n = 13). The level of reduction, expressed in terms of the 'logarithmic reduction value' (LRV), was calculated for each treatment process and each pathogen group as $\log_{10}(C_{in}/C_{out})$ (Table 2). C_{in} was the level of surrogate before a treatment process (influent) and C_{out} was the level after (effluent). C_{in} and C_{out} were paired by the date of sampling. Only the results from the latter monitoring campaign were used to assess protozoal removal due to the recent improvement of treatment performance.

Three major steps were involved in determining whether the health-based target was met (Table 3). First, an LRV for each major pathogen group was estimated for the water recycling plant based on the surrogate monitoring results. Because the lower range of performance was of interest, the fifth percentile statistic of the LRV results was used in the subsequent assessment stages (Table 2). Use of the fifth percentile is conservative in comparison to the more common approach of using an average LRV.

The second step was the incorporation of default LRVs associated with end-use limitations, as guided by EPHC-NRMMC-AHMC[3] (Table 3). These limitations seek to reduce exposure of the public to recycled water. They are codified as management practices in the water safety plan.

Table 2. Summary statistics of the daily log-reduction.

Pathogen type/ monitored surrogate	Biological digestion	Chlorination	Full treatment plant
Bacteria (*E. coli*)			
Minimum	2.22	2.39	5.47
Fifth percentile	2.27	3.08	6.07
Median	2.98	4.41	7.26
Mean	2.93	4.13	7.11
Maximum	3.80	5.02	7.82
Viruses (bacteriophage MS2)			
Minimum	–0.41	2.16	3.00
Fifth percentile	0.41	2.42	3.50
Median	1.20	2.96	4.14
Mean	1.14	2.90	4.03
Maximum	2.18	3.41	4.62
Protozoa (*Clostridium perfringens* spores)			
Minimum	1.02	NA	1.02
Fifth percentile	1.30	NA	1.30
Median	1.76	NA	1.76
Mean	1.74	NA	1.74
Maximum	2.25	NA	2.25

Removal of protozoa through chlorination is not applicable (NA) due to their resistance to this disinfectant.

The final step involved the comparison of the aggregated LRVs to the default LRV requirements (Table 3). The default LRV requirements were taken from the draft revised *Australian Guidelines for Water Recycling*[13], rather than the current version, for two reasons. First, the revised guidance recognises the decreased incidence and burden of rotavirus infection following the recent implementation of broad community vaccination[13–15]. Second, newer literature relevant to the selection of dose-response models and other assessment

Table 3. Aggregated assessment of pathogen reduction requirements.

Log-reduction value (LRV) type	Notation	LRV			Derivation
		Bacteria	Viruses	Protozoa	
Fifth-percentile total verified treatment reduction	LRV_{P5}	6.07	3.50	1.50	Table 2
Exposure-reduction adjustment: restricted public access during irrigation	E_1	2.00	2.00	2.00	[3]
Exposure-reduction adjustment: spray-drift controls	E_2	1.00	1.00	1.00	[3]
Total scheme LRV claimed		**9.07**	**6.50**	**4.50**	$LRV_{P5}+E_1+E_2$
Required scheme LRV		*4.70*	*5.0*	*4.40*	[13]

assumptions are incorporated[13]. The health-based target was considered met if the aggregated LRV for each pathogen type was greater than or equal to the relevant LRV requirement.

Using the microbial surrogate data and the above method, the recycling scheme was shown to meet the public health requirements for waterborne enteric pathogens (Table 3). The key benefit of the surrogate monitoring approach is its ability to overcome the described limitations inherent to operational water recycling schemes when assessing public health requirements. The approach complements existing methods for the assurance of safe water recycling. Overall, it is crucial that QMRA implementation is subject to ongoing critical evaluation, adapts to new evidence, and incorporates conservative assumptions where appropriate.

Conflicts of interest

The authors declare no conflicts of interest.

Declaration of funding

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Biographies

Dr Christopher Owens is a recent doctoral graduate from the University of New South Wales. This paper stemmed from his doctoral research and his prior professional work as a Senior Analyst in public health at Sydney Water. He is currently a Research Fellow at the Centre for Economic Impacts of Genomic Medicine at Macquarie University.

Dr Peter Cox is the Water Quality Lead at Sydney Water and is the workplace supervisor for this research.

Dr Paul Byleveld is the Manager of the Water Unit at NSW Health and is an academic co-supervisor of this research.

Associate Professor Nicholas Osborne is principally affiliated with the School of Public Health, University of Queensland, and is an academic co-supervisor of this research.

Dr Md Bayzidur Rahman is a Senior Lecturer at The Kirby Institute, University of New South Wales, and is the principal academic supervisor of this research.

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'The awesome power of yeast' in Alzheimer's disease research

Sudip Dhakal

School of Science, RMIT University, Bundoora, Vic. 3083, Australia. Email: sudip.dhakal@rmit.edu.au; dhakal.sudip001@gmail.com

Abstract. The difficulties in performing experimental studies related to diseases of the human brain have fostered a range of disease models from highly expensive and complex animal models to simple, robust, unicellular yeast models. Yeast models have been used in numerous studies to understand Alzheimer's disease (AD) pathogenesis and to search for drugs targeting AD. Thanks to the conservation of fundamental eukaryotic processes including ageing and the availability of appropriate technological platforms, budding yeast are a simple model eukaryote to assist with understanding human cell biology, offering a platform to study human diseases. This article aims to provide insights from yeast models on the contributions of amyloid beta, a causative agent in AD, and recent research findings on AD chemoprevention.

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Alzheimer's disease (AD) is one of the most important progressive age-related neurodegenerative diseases. It accounts for the majority (60–80%) of dementia-related deaths in the elderly[1]. Until recently, AD drugs approved by FDA have been limited to the treatment of moderate symptoms[2]. In June 2021, Aducanumab, a monoclonal antibody acting against amyloid beta (Aβ), was approved by the FDA[3,4]. However, it is not likely to cure the neuronal loss found in AD[5].

The major hallmarks of AD pathology include the presence of extracellular amyloid plaques, intracellular tau neurofibrillary tangles, accumulation of oxidative stress, loss of proteostasis, epigenetic changes, alteration in biometal distributions, lipid imbalances, mitochondrial dysfunction, genomic instability, chronic cellular stress, neuroinflammation, neuronal death, loss of synapses and cognitive deficits[6]. The complexity and difficulty of assessing the brain in living humans makes it difficult to perform experimental research to understand AD pathogenesis and has hindered exploration of therapeutic strategies.

Yeast models and bioassays to study AD

The "awesome power of yeast" has been hailed by numerous researchers, with at least five Nobel prizes awarded to yeast researchers in the last two decades. In studies initiated by Macreadie and colleagues, yeast has offered powerful contributions for studying AD pathogenesis and for finding novel therapeutic agents. There are many reasons for the preference of yeast models in AD research. The most important one stems from the conservation of the molecular mechanisms in yeast that inform about fundamental processes of human biology[7]. Energy metabolism, genetics, vesicle trafficking, cell division, protein homeostasis networks, lipid metabolism, stress response pathways and cell death pathways are some major processes that are conserved between humans and yeasts[8]. The different phases of yeast growth also allow us to understand chronological and replicative lifespans[9]. Stationary phase yeast cells can mimic terminally differentiated human neurons with several neuronal features conserved in these cells. Most yeast species divide by budding. When a budding yeast mother cell produces a bud, chitin-rich bud scars are left behind on the cell surface (Figure 1*a*). Staining of these bud scars provides an excellent method to differentiate the old and young cells (Figure 1*b*), which is instrumental to understand the ageing process. In addition, the availability of several analytical platforms to analyse single cells or large populations, robust growth, facile genetic modification and the existence of numerous yeast species and gene deletion libraries improves our ability to use them as models for several chronic diseases including AD[8].

It is now clear that the protein homeostasis network is involved in the pathogenesis of AD, and proteostasis failure a major cause of AD. Proteostasis failure in AD is present at all levels of the protein quality control system inside a cell including unfolded protein response, ubiquitin proteasome system and autophagy[5]. Some of the most important evidence depicting the proteostasis failure are the presence of misfolded proteins, calcium dyshomeostasis, defective proteaphagy, impaired mitophagy, mutations of ubiquitin, oxidation of deubiquitinating enzymes, vacuolar-ATPase assembly defects and presence of several types of uncleared autophagic vesicles containing the toxic amyloid beta (Aβ) protein in the neurons of AD patients[5]. Most importantly, the persistent misfolded proteins

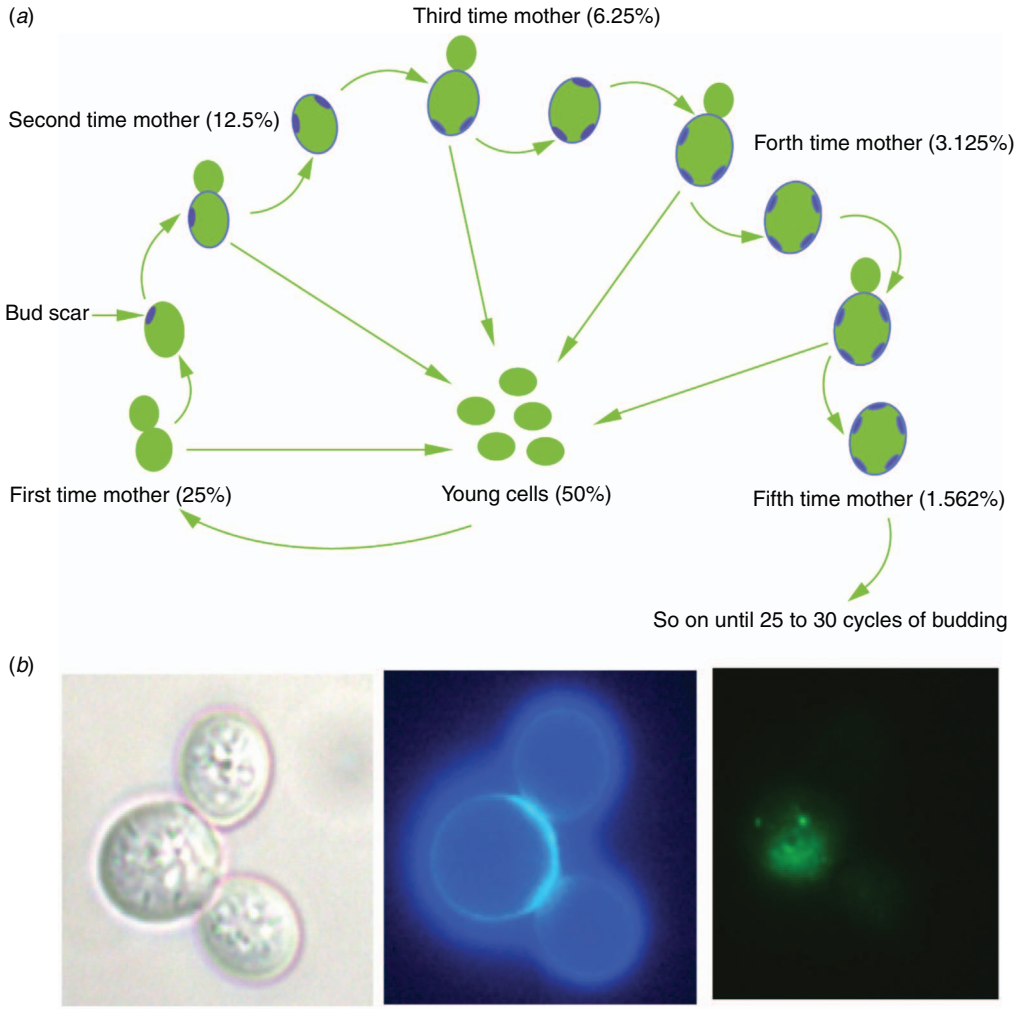

Figure 1. Yeast showing loss of proteostasis in ageing cells. Part a shows yeast cell budding to produce daughter cells. Part b, left panel shows a phase contrast micrograph of budding yeast cells expressing green fluorescent protein (GFP) fused to Aβ: the central panel shows blue-fluorescent chitin-rich bud scars present in the mother cell stained with Calcofluor White; right panel shows GFP-Aβ associated green fluorescence limited to older mother cell. The GFP-Aβ was produced in all cells (under the control of a constitutive promoter) but younger cells removed the protein, indicating loss of proteostasis in older cells (Figure 1*b* is adapted from Macreadie and Luu[10]).

and protein aggregates of Aβ and tau in AD patients continuously activate the heat stress response in the neuronal cells[11]: Aβ and tau are the proteins most associated with AD and are likely to be the causative agents. Meanwhile, the chronic stress response activation and inability of the protective stress response to recover the cells from detrimental effects of these toxic proteins render cells prone to activation of destructive mechanisms[12]. Disrupted proteostasis and protein aggregation is common in ageing yeast, similar to that of ageing neurons. Considering such similarities with human cells, several yeast assays have been developed to monitor these fundamental eukaryotic processes or to find chemicals that can protect cells from the toxicity of protein misfolding and ageing.

A number of assays have been developed to study autophagy in yeast[13]. Yeast expressing amyloid precursor protein, Aβ, GFP-Aβ and tau protein have been engineered to study the effects of these proteins on cells[8,14,15]. Such yeasts have shown that Aβ causes mitochondrial dysfunction, and increases intracellular reactive oxygen species, cell stress and cell death[15,16]. Interestingly, ageing yeast cells harboured Aβ while young cells removed it, mimicking what is observed to happen to Aβ in human ageing (Figure 1*b*). Being able to evaluate the turnover of GFP-Aβ measured by its fluorescence has revolutionized the drug screening process[17]. The punctate patterns of the GFP-Aβ fusion protein observed in the microscopic images (Figure 1*b*) illustrate the aggregating nature of the fusion protein and provide evidence of the utility of the yeast model[15].

Bioassays to find chemoprotective agents against AD

Yeasts have not only been used as a platform to understand how Aβ and tau proteins are involved in cellular destruction, but have also been used to study drugs and bioactive compounds that can prevent detrimental effects caused by Aβ and tau[17–19]. For example,

simvastatin (the best chemopreventative for AD[20]) has been shown to reduce levels of both GFP-Aβ and native Aβ from yeast cells. This reduction was independent of the effect of statins on lowering ergosterol, a functional equivalent of cholesterol in yeast[17]. It is considered that simvastatin might act on other pathways in addition to that involving cholesterol biosynthesis. One such mechanism that is of high importance is protein prenylation[21]. Importantly, protein prenylation is involved in regulating autophagy, inflammatory responses, oxidative stress and synaptic/cognitive function[22]. In addition, to determine the stress response activation, a stress reporter yeast has been developed[23]. The reporter yeast is designed such that the mCherry fluorescent protein is expressed under control of a heat shock promoter[23]. The promoter is activated once the heat shock factor 1 (HSF1) transcription factor was translocated into the nucleus as a result of stress, thus activating the heat stress response. This yeast model could be an important method for rapid screening of compounds that can induce stress response and simultaneously decrease levels of Aβ in cells. In addition, when used in conjunction with other assays such as reactive oxygen species (ROS) measurement, toxicity assays and growth inhibition assays, yeasts may provide unprecedented benefits. So far, the results obtained from such studies have been promising and have demonstrated that these assays could lead to excellent discovery of novel therapeutics[17–19,24].

Bioassays to find compounds that enhance Aβ toxicity

Engineered yeast have also been used to identify compounds that exhibit toxic synergies with Aβ. In separate recent studies, both tyramine and aluminium exacerbated Aβ toxicity by enhancing the ROS inside cells[25,26]. Aluminium is the most abundant neurotoxic metal present in our surroundings including our food and consumer products. Although the toxic effect of aluminium in AD has been proposed for decades with limited evidence, the recent yeast study illustrated its ability to enhance Aβ toxicity suggesting its potential role in AD[25]. Similarly, tyramine and Aβ's synergistic toxicity indicates tyramine's potential role in AD[26]. It is difficult to perform similar studies to understand the involvement of biogenic amines in humans as they are present in very low amount and they are rapidly metabolized. In contrast, the yeast models hint at the potential role of trace amines in AD. Furthermore, trace amine associated receptor (TAAR) signalling could also be involved in AD as it has been found to be associated with various molecular pathways involved in AD[27].

Mitochondrial dysfunction and impaired mitophagy are common features of AD[28]. The fact that mammalian cells are not able to survive with defective mitochondria makes it impossible for studies involving investigations on mitochondrial health. On the contrary, the ability of yeasts to grow without functional mitochondria allows discovery of chemicals or biomolecules that directly affect mitochondria, its turnover and biogenesis[9]. For example, the respiratory growth (growth supported by mitochondria) of the yeast cells was inhibited more in GFP-Aβ transformant yeast cells compared to those in GFP transformants in the presence of tyramine and aluminium indicating increased mitochondrial and mitophagy defects in these cells[25,26]. These studies illustrated that yeast provided a unique platform to investigate compounds that affect mitochondrial health, which is impossible in higher eukaryotes.

The potential role of these novel modifiers of Aβ toxicity, that are present in human body, can be identified using the yeast models. This opens up avenues for new dimensions in AD research[25,26]. These studies are important because the sporadic nature of AD suggests factors in addition to Aβ may be involved in AD. These factors could be compounds or elements discovered in recent studies using yeast models.

Conclusion

In summary, yeast models have provided a very attractive platform to study the toxic proteins involved in AD and compounds that can modify the effects of these proteins. Intriguingly, yeast mitochondrial function is not essential for yeast survival, so it is the only available model that can grow with defective mitochondria enabling studies on drugs that specifically target mitochondrial health. Despite the enormous potential of yeast as model for AD research, the lack of proper nervous system, endocrine system, circulatory system, immune system, and other systems that are present in humans limit its applications. However, with careful manipulation of the yeast genome and appropriate alterations in culture conditions it is possible to mimic the human cellular environment. Importantly, yeasts have the potential to enable us to answer significant research questions about AD including those related to cell–cell communication. New dimensions in AD pathogenesis and pathology along with the discovery of novel bioactive compounds beneficial for prevention or cure of AD are being discovered thanks to "the awesome power of yeast".

Conflicts of interest

The author declares no conflicts of interest.

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Biography

Sudip Dhakal is a PhD researcher and a tutor/instructor at School of Science, RMIT University, whose research focuses on investigating therapeutic strategies against Alzheimer's disease using yeast models.

COVID-19 myths busted

Correct information on some of the COVID-19 myths can be found through WHO links:

https://www.who.int/emergencies/diseases/novel-coronavirus-2019/advice-for-public/myth-busters#misinformation

- 5G Mobile networks
- Alcohol
- Antibiotics
- Bleach
- Cold weather, snow
- Dexamethasone
- Drugs
- Garlic
- Hand dryers
- Holding your breath
- Hot and humid climates
- Hot baths
- Hot peppers
- Houseflies
- Hydroxychloroquine
- Masks, CO_2 intoxication
- Masks, exercise
- Medicines
- Methanol, ethanol
- Misinformation
- Mosquitoes
- Older people, younger people
- Pneumonia vaccines
- Recovery
- Reduce risk of infection
- Saline
- Shoes
- Sunny and hot weather
- Supplements
- Swimming
- Thermal scanners
- Ultra-violet (UV) lamps
- Viruses, bacteria, antibiotics

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Viral fossils in marsupial genomes: secret cellular guardians

Emma F Harding[A,B], *Grace JH Yan*[A] *and Peter A White*[A,C]

[A]School of Biotechnology and Biomolecular Sciences, University of New South Wales
[B]Email: e.harding@unsw.edu.au
[C]Email: p.white@unsw.edu.au

Abstract. Genomic viral integrations, termed endogenous viral elements (EVEs), are fragments of viruses in host chromosomes that provide information about viral evolution and could even help protect the host from infection. In the present study we examined EVEs in thirteen different Australian marsupial species to identify trends in their integration, commonality and to investigate their possible cellular function. We found that marsupial EVEs are commonly derived from viruses of the *Bornaviridae, Filoviridae* and *Parvoviridae* families, and circulated up to 160 million years ago. We also show the EVEs are actively transcribed into both long and short RNA molecules in marsupials, and propose they are involved in a cellular defence mechanism to protect the germline from viral genomic invasion.

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Every time you get infected with a virus, there is a chance it will leave some of its genetic material behind in your DNA. This happens commonly during infection with retroviruses, like human immunodeficiency virus (HIV), which have a reverse transcriptase and integrase enzymes that allows them to integrate into our chromosomes. Other viruses, including filoviruses like Ebola and bornaviruses like Borna disease virus, can also be integrated into chromosomes by host retrotransposons[1]. If these integrations occur in germline cells, they are inherited by progeny and can spread through a population. These viral remnants, also known as endogenous viral elements (EVEs), are in every known vertebrate and invertebrate and provide an extensive history of viral evolution[2,3].

Few functions have been proposed for EVEs, with the common consensus that they are accidental integrations which degrade through slow mutagenesis over time. EVEs from structural viral genes rapidly lose their coding ability through negative selection to avoid immunological stimulation due to the production of viral capsids or envelopes. However, some EVEs are preserved and exapted for a new cellular function. For example, the syncytin gene, integral to placenta development, is a captured retroviral envelope gene from an ancient integration[4]. Similarly, a nucleoprotein gene from a bornavirus is translated into a protein in an American squirrel to impede viral infection[5].

Our study conducted at UNSW Sydney by PhD students Emma Harding, Grace Yan and Alice Russo, and led by Professor Peter White, investigated EVEs in Australian marsupials[6]. With the exception of rodents, marsupials dominate the mammalian fauna of Australia, in contrast to every other terrestrial ecosystem. We looked at 13 different species – the Tasmanian devil, koala, bare-nosed wombat, sugar glider, long-nosed bandicoot, Southern brown bandicoot, Western pygmy possum, brushtail possum, striped possum, tammar wallaby, fat-tailed dunnart, false antechinus and yellow-footed rock wallaby (Figure 1). The study examined EVEs to trace virus evolution and investigated if EVEs served any functions in marsupial cells.

RNA-Sequencing datasets were screened for the presence of EVE-derived transcripts – RNA transcripts with identity to viral genes which were endogenous in origin. Transcripts were classified as endogenous if they mapped back to the representative marsupial genome (where available), contained premature stop codons or were substantially shorter than the viral counterpart (<50% length).

All viral families were searched for, however only *Filoviridae, Bornaviridae, Parvoviridae* and *Retroviridae* EVE transcripts were identified. Whilst many viruses commonly integrate into host DNA during infection, only the small percentage that integrate into germline cells are passed on to progeny. Subsequently, only a small percentage of those become fixated in a population due to selection pressures, leading to the loss of many endogenized sequences in a short space of time. EVEs that successfully 'survive' this infancy and become fixated can endure within the genomes of a species for millions of years.

EVEs from *Bornaviridae, Filoviridae* and *Parvoviridae* viruses were ubiquitous throughout all of the animals sampled (Figure 2*a*). These viral families have been shown previously to be endogenised and are seen in almost every lineage of eutherian mammals. *Bornaviridae* EVEs, in particular, are ubiquitous in

Figure 1. A subset of the Australian marsupials included in this study. Top left to right: koala, tammar wallaby, Tasmanian devil. Bottom left to right: sugar glider, bare-nosed wombat, brushtail possum. Photo credits: David Clode on Unsplash and Ian Macreadie.

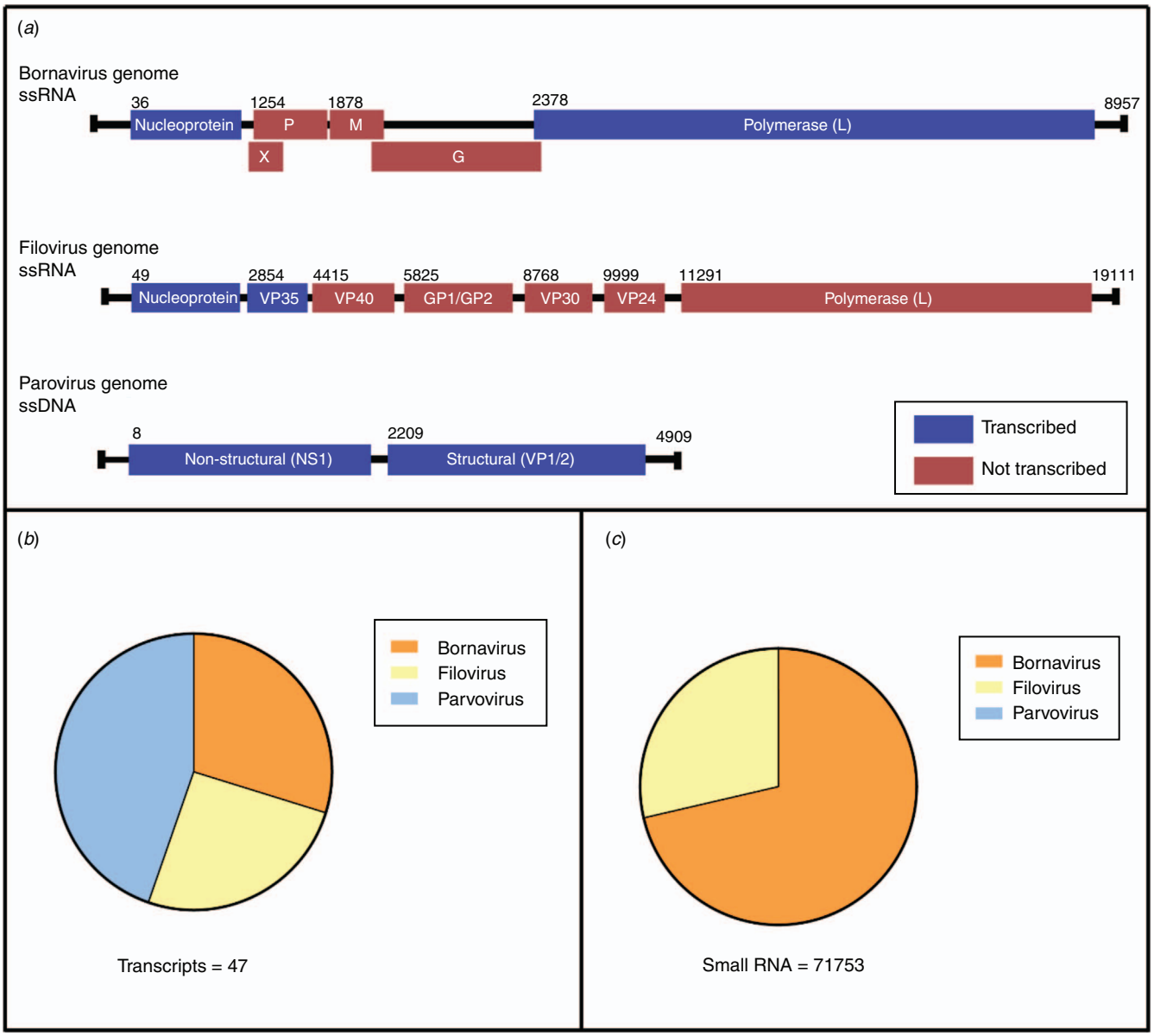

Figure 2. Transcribed endogenous viral elements in Australian marsupials. (*a*) The three viral families transcribed in marsupial tissues. Blue represents one or more transcripts were identified in this study and brown represents no evidence of transcription. (*b*) Long EVE-derived transcripts identified in koala tissue. Four koala RNA-Sequencing datasets were screened for the presence of transcripts from Bornaviridae, Filoviridae and Parvoviridae families using a BLAST-based approach. (*c*) EVE-derived PIWI-interacting RNA identified from koala testis. Four testis miRNA sequencing datasets were screened for the presence of PIWI-interacting RNA molecules mapping to koala EVEs. PIWI-interacting RNA were defined as small RNA molecules between 21 and 29 nucleotides in length.

vertebrates and are estimated to have been infecting animals for at least 100 million years. These viral families, two of which are RNA and one of which is DNA, have never been sequenced from marsupials, however the widespread presence of EVEs indicates a shared history. The EVEs are extremely divergent from any extant eutherian counterparts – their closest relative viruses are avian and reptilian-infecting rather than those that infect mammals like us.

In addition to their presence in marsupial chromosomes, many EVEs are actively transcribed into RNA (Figure 2*b*). While transcription is a likely key step for performing a cellular function, the EVEs in marsupials were not being translated into protein, or if they are, the proteins are very short. Each EVE transcript was riddled with stop codons, making the likelihood of its translation into anything functional very low. Instead, we set our sights on their role as non-coding RNA.

Non-coding RNA is present in every cell and contributes to many vital cellular functions, including transcriptional regulation, development and RNA defence. In plants and invertebrates, the primary immune defence against viruses is a system of non-coding small RNA molecules involved in the RNA interference system[7–9]. We considered, instead of being translated into an antiviral protein, that perhaps the EVE transcripts were being spliced into small RNA to bind and destroy incoming target viral nucleic acid.

To investigate this hypothesis, we looked at small RNA sequencing data from the koala. We found that EVEs were indeed giving rise to small RNA molecules – both small interfering RNA (siRNA) and P-element-induced wimpy testis protein-interacting RNA (piRNA) – associated with antiviral defence in invertebrates (Figure 2*c*)[10]. In addition, these molecules were enriched in the testis tissue – a prime location to protect gametes and hence protect offspring from genetic invasion by viruses. This suggests the tantalising possibility of this RNA defence system, previously thought to be abandoned in mammals in favour of the interferon system, still being active and protecting marsupial cells.

Of interest was the common trend that only two types of EVEs were contributing to the small RNA produced in the testis; bornavirus polymerase and filovirus nucleoprotein EVEs. Both *Bornaviridae* and *Filoviridae* viruses are single stranded, negative sense RNA viruses with genomes 7–10 kb, encoding 5–8 proteins (Figure 2*a*). Of these proteins, two were consistently represented in RNA sequencing data – the nucleocapsid, or shell, and the polymerase or replication gene. Bornavirus and filovirus nucleoprotein EVEs are commonly found throughout mammals; however, the bornavirus polymerase is much rarer – bats are the only other mammalian group with this type of EVE[3]. In contrast to trends in other mammals, marsupials had high amounts of RNA derived from these bornavirus

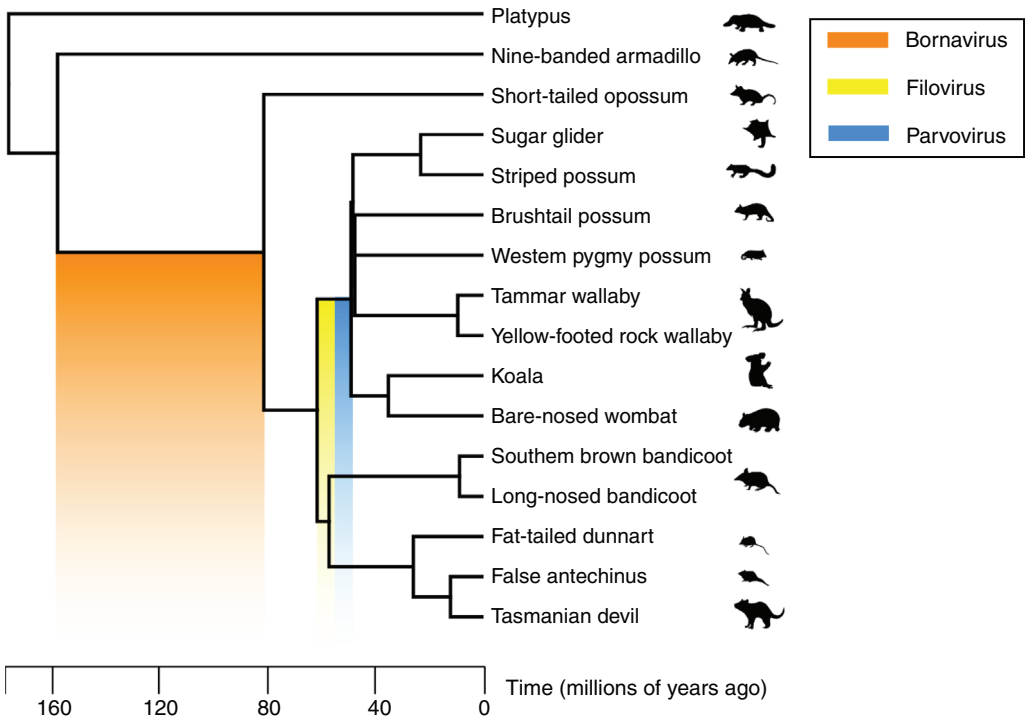

Figure 3. The estimated time of integration for transcriptionally active marsupial EVEs. Transcriptionally active EVEs were mapped (where possible) to representative marsupial genomes. Available genomes included the Tasmanian devil, bare-nosed wombat, koala, tammar wallaby and brushtail possum. The integration time for each EVE was estimated through identification of orthologous EVE insertions in each species. The short-tailed opossum, nine-banded armadillo and platypus were used as outgroup species. The colours represent estimated integration time of the oldest transcriptionally active EVE for each viral family.

polymerase EVEs, and the small RNA was predominantly from these. One theory suggests the nucleocapsid and polymerase proteins are prime targets for negative sense ssRNA viruses[11]. In these viruses, the nucleocapsid encapsulates the other viral proteins, and any degradation of this protein will lead to the exposure of pathogen-associated molecular patterns (PAMPs) and inhibition of viral replication[12]. Following this, it would make sense that these EVEs from these two viral genes are retained in host chromosomes to act as an effective antiviral defence against invasion from similar negative sense RNA viruses.

For additional evidence that this was a conserved protective system, we looked for clues on how old these EVEs were. By looking for orthologous integrations – EVEs in the same genomic loci – in different marsupials, we were able to estimate the age of each EVE that was making RNA transcripts. Most of the transcriptionally active EVEs were estimated to have integrated into marsupial genomes ~40 million years ago, however one bornavirus polymerase EVE was aged between 80 and 160 million years old (Figure 3). This EVE was present in all Australian marsupial genomes, as well as the American opossum genome, indicating it was an ancient virus that circulated during the time of the dinosaurs. We believe the conservation of these EVEs is no accident.

Whilst our study uncovered some very interesting observations, it is far from explaining their occurrence. It has led to many new questions, including why these three viral families (*Bornaviridae, Filoviridae, Parvoviridae*) are so common in marsupial genomes, why they are still being transcribed and if they do act to protect germline cells. If they protect marsupials, could they hint at a defensive system conserved throughout mammals? Could similar integrations in our DNA be protecting us from infection?

Conflicts of interest

The authors declare no conflicts of interest.

Declaration of funding

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Biographies

Emma Harding is a PhD candidate in Professor Peter White's laboratory in the School of Biotechnology and Biomolecular Sciences at UNSW Sydney. Her research investigates endogenous viruses in mammalian DNA, their evolution and possible cellular functions.

Grace Yan is a PhD candidate under the supervision of Professor Peter White at the School of Biotechnology and Biomolecular Sciences, UNSW Sydney. Her research combines the use of clinical and sewage samples to study the molecular epidemiology of viral gastroenteritis.

Peter White is a Professor in Microbiology at the School of Biotechnology and Biomolecular Sciences at UNSW. His research interests are currently viral gastroenteritis, viral discovery and evolution, and the development of antiviral agents.

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Perspective

Reflections on the COVID-19 pandemic from a university academic

Ian Macreadie

School of Science, RMIT University, Bundoora, Vic. 3083, Australia. Tel.: +61 402 564 308; Email: ian.macreadie@rmit.edu.au

The COVID-19 pandemic has affected all of us, and we all have different stories. Here are some of my reflections from academia to forced 'retirement'.

At the start of the pandemic I was employed 3 days per week as a course coordinator at RMIT University. I was leading a great life with my wife and spending what time we could with our four married children, their spouses and our 12 grandchildren. My other passion was editing *Microbiology Australia* and overseeing its reach to Australian microbiologists as well as the worldwide community of readers. I had an exciting year planned, including a holiday to Iceland. At the start of 2020, little did I know what was coming.

Although we have experienced outbreaks of major infectious diseases around the world in recent history, including Ebola, Hendra, SARS, MERS and others, it has been more than 100 years since the last big one, the Spanish influenza pandemic, which is estimated to have killed 20–100 million people[1]. While we have controlled many pandemic-capable diseases through our knowledge of OneHealth, hygiene and infection control, human-to-human infections caused through airborne transmission remain a major concern.

In the early part of 2020, as the world came to realise that COVID-19 needed to be isolated, we started to see restrictions on international travel, initially from Wuhan, but becoming more general. International students form a large component of the student cohort and we saw many of them not enrolling or re-enrolling in courses for 2020. The impact of this was a huge blow to many universities whose budgets heavily depended on income from international students. RMIT University worked through the projected revenue losses by offering redundancies to staff, ceasing casual employment, stopping all travel and not renewing contracts. I was affected by the latter situation and ceased employment at the end of my contract in 2020. Many other universities reacted in a similar manner. It is sad to think of how the talents and careers of so many young scientists have been affected by these budget cuts: this will impact the next generation of educators and researchers who will be needed to maintain Australia's high reputation in education, training and research. The situation for sessional staff was particularly tragic since they did not qualify for JobKeeper.

The indefinite shutdown of face-to-face teaching at universities started in late March 2020, soon after the start of semester, leaving a challenge of how to enable the delivery of teaching and learning. Fortunately, at RMIT University, there was a long practice of recording lectures (in some cases tutorials too): indeed, for many students, listening to recordings in their own time was their preferred learning mode, while other students were strong adherents to face-to-face learning. One fall-back position was to use recordings from 2019, which could be re-played (if the curriculum had not changed). This was a good option for the immediate transition and was ideal for replacing expert guest lectures. However, it soon became essential to deliver lectures live from one's home. This reassured students that lecturers were still present and with them, but it made the engagement more difficult since it was not possible to respond to the reactions of students: to answer questions, to read their faces, to gauge their understanding. I tried to stimulate more interest using a background with a bird feeder, so occasionally students could see galahs or parrots, and sometimes even see kangaroos in the background. I know they remembered those moments!

The biggest challenge with microbiology though is the practicals. How does one manage without practicals? My 3rd year Industrial Microbiology course involved four multi-week practicals that ran, often simultaneously, throughout the semester. They enforced basic microbiology skills but also taught new skills like PCR, DNA sequencing and microbial identification using mass spectrometry. When we went into lockdown students had participated in three practical sessions only and still needed to learn and practice skills. Practicals were the favourite parts of the course and there is no substitute for hands-on activity. In addition, the work included a real research component that involved discovery of novel microbes, leading to publishable outcomes (e.g. discovery of *Cedeca colo*[2]). However, with no lab access the options were to delay the courses or to compromise through simulations.

The more widespread problems of remote teaching were discussed at ASM's EduCon meeting, held using Zoom and reported by ASM's EdSIG Convenor, Megan Lloyd[3]. Huge challenges are the mental health needs of students, engagement, and arranging

authentic assessment. In addition, some students do not have good internet access, limiting their online learning.

With regard to research outputs, my students and I spent more time reading and writing. In fact, 2020 was one of our most productive years. However, while this may look good on CVs, it may not be a good indicator of impact.

The time of lockdown restrictions provided more time in a day since there was no longer a need to travel and get fully dressed! For me it meant that I could split my time over every day. My wife and I enjoyed the permitted 2-hour exercise break each day with lovely nature walks and riding our e-bikes. However, she had a bad crash on her bike on 21 April, breaking her humerus into four pieces. She was taken by ambulance to hospital; however, with all the COVID-19 restrictions taking place she was sent home the next day to wait a week for surgery. After a difficult 5-hour operation the humerus was re-joined with a plate and 12 pins. Recovery was slow and still continues, but she has returned to the e-bike and had hoped to snow ski this year, but Victoria's 5th lockdown put an end to that. Personally, major fallouts from COVID-19 have been my wife's injury, plus denial of being able to travel to visit half of our family members spread out from Western Australia and Germany. I know others have done it much tougher, including editorial board members who have been separated from family members, and tragically some who have lost parents overseas.

As the year continued, there was some hope for return of face-to-face teaching but ultimately it was restricted to catch-up practicals for a minority of students. Some international students continued their courses and perhaps it suited them better being able to stay in their home countries. However, for many, the chance to physically study in Australia remains a major reason for choosing an Australian university for studies. An additional challenge for many of the international postgraduate students was loss of income and training due to not being able to work as tutors and demonstrators. The impoverished state of many of our international students came to the attention of some charitable organisations who provided some support.

Many Australians do not know anyone who has had COVID-19 and feel somewhat distant from the horrible deaths and morbidity that it causes. However, for many of us working with international students and international colleagues we know there is a huge impact beyond our borders. Our international students have all been affected by COVID-19. Some of our students have had COVID-19 in Australia, and some have had it in their home country. Sadly, many have lost loved ones to COVID-19. One of my students lost both parents in their home country and was unable to return to home to grieve with their families. Another is a front-line worker in a small country with an internal border restriction to limit COVID-19 spread: his work has required him to live in a hotel and to isolate from his family since March 2020. Many of us know Arny Demain, the grandfather of industrial microbiology, who died from COVID-19 complications on 3 April 2020. Arny was known and loved by many Australian microbiologists: he was ASM's Rubbo orator in 1979 and was given a tribute in *Microbiology Australia* in 2010[4].

Microbiology Australia has endeavoured to provide updates on COVID-19. The first issue (March 2020) after the start of the outbreak featured one of the first electron micrographs of SARS-CoV-2 on the front cover (Figure 1) and a Hot Topic report from Mackenzie and Smith[5] on the outbreak. Since then, every issue has provided further insights on COVID-19 and the entire first issue of 2021 (https://www.publish.csiro.au/ma/issue/10435) was devoted to COVID-19. I thank all of those experts who have contributed peer-reviewed articles to inform our community of the facts. The provision of correct information has been so important during the pandemic, when so many people are accessing misinformation. We can be encouraged that *Microbiology Australia* adheres to the strictest publication standards and is a member of COPE (Committee on Publication Ethics). All articles are peer-reviewed and all articles report conflicts of interest (if any) and disclose funding (if any). Vertical Transmission columns from our President have acknowledged and updated ASM members on the challenges that COVID-19

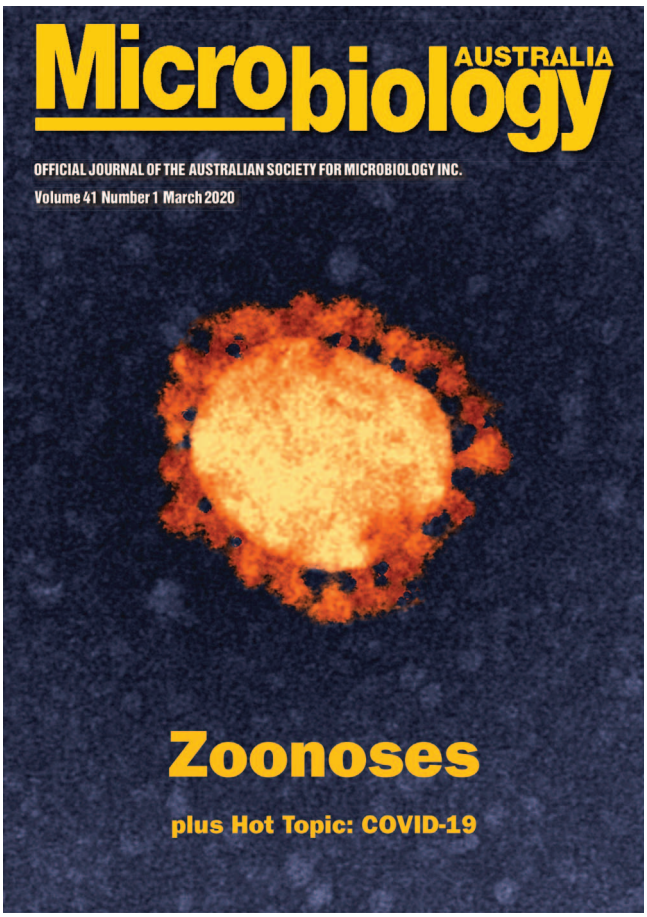

Figure 1. *Microbiology Australia* March 2020 cover.

has inflicted on ASM and its members, who include frontline workers, researchers of COVID-19 and those involved in training and teaching of future microbiologists who are being trained to respond to COVID-19 and future pandemics, whether they come from viruses, bacteria, fungi or parasites.

At the end of 2020 I left my RMIT University employment but have remained involved with RMIT University in an honorary capacity. 2021 has been a mix in Australia and continues to be a difficult year due to the slow vaccine rollout, and sporadic but highly threatening outbreaks. In Australia, our elimination strategy has seen relatively small numbers of people infected, and relatively few deaths. However, repeated lockdowns, especially in Victoria and NSW, have impacted many people in multiple different ways. Our 'zero' strategy is a stop gap strategy: it is unsustainable in the long term due to its toll on the economy and people's well-being. Most of us expect to be able to live life with COVID-19 once we have the vaccine in a large proportion of the population. However, there will be uncertainties due to the mutations of SARS-CoV-2, which could lead to greater evasion of antibody responses. Fortunately, our experts are anticipating the future needs and are poised to respond. Unlike the ongoing challenges that medical researchers face in obtaining funds for other infectious disease, COVID-19 research has received good support.

A further worry will be the unvaccinated portion of our population. As being reported by the media here and elsewhere in the world, as restrictions are lifted, the people filling hospital beds and accounting for the majority of COVID-19 deaths are from the unvaccinated population. This knowledge should spur unvaccinated Australians to seek vaccines as they become available. For some people, there are objections to COVID-19 vaccines due to the involvement of fetal cell lines, produced many decades ago, in some COVID-19 vaccines. There is no complicity of vaccine manufacturers in the production of these cell lines, so we can receive these vaccines with a good conscience: a good discussion on the ethics of these vaccines can be found in an article by Richard Zimmerman[6]. A further question will involve our approaches to vaccinate children, an increasing point of discussion in the scientific community.

The quest of vaccine workers is one of tremendously hard work, dedication and sacrifice to achieve control of deadly infectious diseases. The journeys on Australia's COVID-19 vaccine were reported by Paul Young[7]. Sarah Gilbert and Catherine Green, who led work resulting in the highly successful AstraZeneca vaccine, reported their story in their new book, *Vaxxers: The Inside Story behind the AstraZeneca Oxford Vaccine and the Race against the Virus*. The book is reviewed by Cheryl Power in this issue of *Microbiology Australia* (volume 42, issue 3).

By the end of 2021, it is expected that ~20% of the eligible Australian population will be unvaccinated. This population is of concern. Despite some protection via herd immunity, we need to continue research on efficacious drugs that can be used to treat COVID-19. The progress on COVID-19 drugs was recently reported in two *Microbiology Australia* articles[8,9].

I thank those who have allowed me to share their experiences, as well as those who have provided advice for this article: Anthony Baker, Sudip Dhakal, Tshering Dorji, Megan Lloyd, Jo Macreadie, Peter Macreadie, Ipek Kurtböke, Dena Lyras, Wieland Meyer, Cheryl Power and Ramtin Radman, Roy Robbins-Browne and Mark Shembri.

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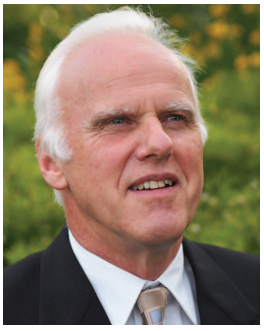
Biography



Ian Macreadie is an Honorary Professor at RMIT University and Editor-in-Chief of *Microbiology Australia*. His research and expertise are in diverse fields of biosciences ranging from industrial microbiology to biomedical research.

ASM2021: event report

Karena Waller
Chair, Local Organising Committee (LOC), ASM2021

The Victorian Local Organising Committee (LOC) and the Australian Society for Microbiology (ASM) Executive are truly delighted to have been able to hold the 2021 ASM Annual National Scientific Meeting and Trade Exhibition (ASM2021), from Monday 31 May to Thursday 3 June 2021. Although we collectively had high hopes (especially after the postponement of the ASM2020 Meeting) of delivering this year's Meeting in hybrid (in-person and online attendance) format from the Melbourne Convention and Exhibition Center, the imposition of COVID-19 restrictions in Melbourne just 4 days prior to the commencement of the Meeting required rapid transitioning of the Meeting to wholly online delivery.

This year's Meeting delivered a diverse and stimulating scientific program, which featured many leading microbiologists from around Australia and globally presenting their latest research findings, as well as 2 years' worth of ASM award winners' presentations. The program was rounded out by inclusion of the online poster sessions, Rubbo Oration and celebration, Student and Early Career Researcher events, and the trade exhibitions and social functions. Despite the online format, selection and building-in of the delegate-focussed online delivery platform during the early stages of planning by the LOC for the Meeting ensured there was plenty of opportunity for discussion, interaction and re-connection with colleagues.

The Meeting kicked-off at noon on Monday with a Public Lecture by Nobel Laureate Professor Peter Doherty, who insightfully explored *The journey so far with COVID-19, and where [to] next?* The public lecture can be viewed at ASM's link: www.theasm.org.au. Professor Doherty's lecture was then followed by the formal Meeting Opening Address and the presentations of the 2020 ASM Fenner, Pittard and Gilbert Award recipients, as well as the presentations of the 2020 Nancy Millis Award recipients. The evening's Bazeley Oration by Professor Daniela Ferreira, in which she explored the makings of the Oxford COVID-19 vaccine, rounded out a stellar sequence of presentations on the opening day of the Meeting.

Tuesday's program featured the Student and Early Career Researcher (ECR) events, including the presentations of the 2021 Nancy Millis Student Awards and the Nancy Millis Student and ECR lunch. The day's program was rounded out by presentations from the 2021 Fenner and Pittard Award recipients, and an opportunity to interact and re-connect with colleagues via the super funky *SpatialChat* platform used in the concluding social function of the day.

Wednesday's program featured the Annual General Meeting (AGM) of the ASM, and the Rubbo Oration, by Professor Eddie Holmes. Professor Holmes' presentation was an excellent and engaging overview of the evolution and emergence of RNA viruses, including the SARS-CoV-2 (COVID-19) virus. The evening wrapped up with the Rubbo Celebration, which allowed those keen to test their trivia knowledge via a *Kahoot!* quiz before proceeding to the concluding social function.

In addition to the many opportunities for discussion, interaction and re-connection with colleagues embedded into the Meeting's program, opportunities to interact and engage with the many trade exhibitors and sponsors was also provided via the online delegate platform. We sincerely thank our trade exhibitors and sponsors for their ongoing support and participation in the Annual Meeting.

Delivery of this year's Meeting, the first-ever wholly online ASM Annual Meeting, represents the culmination of almost 3 years of work for the members of the LOC (inclusive of the LOC's work developing the ASM2020 Meeting which was postponed due to the pandemic) - this truly has been a heroic, team effort! I would like to take this opportunity to thank each team member for their 'above and beyond' efforts over the last few years, in developing and delivering (the ASM2020) and ASM2021 Meetings: Maria Liaskos, Steve Petrovski, Catherine Satzke (Scientific Program Committee), Christine Seers (Workshops), Sarah Baines (Students & ECRs, Social/Bits'n'bobs/Advertising), Jacqueline Heath (Posters & Awards, Workshops), Mary Valcanis (Posters & Awards, Social/Bits'n'bobs/Advertising) and Lauren Zavan (Students & ECRs).

On behalf of the LOC, I also thank and acknowledge the support of the ASM Executive and in particular Dena Lyras, Kate Seib and Anthony Baker. I also thank the team from ASN Events (especially Kara Barker, Alycia Manuel, Gemma-Ann Taylor and Nitesh Patel) for their enormous efforts in organising this conference and flipping us so seamlessly wholly online just 4 days prior to the commencement of the Meeting!

We did it!

ASM Summer Student Research Awards: 2021

Priscilla Johanesen

Chair, ASM Standing Committee for Professional Development

There is no time like the present to foster the development of future microbiology researchers. The ASM Summer Student research awards are a fantastic opportunity for students to join a research group and experience what is like to work in a research environment. This year the society awarded more summer student awards than ever before with national office and the state branches together offering seventeen ASM Summer Student Research Awards across Australia. As in previous years the microbiology research projects that students undertook represented the diversity of the discipline across food, veterinary and medical microbiology. This year the successful awardees were: Callum Kay, Daniel Neville, Lucy O'Shannessy and Tsung-Yu Pai from New South Wales; Daniel Ellis, Jin Gu and Stephen Carpenter from Queensland; Jack Waters from South Australia; Naomi Foo and Primrose Mandalawatta from Tasmania; Jacinta Agius, Taylah James, Sameer Mohammad Khan and Cecil Wheeler from Victoria, and Jake Cummane, Merrin Mary Eapen and Beatrice Alexandra E. Panganiban from Western Australia. The society would like to congratulate all of the winners of the 2021 Summer Student Research Awards and wish them all the best for the future.

New South Wales

NLRP3 inflammasome activation by the bacterial toxin phospholipase C

Callum Kay[A,B] and Si Ming Man[A]

[A]Department of Immunology and Infectious Disease, The John Curtin School of Medical Research, The Australian National University, Australian Capital Territory, Australia
[B]Medical School, The Australian National University, Australian Capital Territory, Australia

Inflammasome signalling is a central pillar of innate immunity leading to inflammation and cell death. Identifying and characterising new activators of the inflammasome is critical in elucidating the molecular basis of innate immune recognition of pathogens and to inform the development of novel therapeutics. Previously, I screened a panel of toxins from phylogenetically diverse organisms and identified phospholipase C (PLC) from the bacterial pathogen *Clostridium perfringens* as an activator of the NLRP3 inflammasome. My existing data suggest that phagocytosis and endo-lysosomal trafficking of the toxin are required for PLC-mediated inflammasome activation. PLC then likely induces lysosomal membrane disruption and potassium efflux as a signal to trigger NLRP3 inflammasome assembly. In this study, I obtained new data to strengthen this model. In particular, I used correlative light and electron microscopy to demonstrate that PLC localised with vesicular structures resembling lysosomes in macrophages. I also verified PLC as a *bona fide* NLRP3 activator using recombinant PLC produced by an alternative commercial source. Additionally, I examined the role of the plasma membrane rupture mediator protein NINJ1 in PLC-mediated cell death. Together, these data further elucidate the mechanism by which a pathogen-derived phospholipase can be detected by the mammalian innate immune system.

The alcohol dehydrogenase adhE encodes a regulatory small RNA within the 3′UTR that is induced by heat shock in enterohaemorrhagic *E. coli*

Daniel Neville, Brandon Sy and Jai Tree

School of Biotechnology and Biomolecular Sciences, UNSW, New South Wales, Australia

Enterohaemorrhagic *E. coli* (EHEC) is the most prevalent etiological agent of infectious haemolytic uremic syndrome. EHEC elaborates a Shiga toxin that antagonises the renal endothelium, preventing protein synthesis that leads to cell apoptosis and potentially renal failure. Post-transcriptional regulation plays an important role in EHEC pathogenesis and is controlled by regulatory small RNAs (sRNA). A novel sRNA (here termed AdhU) was identified within the 3′UTR of the highly conserved bifunctional aldehyde/alcohol dehydrogenase AdhE. Deletion of *adhE* attenuates EHEC pathogenesis. The mechanism of this attenuation is unknown, and we hypothesise that AdhU may play an important role in virulence gene regulation. Here we have demonstrated that RNase E is not required for the AdhU biogenesis but in doing so, observed heat shock-dependent induction of AdhU expression in EHEC. This indicates that AdhU is controlled by a heat shock responsive promoter. We have used RLM-RACE to verify the presence of multiple RNA 5′-ends upstream of the *adhE* 3′UTR and RNA-seq experiments to identify the regulatory

targets of AdhU are ongoing. These are expected to further elucidate the role of AdhU in EHEC pathogenesis.

Investigation of mechanism of phagocytosis of *Coxiella burnetii* by bovine mammary epithelial cells

Lucy O'Shannessy, Katrina Bosward and Paul Sheehy

The University of Sydney, New South Wales, Australia

Q fever is a globally important zoonotic disease caused by the bacterium *Coxiella burnetii*. Livestock are reservoirs for human infection, with shed bacteria transmitted primarily *via* inhalation of contaminated aerosols, while ingestion is a less common and poorly understood transmission route. *Coxiella burnetii* shows high prevalence in unpasteurised dairy products worldwide, thereby presenting a possible public health risk. Bovine mammary epithelial cells (bMECs) have been hypothesised to be a replicative niche in the mammary gland, hence this study aimed to characterise the mechanism mediating their uptake of *C. burnetii*. Expression of Leukocyte Response Integrin ($\alpha_V\beta_3$) and Complement Receptor 3 (CR3) (receptors mediating uptake in phagocytic cells) was confirmed on the bMEC line, MAC-T, in 2D and 3D organoid culture by immunohistochemical analysis, using monoclonal antibodies targeting CD61 and CD11b (integrins forming $\alpha_V\beta_3$ and CR3, respectively). A phagocytosis assay was then conducted by pre-incubating MECs with antibody to block $\alpha_V\beta_3$ and CR3, followed by inoculation with fixed and fluorescently labelled Nine Mile phase II (Clone 4) *C. burnetii*. Despite co-incubation with antibody, *C. burnetii* was still taken up, highlighting the complexity of the uptake mechanism. Future work is required to optimise the competitive inhibition model and allow characterisation of uptake mechanisms.

Metal-based antifungal drug testing in insect model (*Galleria mellonella*)

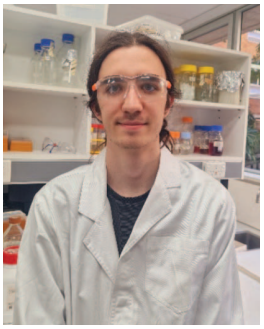
Tsung-Yu Pai[A,B], Wieland Meyer[B] and Alex Kan[B]

[A]University of Technology Sydney, New South Wales, Australia
[B]Molecular Mycology Research Laboratory, Centre for Infectious Diseases and Microbiology, Faculty of Medicine and Health, Sydney Medical School, Westmead Clinical School, Marie Bashir Institute for Infectious Diseases and Biosecurity, The University of Sydney, Westmead Hospital, Research and Education Network, Westmead Institute for Medical Research, Westmead, New South Wales, Australia

Upon the search for novel antimicrobials due to the urgent need for new therapeutic mechanisms to address the pressing issue of antibiotic resistance, cobalt-based complexes were found to present antifungal activity *in vitro* in previous work. Various cobalt complexes were tested by Tsung-Yu Pai together with Alex Kan under the supervision of Prof. Wieland Meyer (Molecular Mycology Research Laboratory, Centre for Infectious Diseases and Microbiology, Westmead Institute for Medical Research) for the therapeutic effect against four clinically important fungal species (*Candida albicans, C. auris, Cryptococcus gattii* and *C. neoformans*) in the moth larval model, *Galleria mellonella*. Due to the short timeframe and the limited moth larvae supply, only two test compounds could be tested once. The result showed no significant difference in the survival rates of moth larvae between fluconazole treated and non-treated groups. Therefore, in subsequent experiments the optimal inoculating concentrations of the fungal strains need to be refined, more replicates will need to be conducted, and additional compounds will be included.

Queensland

Genomic diversity and potential antimicrobial targets in non-typeable *Haemophilus influenzae*

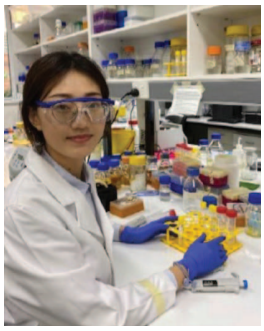


Daniel Ellis and Ulrike Kappler

School of Chemistry and Molecular Biosciences, The University of Queensland, Queensland, Australia

Antimicrobial resistance is an increasing concern in the human specific respiratory pathobiont, *Haemophilus influenzae* (NTHi), with the WHO naming NTHi as a priority pathogen for the development of new antimicrobial therapies. A possible new antimicrobial target in NTHi may be the sulfoxide reductase *Hi*DmsABC, which has previously been shown in our lab to be important for host-pathogen interactions despite its substrate being unknown. Here we have looked at the genomic diversity of NTHi and the distribution of *dmsABC* genes within NTHi genomes, as well as characterised the activity of *Hi*DmsABC with several potential substrates that occur in the human respiratory tract. The results revealed that while NTHi is highly genetically diverse, with a core genome of 1258 genes and an accessory genome of 3453 genes, but *Hi*DmsABC was found to be conserved across all genomes. *Hi*DmsABC showed promising activity with several of the substrates tested with K_M values in physiologically relevant ranges, suggesting several possible roles for *Hi*DmsABC in supporting virulence.

Investigation into the role of metal homeostasis in *Haemophilus influenzae*



Jin Gu and Ulrike Kapplert

School of Chemistry and Molecular Biosciences, The University of Queensland, Queensland, Australia

Haemophilus influenzae (Hi) causes a variety of respiratory diseases and is highly resistant to reactive oxygen species which are generated by human immune cells. Mn ions have been implicated in oxidative stress resistance, and Mn uptake in *H. influenzae* relies on the PsaABCD, ABC-type Mn transporter. Mn (II) ions serve as a cofactor of superoxide dismutase (SOD), and the cellular abundance of manganese regulates the activity of SOD. In the project, a mutation in the *psaA* gene was complemented, which reversed the sensitivity to superoxide stress observed for Hi2019$^{\Delta psaA}$ to wildtype (WT) levels, proving that Mn uptake is essential for oxidative stress resistance in *H. influenzae*. Despite this, following growth on Brain-Heart Infusion medium, no difference in SOD activity was observed for the WT and mutant strain. We propose that this may be due to relatively high content Mn in this medium that may have compensated for reduced uptake rates. It is likely that Mn may have been taken up from BHI medium via other, low specificity transport mechanisms.

Exploring bacterial transporters in antibiotic resistance in mycobacteria

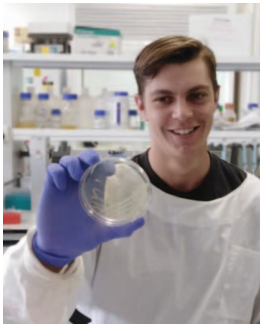
Stephen Carpenter, Giorgia Mori and Antje Blumenthal

UQ Diamantina Institute, The University of Queensland, Queensland, Australia

Tuberculosis (TB) remains a major public health concern around the globe, with the prevalence of drug-resistant tuberculosis increasing at alarming rates. Efforts in defining improved and new treatment opportunities for TB include not only the search for new antibiotics, but also strategies for increasing the sensitivity of *Mycobacterium tuberculosis* (*Mtb*) to existing antibiotics. This project focused on molecular mechanisms by which antibiotics are transported into *Mtb*, specifically the contributions of proteins that actively transport cargo into bacterial cells. To this end, plasmids for the expression of *Mtb* candidate genes were generated and transformed into *Mycobacterium bovis* Bacille Calmette Guerin (BCG) to achieve bacterial clones that overexpress putative importers. *Mtb* gene expression in BCG clones was confirmed by qPCR. Select BCG clones were then assessed for their sensitivity to current TB antibiotics by determining minimum inhibitory concentrations (MIC) in a liquid culture assay. The preliminary observations arising from this summer project encourage further exploration of importers in the uptake of antibiotics and antimicrobials by *Mtb*.

South Australia

Regulating fatty acid acquisition in *Acinetobacter baumannii*



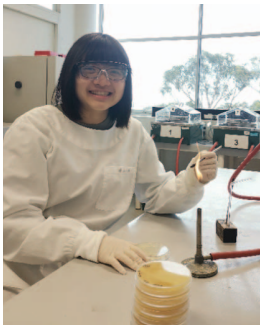
Jack Kenneth Waters and Bart Antonie Eijkelkamp

College of Science and Engineering, Flinders University, South Australia, Australia

Acinetobacter baumannii is an opportunistic nosocomial pathogen responsible for highly drug resistant infections. The World Health Organization has placed *A. baumannii* at the top of its list for the urgent development of new antimicrobials, highlighting a need to fully understand this unique pathogen. The bacterial membrane, which presents a primary barrier to antibiotic entry, has been overlooked in its role in resistance. In Gram-negative bacteria, environmental fatty acids are acquired foremost through the outer membrane protein FadL, but how this system is regulated, and its substrate preference is poorly understood. Analyses of distinct fatty acids in wild-type and a *fadL* mutant derivative confirmed a role in the acquisition of long-chain fatty acids in *A. baumannii*. The development of a fluorescent reporter system has provided novel insights into *fadL* regulation. Preliminary data suggests that *fadL* is regulated at the translational rather than transcriptional level, owed to a series of stem loops found in its upstream region. Overall, this placement project has identified that *fadL* is subjected to multilevel regulation, which is, at least in part, attuned to its substrate range.

Tasmania

Isolation and identification of bacteria and yeasts in kefir grain and milk kefir

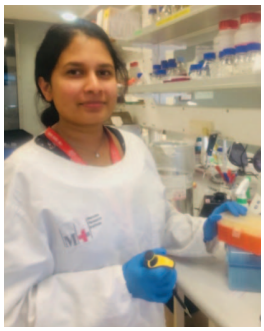


Naomi Foo, Faisal Abdulrahman J Alraddadi, John Bowman and Shane Powell

Centre for Food Safety and Innovation, Tasmanian Institute of Agriculture, University of Tasmania, Tasmania, Australia

To elucidate the composition and diversity of kefir grain microbiota, this study employed selective culturing techniques to isolate lactic acid bacteria (LAB), acetic acid bacteria (AAB) and yeast from kefir grains and at different stages of milk kefir fermentation, to be utilised in subsequent experiments. 16S rRNA gene sequencing was used to aid in the identification of the said isolates. Bacterial species known to be prevalent in kefir grains and milk kefir, *Lactobacillus kefiranofaciens*, *Leuconostoc mesenteroides* and *Lactococcus lactis* were detected. The primary lactose-fermenting and non-lactose fermenting yeasts *Kluyveromyces marxianus* and *Torulaspora delbrueckii* were also identified. These findings were in line with the results of previous studies, indicating that a portion of the kefir grain ecosystem is relatively stable. Minor bacterial species like *Pseudomonas putida* and *Cephalothrix lacustris*, which are believed to be environmental contaminants, were also isolated. However, no AAB were isolated possibly owing to low cell viability or lack of use of suitable selective media. Future studies employing a polyphasic approach to microbial detection and identification are necessary to overcome the limitations of culture-based techniques.

Pathogenic potential of *Pandoraea fibrosis*



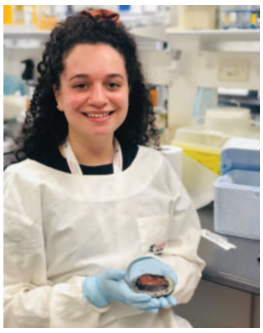
Primrose Mandalawatta and Louise Roddam

School of Medicine, University of Tasmania, Tasmania, Australia

Cystic fibrosis (CF) is an inherited life-limiting single gene disease in Caucasian populations and is caused by dysfunction or absence of the CFTR protein. This results in thickened mucus lining the airways and a propensity for people with CF to have lung infections with a variety of respiratory pathogens and inflammation. *Pandoraea* were recently described and are being increasingly isolated from CF clinical samples and are able to cause chronic infections, bacteraemia and probably contribute to damage and pulmonary decline. To-date, there have been 3 Tasmanian CF patients with documented *Pandoraea* lung infections. The first patient had two isolates collected and these isolates represented a new species, *P. fibrosis*. The newer isolates are yet to be assigned to a species. In this study the bacterial *gyrB* gene was used to determine species of these new isolates. The *gyrB* gene of isolate 366 has 98.81% sequence similarity to *P. fibrosis* and *gyrB* gene of isolates 511 and 329 has 99.45% sequence similarity to *P. apista*. 511 and 329 were 100% identical to each other, supporting these isolates as being clonal and associated with a chronic infection. Overall, this study also investigated biofilm formation and regulation by *Pandoraea* isolates and our results show that *Pandoraea* responds to exogenous QS signals, such as those produced by other CF pathogens even if unable to synthesise their own.

Victoria

Immune priming strategies to combat herpesvirus infection in the abalone



Jacinta Agius and Karla Helbig

Department of Physiology, Anatomy and Microbiology, La Trobe University, Victoria, Australia

Molluscan herpesviruses infect species of oysters and abalone resulting in mass mortality events that have been witnessed worldwide, including in Australia. Australian abalone are highly susceptible to Haliotid herpesvirus (HaHV-1) with a 90% mortality rate upon infection. Despite the impact of this virus, practical preventative and treatment options do not exist. In this study, an *in vivo* HaHV-1 infection model was optimised through the successful production of infectious HaHV-1 viral water. It was demonstrated for the first time, that intramuscular poly(I:C) priming has a potential to protect HaHV-1 challenged abalone when compared to phosphate-buffered saline (PBS) primed abalone. This study presents a novel method to prevent HaHV-1 infection in the abalone and therefore provides insight into future immune priming studies.

Examining the movement and diversity of *Streptococcus pyogenes* mobile genetic elements within remote Indigenous Australian communities

Taylah B James and Jake A Lacey

Department of Infectious Diseases at the Peter Doherty Institute for Infection and Immunity, The University of Melbourne, Victoria, Australia

Indigenous communities within remote northern Australia are disproportionately affected by *Streptococcus pyogenes* (Strep A) infections, with impetigo and immune-mediated diseases persisting at hyperendemic rates. In non-endemic settings, specific Strep A *emm* types have been linked to specific disease outcomes. However, in endemic settings like northern Australia where multiple *emm* types circulate, associations do not match those observed in non-endemic settings. We aimed to inform the epidemiology of Strep A disease by investigating the relationships of virulence factors, mobile genetic elements (MGEs) and *emm* types from two endemic communities within northern Australia via whole genome sequencing. We

found virulence factors persisted for three times longer that *emm* type in each community and were not restricted to a particular serotype. Isolates of the same serotype, sampled from the same date in the same household were found to differ in their accessory genome relating to bacteriophage elements. Phage mobility, particularly phage transduction may be responsible for virulence factors persisting for longer periods in both communities despite the rapid turnover of *emm* types. We concluded this study provides a strong baseline for future work to re-examine the association of virulence factors and disease presentation.

IFNɣ reduces T3SS effector translocation and suppresses MNGC formation in macrophages infected with *Burkholderia thailandensis*

Sameer M Khan[A,B], Garrett Z Ng[B] and Elizabeth L Hartland[B,C]

[A]The University of Melbourne, Victoria, Australia
[B]Centre for Innate Immunity and Infectious Diseases, Hudson Institute of Medical Research, Victoria, Australia
[C]Department of Molecular and Translational Science, Faculty of Medicine, Nursing and Health Sciences, Monash University, Victoria, Australia

Melioidosis is an often fatal disease caused by the intracellular pathogen *Burkholderia pseudomallei*. *B. pseudomallei* invades the cytosol of host cells using the *bsa* encoded type 3 secretion system (T3SS), and subsequently spreads intercellularly, causing the formation of multinucleated giant cells (MNGCs). These processes are similar in *B. thailandensis*, which is used to model *B. pseudomallei* infection. Interferon-γ (IFNγ) is critical for surviving *Burkholderia* infection, though how it restricts infection is unclear. Our aim is to examine how IFNγ affects fundamental aspects of pathogenesis: the translocation of T3SS effectors and formation of MNGCs. MNGCs were quantified by counting cells with multiple nuclei. IFNγ stimulated RAW264.7 cells formed fewer MNGCs when infected with *B. thailandensis*. To assess effector translocation, *B. thailandensis* strains expressing a BopE- β-lactamase (TEM1) fusion protein were created. Translocation was then quantified by staining infected RAW264.7 cells with the green cellular dye, CCF2, which fluoresces blue when cleaved by TEM1. This revealed that IFNɣ reduced BopE-TEM1 translocation by wildtype *B. thailandensis*. Confoundingly, IFNɣ increased blue CCF2 signal when a T3SS-deficient *B. thailandensis* was used, perhaps indicating increased vacuolar instability of the T3SS mutant under IFNɣ stimulation. These results suggest that IFNɣ may inhibit the earliest stages of cellular pathogenesis to suppress *Burkholderia* infection.

Prevalence of internal parasites in captive dingoes

Cecil Wheeler and Richard Bradbury

Federation University, Victoria, Australia

Dingos have the ability to carry zoonotic parasites however, the prevalence of *Strongyloides stercoralis* is uncertain. The aim of the study was to assess the prevalence of *S. stercoralis* in a sample of captive dingos. Forty faecal samples were assessed for parasites using Sheather's sucrose solution egg flotation tests, Koga agars and the Baermann technique. Flotation tests showed eggs of *Toxocara canis* (15%), *Trichuris vulpis* (2.5%), hookworm (10%), *Taeniids* (7.5%), Strongylid eggs (5%) and oocysts of *Cytoisospora canis* (17.5%) and another *Cytoisospora spp*. (8%). The Koga plates and Baermann's sedimentation recovered hookworm larvae but did not identify the Strongylids. The addition of 10 mg/L of amphotericin B to Koga agar was effective in preventing fungal overgrowth and allowed recovering of larvae. The standard Baermann was found to be more effective at recovering hookworm larvae than the modified method. Further investigations using PCR and sequencing to confirm species and larger sample numbers will assist in clarifying the dingo's status as an intestinal helminth reservoir.

Western Australia

Optimisation of protein crystallisation for X-ray structure determination of FseA, a novel and widespread DNA-binding protein controlling gene transfer throughout the proteobacteria

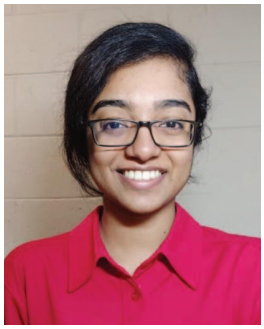
Jake Cummane, Karina Yui Eto and Joshua Ramsay

Curtin Health Innovation Research Institute, Curtin University, Western Australia, Australia

The protein domain-of-unknown-function-2285 (DUF2285) is present in both transcriptional activator and quorum-sensing antiactivator proteins and is widely distributed in the proteobacteria. FseA is a DUF2285-domain protein of *Mesorhizobium japonicum* that binds DNA and stimulates horizontal gene transfer. FseA shares no sequence similarity with structurally characterised DNA-binding domains, suggesting DUF2285 members represent a new family of transcriptional activators. In this work we purified a maltose-binding (MBP) fusion derivative of FseA, 6H-MBP-FseA, and using size exclusion chromatography confirmed 6H-MBP-FseA

was predominantly present as a 150-kDa dimer in solution. Incubation of 6H-MBP-FseA in the presence of its 32-bp DNA-binding target, led to an increase in the molecular weight of 6H-MBP-FseA and confirmed formation of the nucleoprotein complex that can be prepared for crystallisation. The purified 6H-MBP-FseA was used in 96-well crystallisation conditions and fine screening of two conditions. Although the obtained crystals did not diffract at the X-ray beam line, we uncovered new crystallisation conditions that could be further optimised. Optimisation of 6H-MBP-FseA crystallisation will advance efforts to obtain the three-dimensional structure of FseA through X-ray crystallography and provide structural insight into the DUF2285-domain family.

Identifying how the Sil1 nucleotide exchange factor can regulate the permeability of ER translocase in *Saccharomyces cerevisiae*



Merrin Mary Eapen, Mark Bienawski and Carl Mousley

Curtin Medical School, Curtin University, Western Australia, Australia

Sec61 complex is a heterotrimeric translocon on the ER membrane of *Saccharomyces cerevisiae*. The Sec61 complex gates the movement of GSH through its interaction with Kar2, an Hsp70 protein folding chaperone. Sil1 is a regulator of ATPase activity in Kar2. This project aimed at identifying how the redox state of the endoplasmic reticulum can affect the glycosylation of Sil1 and also examined its effects on the ER membrane permeability. Unglycosylated Sil1 was seen to accumulate in the *sss1-7* strain, as its mutation stabilises the Sec61 complex in its open conformation which allows GSH influx into the ER lumen. In order to check how the modifications in GSH concentration can affect Sil1 glycosylation, wild-type cells that over-express HGT1 were incubated in increasing concentrations of GSH, followed by protein extraction and immunoblotting of Sil1. Cells incubated in high concentrations of GSH were seen to accumulate uSil1. In both the cases, GSH influx would have created a reductive stress which then altered the glycosylation of Sil1. This project demonstrates that Sil1 coordinates the regulation of ER membrane permeability in conjunction with changes in its redox environment.

Deciphering the chemical crosstalk between bacterial cell–cell signalling systems

Beatrice Alexandra E. Panganiban, Tahlia Bastholm and Joshua Ramsay

Curtin Health Innovation Research Institute, Curtin University, Western Australia, Australia

Bacterial populations detect high cell density using a chemical communication system called quorum sensing (QS). LuxRI-family QS systems produce and respond to membrane-diffusible molecules called *N*-acyl-homoserine-lactones (AHLs). Diverse LuxR proteins respond to distinct AHL molecules produced by cognate LuxI proteins. The *Mesorhizobium* MqsRIC system is a novel LuxRI-family system that produces an unsaturated AHL 5-cis-C12-HSL and uniquely requires a second QS-regulated AHL synthesis protein, MqsC. It is unclear how specific the MqsR receptor is for 5-cis-C12-HSL or if other LuxR receptors can recognise 5-cis-C12-HSL. In this work we used bioassay reporter systems to compare specificity of *Mesorhizobium spp*. LuxR-family proteins MqsR and TraR and the *Pseudomonas* LasR and QscR. MqsR only responded strongly to 5-cis-C12-HSL, and only slightly to C12-HSL, and to the AHL extracts from *Mesorhizobium* strains encoding both the MqsI and MqsC proteins. In contrast, LasR and QscR proteins were activated by all C12-length AHLs tested including 5-cis-C12-HSL, but were not activated by C6-HSL. TraR responded to C6-HSL, 3-oxo-C6-HSL and 3-oxo-C12-HSL but not C12-HSL. In summary, the experiments suggest that MqsRIC system is highly specific for the unsaturated 5-cis-C12-HSL molecule, while the LasR, QscR and TraR proteins are less discriminant and recognise a combination of acyl-chain length and third-carbon modification.

Future issues of *Microbiology Australia*

November 2021: South-East Asia and Pacific infectious diseases
Guest Editors: Sam Manna, Cheryl Power and Catherine Satzke

March 2022: Advanced microscopy and Novel methods in microbiology
Guest Editors: Linda Blackall, İpek Kurtböke and Wieland Meyer

May 2022: Food microbiology
Guest Editors: Tom Ross and Prue Bramwell

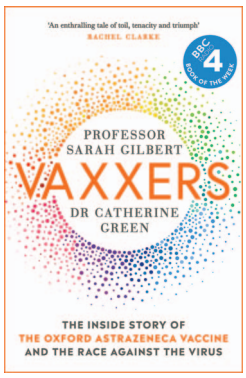
Book reviews

Vaxxers: The inside story of the Oxford AstraZeneca vaccine and the race against the virus

Authors: Sarah Gilbert and Catherine Green, with Deborah Crewe
Publisher: Hodder and Stoughton, 2021
Paperback ISBN 9781 529369878. 336 pages, including 3 appendices, comprehensive notes and an index. Also available as a hardback.

In the Foreword of this book is the following quote:

Better to light a candle than curse the darkness — Anon.



Sarah Gilbert, Professor of Vaccinology at Oxford University, and her colleague, Catherine Green, certainly followed this adage, creating a vaccine that has given the world hope and the means to save lives and livelihoods. Furthermore, its accessibility is ensured by a commitment by AstraZeneca and Oxford University to provide the vaccine on a not-for-profit basis for the duration of the pandemic, and to low- and middle-income countries at no profit in perpetuity.

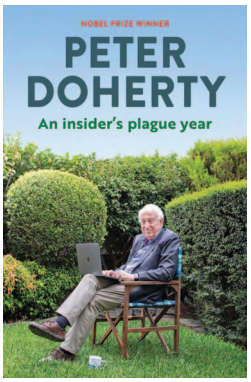
Gilbert and Green have written a truly thrilling and intimate record of how they created a highly effective vaccine in record time against a virus that has brought the world to its knees. For fellow scientists it is a fascinatingly detailed account of how the vaccine was conceived, developed, and produced, then trialled and manufactured and finally distributed. For readers from other backgrounds, particularly those labelled 'vaccine hesitant', the book carefully addresses every possible source of concern about the testing and trialling processes and the ultimate safety of the vaccine. A non-scientist friend to whom I lent my copy of the book said it demystified their research and that the explanations were clear and logical. She found the book informative and very accessible.

Gilbert and Green see their triumphant effort simply as the work that they needed to do to protect their fellow humans, certainly not as a route to fame and fortune. In fact, they both state that they look forward to the time when their lives and laboratory work regain their usual momentum. As they shared the task of bringing the vaccine to the world, Gilbert and Green also shared the task of writing the book, covering their area of input and often giving glimpses into very personal life experiences. Gilbert's work coffee mug, typically an interesting insight into the user's priorities and personalities, has the message, 'Keep calm and make Vaccines'. At a time when we are being told to 'Keep calm and get vaccinated', it is sad that their Herculean effort is not seen more generally as the gift that it is, and its outcome, a safe and effective vaccine, not gratefully taken up by everyone without irrational and selfish objections.

Reviewed by Cheryl Power, Department of Microbiology and Immunology, Peter Doherty Institute for Infection and Immunity, The University of Melbourne

An insider's plague year

Author: Peter Doherty
Publisher: Melbourne University Press, 2021
ISBN 9780522877519. 256 pages



A large section of this book consists of a series of essays that were first published on the Doherty Institute website in Peter Doherty's weekly column 'Setting it straight', but he has added to these and extrapolated into the wider context that the current pandemic predicament sits. Along the way this Nobel laureate gives insight into the work of current and past Scientists; the insights gleaned from newer available technology; explains the current knowledge base and how these fit into the apparent speed of the institutional and scientific response to the COVID-19 pandemic. The last section of the book anticipates the future.

The intention of the original articles was to introduce in an 'amiable way' the whole area of infection and immunity to a lay reader, but in particular to shed some light for others not so intimately aware of the scientific response and the newer enabling technology that is behind the scenes of 'what's happening in this difficult time'.

Doherty reminds us of how the current COVID-19 pandemic fits in the human historical context, relates the current understanding of viral infections and of how vaccines work. He has made considerable effort to provide a gentle introduction to some very heavy science in a nonpatronizing and relaxed way. The scientific jargon is kept to a minimum, and what is included is explained in a rational way. The informal cross-referencing in the text keeps his readers on the right track when a clear understanding of a previously made concept comes into play again.

The reader obtains specialist information in small, guided parcels that engages interest through discussion of a very wide range of topics: personal insights into the life of a Nobel laureate, the history of science, people behind the scenes, and the work being done at the Peter Doherty Institute and elsewhere past and present.

The book provides an overview of a specialist scientific area in a way that the reader should be able to draw their own conclusions on events and decisions made in the public arena of this current pandemic. In the later chapters he links environmental and social aspects of the 'why' of such pandemics; and doesn't shy away from the approach needed, and other problems that are intertwined with getting out of the current crisis and of avoiding others.

Peter also asks that we use science intelligently to solve other major problems without politicising the questions. He links the current predicament to big picture issues such climate change, biodiversity loss, as well as to social problems including housing and insecure incomes. He makes a comment on what should be the foreseeable damage done to Australia's future due to the lack of support for the University sector (and not just for the sciences).

I thoroughly recommend that you read this book and think on the many issues, and the whole of the message it quietly conveys.

Reviewed by Barbara Porter, retired microbiologist but still an active reader and follower of all things microbiological

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