The Broad Street Pump

Advancing the One/Eco Health agenda in Australia

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The idea that humans, animals, plants and the environment are inextricably linked is not new, but in recent years its profile has been raised as “One Health” (also “Eco-Health” and “Planetary Health”). The concept is simple – that the health of humans is dependent upon healthy animals, healthy plants and a healthy environment and together they must be considered as an integrated ecosystem.

The 21st century presents us with profound global challenges – including food and nutrition insecurity, emerging infectious diseases and antimicrobial resistance. The World Economic Forum ranks the spread of infectious diseases second only to water crises in its list of global risks with the biggest potential for impact. Despite its importance to sustainable health and well-being, the concept of One/Eco Health has little traction in Australia. It is important to understand the reasons for this lack of traction and to facilitate engagement between the scientific community, policy makers and the public to develop a strategic roadmap for politicians and other influential stakeholders. In particular, researchers and service providers across the spectrum of human, animal, plant and ecosystem health must work together to define a common vision and forge new funding pathways.
In 2010 the U.S. Centers for Disease Control and Prevention held a meeting on operationalizing One Health at Stone Mountain (Georgia, USA). Members from the World Organisation for Animal Health (OIE), the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (among others) met to discuss the steps required to develop Global One Health actions and policies, working through individual countries. It was determined that each country would need to undertake: 1) a needs assessment to identify specific areas for targeting improvement, 2) capacity building to identify how to best leverage existing programmes, 3) a proof of concept study to show that One Health interventions lead to better cross-species health outcomes, and 4) a business plan to clearly articulate this information to policymakers and potential donors.

Sydney’s Marie Bashir Institute (MBI) has been at the forefront of an interdisciplinary approach to research, capacity building and advocacy for human, animal and environmental health. In September 2015, MBI hosted a whole-day workshop at the University of Sydney, entitled “Taking a wider view: advancing the One/Eco Health agenda in Australia” which was attended by the Chief Veterinary Officer and representatives from the Commonwealth Chief Medical Officer’s office, the Department of Foreign Affairs and Trade, the National Health and Medical Research Council (NHMRC), the NSW Department of Primary Industries, the NSW Ministry of Health, the Office of the Chief Scientist, from Universities and research institutes across the country and from Industry, amongst others.

The workshop was focussed on how Australia can engage with, and become a world-leader in One/Eco Health research and program delivery. Three multidisciplinary expert panels were asked to consider a suite of open-ended questions or statements on food security, emerging infectious diseases or antimicrobial resistance. Presentations were followed by multidisciplinary group discussions and feedback. Information was collated and will be submitted for publication in an internationally prestigious peer-reviewed journal. This publication will serve as part of the needs assessment to advance the One Health agenda.

Of the three topics discussed, it was agreed that particular attention should be paid to emerging infectious diseases, since a national strategy for containing and reducing antimicrobial resistance (AMR) is well-advanced (implementation of this strategy commenced recently). Food and nutrition security, though integral to Eco/One health, was felt to be a large topic in itself, worthy of separate consideration.
Indeed, Australia’s AMR strategy, released in June 2015, is one of the first national policies created through the collaborative efforts of stakeholders across the animal and human health, food and agriculture sectors, based on the understanding that containing the threat of antimicrobial resistance needs a One Health approach. The same understanding and collaboration is needed to address emerging infectious diseases. With the key stakeholders at the One/Eco Health workshop, MBI continues to working towards raising the profile of One Health and its importance as part of the National Agenda. Furthermore, as a country, Australia should be promoting and leading One Health approaches in the Asia Pacific region.

A number of relevant developments and forums involving MBI members have occurred or are planned in 2016:

♦ The leadership of MBI is continuing discussions with the Chief Medical and Chief Veterinary Officers and other key stakeholders.

♦ Two relevant NHMRC Centres of Research Excellence (CREs) were awarded this year. MBI Director, Professor Tania Sorrell with MBI members A/Professor Vitali Sintchenko, Professors Lyn Gilbert, Jon Iredell, Eddie Holmes, Cheryl Jones and both NSA and interstate investigators, leads the first, entitled “Protecting the public from emerging infectious diseases – genomics, informatics and ethics research for more effective public health action and policy” (CREID). Professors Sorrell (CIB) and Gilbert (CIH) are also CIs on a second CRE entitled the “Australian Partnership for Preparedness Research on Infectious Diseases Emergencies” (APPRISE), which brings together investigators from all states and territories (Professor Sharon Lewin, Doherty Institute, Melbourne, is CIA). Together these CREs will greatly assist Australia’s efforts to prevent, detect and respond to emerging infectious diseases in a multidisciplinary, timely and collaborative manner.

♦ The Australasian Society for Infectious Diseases (ASID) is holding a dedicated Zoonoses program stream at the International Congress for Tropical Medicine and Malaria, Brisbane, 18-22 September 2016.

♦ The 4th International One Health Congress & 6th Biennial Congress of the International Association for Ecology and Health are to be held in Melbourne, in December, 2016. Key themes include: creating a healthier world; pathways to a sustainable world – animal, human and environmental health; food, land and water systems: future challenges and pathways; responding to emerging diseases and invasive species; and integrating science, policy and action. MBI, led by A/Prof Robyn Alders, is playing an active role in this congress.
Due to over use and misuse, antibiotic resistance has become an increasingly urgent global problem that threatens human, animal and environmental health - a ‘slow-onset’ crisis akin to that of global climate change. Australia is one of the highest users of antibiotics among developed countries and it is likely that a significant proportion of antibiotics used in the community are unnecessary. Countries including Australia now need to tackle the difficult path of modifying human behaviour and consumer expectation in relation to antibiotic use. Understanding the socio-cultural dimensions associated with perceptions about antibiotic resistance is necessary to inform the development and implementation of education strategies underpinning behavioural change.

As part of an Honours year in Human Geography in 2015, under the supervision of Associate Professor Dale Dominey-Howes from the School of Geosciences at the University of Sydney and Dr Maurizio Labbate and Associate Professor Beata Bajorek of The University of Technology Sydney, Annie Zhuo set out to survey the knowledge and attitudes of the general public in Sydney to the issue of antibiotic resistance. The study also sought to understand the general public’s behavior in relation to the use of antibiotics, what barriers exist to their better use of antibiotics and who they thought were responsible for managing the antibiotic resistance crisis.

In June and July 2015, 583 individuals who resided in the Greater Sydney region completed a self-administered questionnaire either online, in pharmacies or via mail. The study found that while the majority of people had heard of antibiotic resistance, many held misconceptions about the causes of antibiotic resistance and impacts to themselves and the community. The data showed that 45% of people identified ‘my body gets used to them’ as a side-effect of taking antibiotics suggesting that many misunderstood the biological mechanisms behind bacterial resistance to antibiotics and the impact of their use of antibiotics on the effectiveness of antibiotics for other people.

More people believed that antibiotic resistance was an issue in hospitals and at global and national scales rather than for themselves and their family. This perceived personal invulnerability to antibiotic resistance was further mirrored in lower perceived importance of their individual use of antibiotics relative to actions of other stakeholders including doctors’ prescribing and other’s antibiotic use in dealing with the issue of antibiotic resistance.

About a third of people (29%) indicated that they had used antibiotics inappropriately for the common cold or flu which are often caused by viruses, reflecting poor knowledge of differences between viruses and bacteria. Statistical analysis revealed that individuals whose main language spoken at home was not English, and resided in low socio-economic areas were twice as likely to use and expect antibiotics for common viral URTIs and symptoms compared to those whose main spoken language was English and resided in high socio-economic areas. Further differences in knowledge regarding antibiotic use and resistance were found between respondent ethnicities and educational backgrounds, reinforcing the need to tailor awareness and education campaigns for different cultural and social sub-groups.

In addition to holding inaccurate expectations for antibiotics for viral infections, those who believed it was appropriate to use antibiotics ‘just in case’ when they had a sore throat, were also more likely to use antibiotics inappropriately for common colds and flu. This means that efforts are also needed to educate the public about potential harm of taking antibiotics when they are not required.

Our study represents a first step in understanding public perceptions of antibiotic resistance in Sydney and the factors underpinning these perceptions and antibiotic use. Our findings suggest that targeted awareness and education campaigns are needed to address knowledge gaps and attitudes underlying the sub-optimal and unnecessary use of antibiotics and to empower individuals to see that even though antibiotic resistance seems distant from their everyday life, they have a role and responsibility in preserving the effectiveness of antibiotics.

We are currently working to publish these findings and are extending this methodology in a new interfaculty project that explores multiple stakeholder attitudes towards antimicrobial resistance funded by the University of Sydney and involving academics from Medicine, Dentistry and Veterinary Science.
Respondents’ ratings of the importance of various groups’ actions in dealing with the issue of antibiotic resistance (N=581).

Map of survey respondents’ residential postal codes in Sydney, Australia (N=583). Red postal areas on the map or low deciles are classified as areas of relative high socio-economic disadvantage and low socio-economic advantage. Blue areas or high decile areas are classified as areas with generally low levels of socio-economic disadvantage and relatively high levels of advantage. SEIFA data source: Australian Bureau of Statistics, 2014.

For further information about this study, contact Annie Zhuo at annie.zhuo@sydney.edu.au and Dale Dominey-Howes at dale.dominey-howes@sydney.edu.au
Antibiotic resistance rates in *Escherichia coli* have risen steadily over the past decade, even in countries such as Australia where antibiotic usage is regulated. Resistant strains are now increasingly isolated in the community as well as in the hospital setting [1]. Of major concern is the rise in resistance to the widely-used β-lactam antibiotics, which is commonly mediated by mobile β-lactamase genes carried by large transmissible plasmids. These plasmids can rapidly convert bacteria from susceptible to resistant, even over the course of treatment [2].

One of the most common β-lactamase genes is bla\textsubscript{CMY-2}. Globally prevalent and isolated from both humans and animals, bla\textsubscript{CMY-2} provides a wide spectrum of activity against β-lactam antibiotics and is found in *E. coli* more often than in other Enterobacteriaceae occupying the same environmental niche [3]. While the prevalence in most clinical settings remains relatively low (~1.5%) [4 5], the potential for further spread of bla\textsubscript{CMY-2} is demonstrated by its success in livestock where it accounts for the majority of cephalosporin resistance in some populations [6]. However, the factors influencing the success of bla\textsubscript{CMY-2} are not well-understood. Is it found in common pathogenic *E. coli* strains or in diverse commensal strains? Which plasmid types carry bla\textsubscript{CMY-2} and do they reside in different species or just in *E. coli*? Are we likely to see an increase in the prevalence of bla\textsubscript{CMY-2} over time?

To answer these important questions we investigated the epidemiology of bla\textsubscript{CMY-2} in *E. coli* from Sydney hospitals over two time periods. Using a combination of molecular techniques, including PCR, pulsed-field gel electrophoresis (PFGE), Southern blotting and next-generation sequencing, we characterised over 100 *E. coli* strains carrying bla\textsubscript{CMY-2} to determine the genetic context of the gene, plasmid type and *E. coli* sequence type.

The *E. coli* strains in this collection were relatively diverse with over 40 sequence types identified, representing known pathogenic strains and commensal strains associated with both humans and animals [7]. An interesting result was the clear dominance of one particular plasmid type carrying bla\textsubscript{CMY-2}, usually only identified in *E. coli*, *Klebsiella* and *Salmonella* spp. This is in contrast to the more varied pattern of plasmid types carrying this gene in other parts of the world [8]. Perhaps a clonal plasmid lineage is circulating in Sydney? Still too early to tell!

In the later time period, while this plasmid type remained dominant, half of the tested isolates had bla\textsubscript{CMY-2} inserted in the chromosome rather than on a plasmid. This is likely the result of clonal expansion of a single strain given that PFGE analysis revealed a high level of similarity between these isolates (Figure 1) and PCR confirmed that bla\textsubscript{CMY-2} was inserted in the very same chromosomal location in each. The particular *E. coli* clone identified here has been reported in humans and animals in other parts of the world but little is known about its characteristics. Chromosomal bla\textsubscript{CMY-2} represents both an immediate treatment concern and a wider public health threat as we may be heading into an era where antibiotic resistance is not reversible.

In summary, the epidemiology of bla\textsubscript{CMY-2} in Sydney is dissimilar to other parts of the world, where more diversity is seen at the plasmid level. This may be a reflection of the antibiotic usage in Australia or the founding effect of individual importation events. Understanding the ecology and dissemination of mobile resistance genes such as bla\textsubscript{CMY-2} is an essential part of tackling the global antibiotic resistance crisis as it can aid in identifying key intervention points to limit further spread.

**Figure 1.** *E. coli* isolates carrying bla\textsubscript{CMY-2} on the chromosome, digested with XbaI. Lane 1: marker, lanes 2-16: *E. coli* isolates. Isolates in lanes 5 -9 and 14-16 are closely related whereas the isolate in lane 3 appears to be unrelated to the others.

**The epidemiology of antibiotic resistance: an *Escherichia coli* perspective**

Ms Kaitlin Tagg, Dr Andrew Ginn, A/Prof Sally Partridge, Prof Jonathan Iredell

Centre for Infectious Diseases and Microbiology, The Westmead Institute for Medical Research
The added value of genome sequencing in the investigation of Salmonella Agona outbreak in Sydney

Dr Qinning Wang1, Dr Craig Thompson2, Ms Cristina Sotomayor1,7, Mr Peter Howard3, Dr Craig Shadbolt4, Dr Kirsty Hope5, A/Prof Vitali Sintchenko1,6

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Salmonellosis is the second most commonly notified bacterial gastrointestinal disease in Australia. There are currently around 2,500 Salmonella serotypes (serovars) defined according to the Kauffmann-White scheme on the basis of the serologic identification of somatic and flagellar antigens (1). Salmonella Typhimurium is the predominant serotype identified in NSW, accounting for more than half of all Salmonella serotypes, followed by the other serotypes such as S. Enteritidis and S. Virchow. S. Agona is ranked among the top 11 most common Salmonella serotypes in NSW. This serotype had been recognised as a public health challenge in a few developed countries due to importation of contaminated Peruvian seafood in 1970’s (2-3). Since then S. Agona has emerged as a common global cause of salmonellosis in both animal and humans, which is often found in farmed livestock, vegetables and factory-prepared foods (4-7).

The unexpected increase in of S. Agona notifications was identified in Western Sydney in May-June 2015 through routine public health laboratory surveillance. A total of 38 cases were notified in NSW during the first half year in 2015, compared to an average of 28 cases per year for the previous five years (Figure 1). The public health officers from NSW Department of Health interviewed recent cases and identified two food outlets in one large metropolitan shopping centre as the common sources for some cases. Food and environmental samples were then collected from the both venues by the NSW Food Authority and positive S. Agona cultures were subsequently obtained from the food related sources.

To further investigate the link between clinical cases and food sources, whole genome sequencing (WGS) was performed on the isolates obtained between Jan–Jul 2015 in NSW. A total of 48 S. Agona isolates were investigated, including six human isolates from Food Outlets 1 and 2; six food and environment isolates recovered during the outbreak from the Food Outlet 1 and four from other retail outlets during 2015; one from a case with an exposure to the implicated food; and 31 other sporadic clinical isolates of S. Agona.

References

The epidemiology of antibiotic resistance: an Escherichia coli perspective (continued from page 6)
The added value of genome sequencing in the investigation of *Salmonella Agona* outbreak in Sydney (continued from page 7)

The genomic DNA was prepared using an automated work-station Chemagic Prepito-D (PerkinElmer). The DNA sequencing libraries were prepared using the Nextera XT DNA preparation kit (Illumina) and the sequencing was performed on the NextSeq 500 (Illumina) with 2x150 bp paired-end chemistry. Single nucleotide polymorphisms (SNPs) in individual genomes were identified by mapping reads to the reference genome *S. Agona* 460004 2-1 (NCBI GenBank: NZ_CP011259). The significant thresholds for SNP calling were set for a minimum coverage at 20 and a minimum variant frequency at 80%. The generated SNP list from each of the isolates was exported as excel files and a binary type SNP matrices representing present and absent of SNP in each isolates was generated and imported into BioNumerics (Applied Maths). The clustering analysis was performed using a categorical coefficient with UPGMA as dendrogram in BioNumerics (Applied Maths). To validate the clustering analysis, *de novo* assembly was performed with a minimum contig size set at 200bp. The assembly was then mapped against the reference genome for SNP calling and phylogeny inferred using a web-based server (https://cge.cbs.dtu.dk/services/CSIPhylogeny/). Maximum likelihood phylogenetic trees at 100 bootstraps were generated in MEGA6 [8] to estimate resulting genomic distances between sequenced genomes.

SNP analysis grouped the six clinical isolates collected from the cases involved in two food outlets in the same cluster (A, Figure 2), together with one isolate from a single non-contactable case with exposure. Another four isolates collected from the clinical cases notified early between January and March 2015 were also clustered in the same group. The SNP difference within these isolates were between 0 and 1. The six food and environmental isolates obtained from the Food Outlet 1 had no SNP difference to the clinical cases. Two raw retail chicken isolates collected as part of routine screening (unrelated to this outbreak) were closely related to the epidemiologically linked cases and food isolates collected from the food outlet 1 (4-8 SNPs) (B, Figure 2). Another two cases involving a mother and her new-born had the same SNP profile for the isolates collected (C, Figure 2). Some overseas travellers and sporadic cases had demonstrated very different SNP profiles compared to the outbreak cases (Figure 2). Other genomic results were also inferred from the sequencing data. All isolates were ST13 by MLST, a typical ST type for *S. Agona*. The outbreak isolates harboured a salmonella plasmid pVCM01 which was absent in 48% of non-outbreak isolates. Antibiotic resistance genes were not identified in all outbreak related isolates and some sporadic isolates, while three isolates from patients with overseas travel histories and one sporadic harbourised resistance genes to aminoglycoside (strA/B), beta-lactam (blaTEM-1B), fluoroquinolone (*qnr*S1), sulphonamide(*su*B3) and tetracycline (*tet*A, *dfr*A14).

In conclusion, whole genome sequencing of pathogens of public health importance provides a new capacity to examine relationships between antigenically closely related *Salmonella* isolates potentially associated with community outbreaks. Our sequencing results, combined with epidemiological evidence, strongly suggested that the consumption of the food at Food Outlets 1 and 2caused the outbreak and the chicken meat was most likely the source of contamination. Furthermore, WGS findings indicated that the outbreak started much earlier than was previously estimated. This experience further demonstrated the added value of high resolution and high-throughput capacity of WGS in the investigation of *Salmonella* community outbreaks. As the cost of this technology becomes more acceptable and turnaround times are reduced, WGS is expected to be of utmost benefit to communicable disease surveillance and outbreak investigations. This technology will to play an increasingly important role in public health laboratory surveillance and diagnostic microbiology.

References:

Figure 1. *Salmonella* Agona cases notified between January 2011 and September 2015 in NSW (NSW Department of Health).

Figure 2. Single nucleoid polymorphism clustering tree of *S.* Agona isolates collected in NSW between January and July 2015 with sample sources and epidemiological links.
Marian was awarded her PhD in the area of Molecular Genetics in 1996 and after her first post doc at the Victor Change Cardiac Research Institute moved to The Children’s hospital at Westmead. Since then, she has actively contributed to both basic and clinical research in infection and immunity at Prof Cheryl Jones’ Lab at The Centre for Perinatal Infection Research, Kids Research Institute, CHW. She developed a unique murine model of neonatal and adult HSV infection and generated a series of major findings on the immunopathogenesis of HSV in collaboration with Prof Tony Cunningham, Centre for Viral research (WIMR) and Prof Stephen Alexander, Centre for Kidney research (CHW). She has presented her research at many nation and international conferences and as online journal publications.

In addition, Marian holds a Conjoint Lecturer position in Paediatrics & Child Health, Faculty of Medicine, University of Sydney since 2008, co-supervises many lab personnel including undergraduate, postgraduate and PhD students and has received awards for mentorship and teaching. She has been an active member of NSW scientific societies and a representative on many committees at the Westmead research hub.

Marian joined the CREID, MBI at WIMR in March this year. As the CREID Virology Geneticist, her current interest is in molecular epidemiology of infectious viruses using next-gen sequencing and state-of-the-art bioinformatics with Prof Edward C. Holmes and Prof Tania Sorrell.

**Staff Profile**

**Name:** Dr Marian Fernandez MSc MPhil PhD  
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Marian was awarded her PhD in the area of Molecular Genetics in 1996 and after her first post doc at the Victor Change Cardiac Research Institute moved to The Children’s hospital at Westmead. Since then, she has actively contributed to both basic and clinical research in infection and immunity at Prof Cheryl Jones’ Lab at The Centre for Perinatal Infection Research, Kids Research Institute, CHW. She developed a unique murine model of neonatal and adult HSV infection and generated a series of major findings on the immunopathogenesis of HSV in collaboration with Prof Tony Cunningham, Centre for Viral research (WIMR) and Prof Stephen Alexander, Centre for Kidney research (CHW). She has presented her research at many nation and international conferences and as online journal publications.

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**CONTACT US**

*For more information on any articles or CIDM-PH & MBI events, or to join the e-lists and receive regular updates, please contact us at:*

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Moataz is an Egyptian citizen who got his PhD in Immunology from Cairo University in 2007 with practical work performed in Professor Gordon Dougan’s laboratory at the Wellcome Trust Sanger Institute, UK. His project involved the utilization of advanced approaches to develop genetically manipulated live strains of Salmonella (carriers) characterized by their impaired ability to colonize the small intestine and stably express foreign (heterologous) antigens. During his postdoc at the King Abdullah University of Science and Technology (KAUST) he started and led on various genomic and functional genomic studies to characterize the transmission and fitness of bacterial pathogens, especially those causing enteric infections. In collaboration with the Saudi Ministry of Health he started a pioneer large-scale study that uses advanced molecular and genomic approaches to characterize enteric pathogens predominating during the Hajj pilgrimage.

To date the majority of his research focuses on the molecular biology, functional genomics and genetics of bacterial species of public health importance, particularly enterics. He also has experience working in the vaccine industry for more than 5 years in both R&D and the production of enteric bacterial vaccines. He also worked as a molecular virologist in the WHO regional reference laboratory for the diagnosis of enterovirus and poliovirus where he developed extensive experience in virus isolation on cell lines, and typing intratypic differentiation molecularly (RT-PCR, probe hybridization) and antigenically (ELISA).

In November 2015, Moataz joined the Westmead Institute for Medical Research and the Marie Bashir Institute for Infectious Diseases and Biosecurity, The University of Sydney, Australia. He is contributing to the establishment of a pathogen genomics programme that use molecular biology including new whole genome, whole transcriptome and functional genomic technologies to tackle the emergence and transmission of infectious diseases. These include in particular antimicrobial resistance, food security and mass gathering medicine.
Upcoming Events....

Foodborne Diseases: Changing Epidemiology and Disease Control Research Symposium
Co-hosted by CIDM-PH and MBI

Event Details
Date: Thursday, 1 September 2016
Time: 9am – 5pm
Location: Lecture Theatre 3
Westmead Education & Conference Centre
Westmead Hospital, Sydney

Confirmed Speakers:
A/Professor Robyn Alders, AO, Faculty of Veterinary Science, University of Sydney
Mr John Bates, Public Health Microbiology, Queensland Health
Professor Robyn McConchie, Faculty of Agriculture and Environment, University of Sydney
Dr Kirsty Hope, NSW Health Protection
Professor Benjamin Howden, University of Melbourne
A/Prof Martyn Kirk, Australian National University
Dr Craig Shadbolt, NSW Food Authority
Dr Vicky Sheppeard, NSW Health Protection
Professor Peter White, University of NSW

Key sessions include:
• Evolution of strategies for control of foodborne diseases
• Advances in laboratory diagnosis of enteric pathogens
• Genomic surveillance of foodborne pathogens
• Improving the understanding and safety of food systems

Cost: $120 [incl GST Morning/Afternoon Tea and Lunch]
Enquiries: mbi@sydney.edu.au


This event co-hosted by Centre for Infectious Diseases & Microbiology- Public Health (CIDM-PH) and the Marie Bashir Institute (MBI).

Save the date...

19-20 October 2016
Short Course in Critical Infection
Venue TBA

21 October 2016
Whole Genome Sequencing Workshop
Westmead Education & Conference Centre

3 November 2016
MBI Colloquium
New Law School
University of Sydney

25 November 2016
CIDM-PH Colloquium
Westmead Education & Conference Centre

In the media....

Professor Dominic Dwyer: 5 Myths busted and how to stay healthy this winter
20 May 2016: Professor Dominic Dwyer speaks to Siobhan Ryan of USYD TV Network about the facts and fiction of the Flu

Dr Cameron Webb: Summer summary of mosquito media madness
https://cameronwebb.wordpress.com/