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CIDM-PH Overview

CIDM-PH AS A TRANSLATIONAL RESEARCH NETWORK WITH A MISSION TO IMPROVE COMMUNICABLE DISEASE CONTROL IN NEW SOUTH WALES, AUSTRALIA AND OUR REGION

A/Professor Vitali Sintchenko and Professor Lyn Gilbert, CIDM-Public Health

The Centre for Infectious Diseases and Microbiology-Public Health (CIDM-PH) is an interdisciplinary research group within the Microbiology and Infectious Diseases Departments of the Western Sydney LHD and Pathology West-ICPMR. Our goals are to improve prevention and control of communicable diseases of public health importance by:

- developing, evaluating and implementing innovative pathogen detection and typing methods;
- integrating enhanced laboratory surveillance data and bioinformatics analyses into early warning systems and laboratory networks to advance public health research;
- identifying evidence-based, cost-effective strategies to translate research findings into public health and clinical practice.

Senior investigators in CIDM-PH have been successful in building the network of collaborative research (Figure) and in improving surveillance and management of diseases with epidemic potential with invaluable support from the NSW Health Population Health and Health Services Research Support Program. The CIDM-PH Colloquium 2013 aims at (1) showcasing achievements in translational research conducted by CIDM-PH investigators, post-doctoral researchers and students and (b) planning future research directions in accordance with current NSW Population Health Surveillance Strategy (NSW 2011 to 2020). The following abstracts illustrate the spectrum of CIDM-PH research.

Fostering New Partnerships
ENHANCING PUBLIC HEALTH LABORATORY CAPACITY IN NEW SOUTH WALES

Vitali Sintchenko & Lyn Gilbert

CIDM-PH research has focused on (a) enhanced infectious disease case detection and management; (b) discovery of mechanisms of virulence and drug resistance and development of novel methods of their detection and monitoring; (c) advanced surveillance methods and bioinformatics data analysis, and (d) ethical implications of infectious disease control and barriers to implementation. These themes are closely aligned with Strategic Plans of the NSW MoH and NSW Health Pillars. Specifically:

NSW Health & Medical Research Strategic Review 2012 Recommendations: Strategy 1 – Foster translation and innovation from research

CIDM-PH has developed, evaluated and implemented many innovative laboratory diagnostic approaches to detection and characterisation of pathogens with epidemic potential. We have investigated novel mechanisms of drug resistance. For example, Dr Mark Douglas’s group (NHMRC Project Grant APP1003767) recently discovered a novel method of suppression of hepatitis C virus (HCV) replication, providing a novel antiviral treatment strategy, which was the basis of a successful International Patent application (PCT/AU2010/001695).

CIDM-PH is a leader in development and implementation of new microbial strain-typing methods, designed to facilitate interlaboratory harmonisation, to support multijurisdictional outbreak investigations and global surveillance of drug-resistant pathogens. Our researchers are chief investigators in the NHMRC Centre for Research Excellence (CRE) in Tuberculosis (TB) Control and Prevention (APP1043225) with major nodes at CIDM and the Centenary and Woolcock Institutes. This has particular importance because of rapid emergence of drug resistant *Mycobacterium tuberculosis* (MTB) in the Asia-Pacific region, which can only be contained by better understanding of factors that facilitate transmission. CIDM-PH investigators also lead an NHMRC grant (APP1044986) aiming to discover genomic determinants of MTB transmissibility.

NSW Health & Medical Research Strategic Review 2012 Recommendations: Strategy 2 – Build globally relevant research capacity

Integrating an active R&D program within a large service department provides opportunities for research training of laboratory staff via short-term secondments, part-time postgraduate studies or participation in evaluation and translation of new technology. Grant-funded research assistants often transfer into permanent positions in service laboratories where their research training maintains a culture of enquiry and improvement. Our involvement in major outbreak investigations (including the 2009 A/H1N1 influenza pandemic) confirmed our commitment to staff training and maintaining innovation and surge capacity.

CIDM-PH has: established a WHO-funded laboratory “twinning” project with clinical and public health laboratories in The Republic of Maldives to assist in epidemic preparedness; developed a productive training program for scientists and graduate students from Asia, Africa and South America who contribute to CIDM-PH R&D and return home to establish or improve diagnostic or research laboratories in their parent institutions. For example, visiting scholars have contributed to development and translation of:
• novel bacterial genotyping and antibiotic resistance profiling systems for surveillance of invasive pneumococcal, *Campylobacter, Salmonella*, methicillin resistant *Staphylococcus aureus* (MRSA), VRE and *Clostridium difficile* infections;

• multiplex detection of sexually transmissible pathogens and markers of drug resistance;

• molecular epidemiology of the fungal pathogens *Candida, Cryptococcus, Scedosporium* and *Trichophyton*;

• novel rapid methods for genotyping varicella zoster and encephalotropic viruses.

**NSW Population Health Surveillance Strategy (NSW 2011 to 2020): Objective 2 – Strengthened surveillance capacity**

CIDM-PH research activities aiming to strengthen surveillance capacity e.g.:

• National serosurveys, in collaboration with the NCIRS, provide information about the prevalence and distribution of VPDs and vaccine uptake in different populations. Data from sequential surveys are used to model effects of future changes and inform immunisation policy.

• Rapid typing of salmonella and cluster identification of food-borne diseases, in collaboration with NSW MoH and NSW Food Authority, allow faster identification of outbreaks and their sources, reduce case numbers and prevents future outbreaks.

• Development of mosquito population management strategies, in collaboration with NSW and local governments, limits pest and vector mosquito problems.

**NSW Population Health Surveillance Strategy (NSW 2011 to 2020): Objective 3 – Transform data into high quality information**

CIDM-PH is committed to “creating better system interoperability between existing disease-specific surveillance systems”. Several externally and CIDM-PH funded activities address this state State and national priority:

• In 2011, CIDM-PH organised a national workshop, with OzFoodNet, to facilitate harmonisation of *Salmonella* Typhimurium (STM) subtyping and has facilitated harmonisation of MRSA and MTB typing systems across different jurisdictions;

• Australian Biosecurity Intelligence Network (ABIN). Our investigators led the ABIN proof-of-concept project in human health and created the Australian Pathogen Intelligence Community Space (APICS) online. The APICS enables epidemiologists and public health professionals to monitor changes in pathogen subtypes and resistance patterns in different areas over time and in response to relevant interventions, and to facilitate outbreak detection;

• Linkage of strain typing methods to spatio-temporal mapping and clustering improves our understanding of microbial transmission dynamics and the capacity for targeted direct public health responses to limit transmission. For example, our current NHMRC-funded project (APP1050227) will develop a web-based integrated surveillance system to study outbreaks and epidemiology of STM.

**NSW Population Health Surveillance Strategy (NSW 2011 to 2020): Objective 4 – Be ready to monitor new and emerging threats to health**

CIDM hosts the NSW high security laboratory (EIBRU PC3/4), maintaining our capability to respond to emerging and exotic infections and bioterrorism events by constantly extending our diagnostic repertoire and training staff in cutting-edge technology. Our network of collaborators in Australia and overseas (including the US CDC Laboratory Response network) allows us to share research data and expertise to optimise responses to new infectious disease threats – as we have done for SARS, influenza A (H1N1)09pdm, food-borne pathogens in the community and novel antibiotic resistance genes in ICUs. CIDM-PH is a major participant in the University of Sydney’s Marie Bashir Institute for Emerging Infectious Diseases and Biosecurity, which offers new collaborative research and funding opportunities by bringing together researchers in human and veterinary infectious diseases, epidemiology, public health law, ethics and social sciences.
**CIDM-PH Overview**

**MARIE BASHIR INSTITUTE FOR INFECTIOUS DISEASES AND BIOSECURITY – CIDM PH COLLABORATIONS**

*Tania C Sorrell*

The links between MBI and CIDM - Public Health are integral to the remit of MBI (namely, to reduce the health and socio-economic impact of infectious diseases on humans and animals, especially in, but not limited to, the Asia-Pacific Region. CIDM-PH provides an essential link between the academic work of MBI and public health microbiology/infectious diseases research and development, policy and practice. Organisms of mutual interest include viruses of pandemic and public health importance, bacterial causes of pneumonia, septicaemia, CNS infection, TB, food-borne pathogens and multidrug resistant pathogens, and fungi of importance in animals, humans or both. Platforms and research interests in common include genomics and proteomics, the evolution of disease emergence, new diagnostics, therapeutics and vaccines, informatics and modelling, temporo-spatial mapping of infectious diseases, clinical trials and socio-behavioural research of relevance to infectious diseases control and prevention.

Pandemics, antimicrobial resistance and biosecurity including food security can be considered as the major areas of interest (see diagram) – for example AMR research and includes stewardship interventions – hospital, community, veterinary; antibiotic use guidelines at local and national level; infection control initiatives; resistance surveillance; diagnostics: molecular detection of infection, resistance targets; new antimicrobial compounds; genetics of antibiotic resistance; biomarkers to differentiate viral from bacterial infection and building international partnerships in education and laboratory capacity development.
CONTRIBUTIONS OF THE MOLECULAR MYCOLOGY RESEARCH LABORATORY TO CIDM-PH

Wieland Meyer

The Molecular Mycology Research Laboratory forms a link between basic and applied science by performing research directed towards a better understanding of the phylogenetic relationships of pathogenic fungi as basis for molecular diagnostics, to understand the molecular basis of fungal virulence and molecular epidemiology of fungal pathogens to guide public health responses to possible outbreak treats. The phylogenetic analysis has led to the selection of the ITS region as the globally accepted fungal DNA barcode and the establishment of the ITS reference sequence database for human/animal pathogenic fungi within the International Society of Human and Animal Mycology (ISHAM) accessible at www.mycologylab.org. The established reference sequences have been used to develop fast and reliable molecular diagnostic methods for human pathogenic fungi, such as the Scedosporium multiplex PCR and cryptococcal RCA. Matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectroscopy (MALDI-TOF MS) has been adopted for the major human pathogenic fungal species and as a fast and reliable identification method for the major molecular types within the C. neoformans /C. gattii species complex. Animal virulence studies have established new links with specific genotypes of fungal disease agents, e.g. C. neoformans, C. gattii and the emerging pathogenic fungus S. aurantiacum. Using genomics, transcriptomics and metabolomics the molecular basis for the development of virulence within pathogenic fungi, e.g. in C. gattii is currently studied, which will allow for the development of genetic markers to improve treatment decisions. Our MultiLocus Sequence Typing (MLST) and MultiLocus Microsatellite Typing (MLMT) schemes have been globally adopted within the ISHAM framework to investigate the global spread of highly pathogenic strains to enable an appropriate public health response. The C. neoformans/C. gattii MLST scheme has been applied to trace the origin of highly virulent genotypes back to the Amazonas rainforest, causing also a smouldering outbreak within Australia. A four locus MLST scheme is currently being used to investigate outbreaks of Pneumocystis jirovecii on the east coast of Australia. These research projects are funded by two NH&MRC grants from Australia, a NIH R21 grant form the US, a Colciencias grant from Colombian and a CAPES Science without borders grant from Brazil.
MEDICAL ENTOMOLOGY

Cameron E Webb & Stephen L Doggett

Translating research into the current and emerging public health risks associated with medically important arthropods to improved public health outcomes remains a priority of the Department. The key publication produced from the Department this year was “Arthropod pests of public health significance in Australia” (Webb, Doggett and Russell) coordinated by the Environmental Health Committee of Australia (enHealth). This was the first major review of the guidelines in a decade and this document now represents an invaluable resource for health professionals and the community on a wide range of insects, mites, ticks and other arthropods that may pose a pest or nuisance concern.

The management of mosquitoes and mosquito-borne disease in Australia is of growing concern. Medical Entomology continues to coordinate the NSW Arbovirus Surveillance Program, and work closely with NSW Health, numerous Local Health Districts and local government to provide information on mosquito and arbovirus activity so that timely preventative measures can be undertaken. The Department is currently undertaking collaborative research with health authorities in QLD to incorporate new field and laboratory based technologies into the program that may provide greater efficiencies in the future. Data collected in the program is not only valuable to local health authorities to prioritise their public health warnings and seasonal mosquito-borne disease awareness campaigns but also helps further our understanding of mosquito-pathogen-wildlife interactions that can be used to develop more effective strategies to reduce mosquito-borne disease risk.

Urbanisation and wetland rehabilitation continues to create regions of increased risk of nuisance-biting mosquito populations and mosquito-borne pathogens such as Ross River virus. The development of mosquito risk assessment guidelines, mosquito monitoring programs and site-specific mosquito control strategies has been a core activity of the Department. Through partnerships with local health authorities, strategies have been developed that can assist environmental conservation while reducing public health risks. Most recently, together with Sydney West Local Health District Medical Entomology developed a set of risk assessment guidelines to be applied to constructed wetlands. It is hoped that this document can be adapted to other regions in NSW.

The Department continues to provide expert advice on bed bug management both locally and internationally. Doggett has been active in the assessment of industry prevention and control methodologies, and has led the development of “A Code of Practice for the Control of Bed Bugs in Australia” (currently in its 4th edition) with this document being adopted by the European pest management industry. A companion document has also been produced, “A Bed Bug Management Policy and Procedural Guide for Accommodation Providers”. Doggett has been invited to share this expertise on bed bug management at numerous local and international meetings in the past year.

The Department continues to supply disinfected maggots for Maggot Debridement Therapy (MDT) to health care facilities across the country and requests for this service continue to rise. In 2013, MDT program coordinator, Marilyn Geary, was awarded Pathology West STAR award for innovation.
With the increasing concern regarding tick-borne pathogens, Stephen Doggett has been invited to participate in two expert advisory panels; Clinical Advisory Committee on Lyme Disease in Australia and TIARA (Induced Allergies Research & Awareness).

Two post-graduate research students (University of Sydney) are current associated with the department. David Lilly is investigating the role of insecticide resistance in Australian bed bug populations and Liyana Mokhtar is investigating the potential of extracts from Australian native plants as control agents of dengue mosquitoes. In addition, post-graduate student Kai Dang, from Nankai University, China, is also undertaking a research project in Medical Entomology investigating the genetic basis of insecticide resistance in bed bugs.

The Department has maintained a strong publication record with book chapters and published reports, peer-reviewed publications, technical reports, articles in bulletins and trade journals. Members of the Department also gave presentations at workshops, conferences and meetings locally and internationally as well as providing lectures to undergraduate and postgraduate courses at the University of Sydney.

The Department continues to license photographic images for media and commercial use with Doggett’s photography gaining many accolades.
CRE IN CRITICAL INFECTION AND CIDM-PH

Jon Iredell

The CRE in Critical Infection is an infrastructure grant to CIDMPH investigators and collaborators to study critical infections now completing its third of five years. The CRE is focused on several areas, including research in three main themes (septic shock, severe respiratory infection, and encephalitis), in the genetic epidemiology of transmissible pathogens and the application of this for translational diagnostics, and in teaching of postdoctoral Fellows and PhD students and of critical care practitioners. Project grants in all of these areas have allowed CIDMPH researchers to develop new agendas ranging from basic to translational, from common yeast, viral and bacterial infections through to tier 1 biohazardous pathogens.
NOVEL APPROACHES IN HEPATITIS CONTROL AND MANAGEMENT

Mark Douglas

Nearly 300,000 Australians and 170 million people worldwide have been infected with the hepatitis C virus (HCV). HCV is the leading cause of cirrhosis, liver failure and liver cancer in Australia, and now causes more deaths than HIV. Although the recent availability of oral direct acting antivirals (DAA) has significantly improved cure rates, significant challenges remain. In particular, since these drugs directly target HCV proteins, they are only active against certain HCV genotypes and viral resistance can emerge rapidly. Research in our group aims to improve treatment outcomes for patients with chronic HCV infection, using several complementary approaches.

1. Host targeting antivirals (HTA), which target host proteins rather than virus, should be active against all HCV genotypes and reduce the emergence of resistant virus. We have identified several novel approaches to impair HCV replication: inhibiting host lipid metabolism; blocking viral trafficking; and restoring impaired cellular innate immunity.

2. HCV replication is highly error-prone and resistance mutations against antiviral drugs appear rapidly during treatment. We therefore anticipate a clinically important role for viral resistance testing to guide antiviral therapy, as is already the case for HIV and HBV. We have developed a method to detect low level resistance mutations across the whole viral genome, using Next Generation Sequencing. We are currently exploring the clinical utility of these mutations to predict treatment response, and aim to develop a high-throughput assay to identify key resistance mutations.

3. For treatment to be effective, patients need to adhere to and complete their prescribed treatment regimen. Many patients with Hepatitis C come from socially marginalised groups, including people who inject drugs and people living with mental illness. Using a multidisciplinary approach, we have investigated key psychosocial factors that affect patient adherence. We are currently developing a psychosocial screening tool for the clinic, to determine which patients are likely to adhere to treatment, and identify those who would benefit from extra support.
TRACKING MRSA TRANSMISSION IN HOSPITAL

Matthew V. N. O’Sullivan

Background
Identifying transmission of MRSA in hospitals is an important part of infection surveillance and control. However, when a patient is identified as infected or colonised with MRSA, it is difficult to ascertain whether it was acquired in the hospital or in the community prior to admission; even if nosocomial acquisition is suspected, identifying the source from amongst a large number of colonised patients may be impossible. Strain typing can help identify, with reasonable certainty, the source and the recipient of nosocomial transmission events. However, established strain typing methods have been unsatisfactory for routine, high volume use in nosocomial infection surveillance.

Methods
We developed a highly discriminatory, inexpensive, high-throughput and reproducible binary typing system for MRSA using a multiplex-PCR/reverse line blot assay platform, and have routinely typed MRSA isolates from all infection and colonisation episodes at Westmead and Blacktown Hospitals since August 2011. In response to a high number of infections in three surgical wards at Westmead Hospital, environmental and staff sampling was performed and the genotypes of recovered MRSA isolates compared with those from patients.

Results
3572 isolates have been typed using mPCR/RLB, with 418 unique strain types identified. A large number of strain types have been identified within the predominant nosocomial clones AUS2/3 (ST239-III) and UK-EMRSA-15 (ST22-IV).

Twelve of 144 (8%) environmental samples and 1 of 21 (6%) staff swabs were positive for MRSA from the surgical wards, and these were compared with 27 patient isolates. Seventeen binary types were found amongst the 40 isolates; 6 of these 17 types were represented by multiple isolates (range 2-9) while 11 were singletons. Spatial clustering was evident within types, indicating links between patient and environmental isolates.

Conclusions
Rapid, high-throughput and highly discriminatory genotyping can be used routinely to identify nosocomial transmission events and to investigate the role of environmental contamination in MRSA acquisition. Further work is planned to apply this methodology to nosocomial methicillin susceptible Staphylococcus aureus and to investigate the role of whole genome sequencing in infection control surveillance.
PRACTICAL APPLICATION OF DIVERSE METHODS (MRSA MOLECULAR EPIDEMIOLOGY AND VIDEO-ETHNOGRAPHY) TO IMPROVING HOSPITAL INFECTION CONTROL AND STAFF

Lyn Gilbert

Healthcare-associated (HA) MRSA bloodstream infections (BS) are reported to be in decline in several countries, including Australia. Our experience at Westmead Hospital, with HA Staphylococcus aureus BSIs reflects this, but there is still a high prevalence of MRSA colonization and new acquisitions among hospital patients, which have major impacts on patients, hospital practices and workload; the burden of non-bacteraemic HA infections (due to many different hospital pathogens, including MRSA) remains significant but largely undocumented. For the past three years, the CIDM-PH HAI research group has had a major focus on MRSA epidemiology (supported by NHMRC grant #1010542), HA SABSI and improving healthcare worker (HCW) awareness of, and responsibility for, infection prevention and control (IPC) using video reflexive ethnography (with colleagues from UTS NHMRC grant #1009178).

Routine MRSA strain typing of all isolates began in 2011 and has been used to monitor MRSA transmission in NICU (reported in detail last year), ICU (Med Hons project) and in our general surgical and surgical high dependency wards, where several point prevalence surveys (PPS) in 2009-10, complemented by retrospective strain typing, demonstrated a particularly high prevalence of MRSA colonization (25%) and hospital MRSA transmission. A PPS in the surgical wards, in April 2013 showed a further increase in MRSA colonisation prevalence (38% overall; 68% in ward “A”) and an unexpectedly high proportion of MRSA-positive environmental swabs. Urgent infection control team intervention (improving hand hygiene compliance and environmental cleaning using hydrogen peroxide) and strain typing to identify clusters was followed by a second PPS in July, which showed markedly reduced MRSA colonization (13% overall; 32% in ward “A”). A third PPS is being performed in early November. Medical record review for all patients screened (MRSA positive and negative) in the first PPS (and possibly subsequent ones) and analysis of MRSA strain types and patient bed movements, to identify contacts and potential environmental sources, are underway. We aim to identify preventable risk factors that will assist with targeting future IPC strategies.

The period covered by the first two PPSs, coincided with the video reflexive ethnography project teams’ presence in the same wards. Edited video clips of staff engaged in their routine work – such as surgical wound dressing procedure and unit wards rounds - (filmed with their consent) were shown to those involved, during reflexive sessions with an infection control practitioner. This has stimulated discussion among staff and provided unique insights into the complexity and unpredictability of routine hospital practices that contribute to pathogen transmission. It has revealed practical and physical barriers to compliance and some confusion about correct use of personal protective equipment and interpretation of hand hygiene “moments” (partly because of contradictory or unclear guidelines and education). Our challenge is to document these insights and translate our findings into sustained improvement in hospital infection control based on practical, cost-effective measures to overcome barriers and craft clear, consistent infection control messages that allow staff to take personal responsibility for their implementation.
DETECTION AND MANAGEMENT OF EMERGING VIRAL INFECTIONS

Jen Kok

The increasing ease and affordability of travel may result in the rapid spread of newly emerging infections and start pandemics. Mass gathering events further increase the risk of importation and international spread of novel infectious diseases. The key to mitigating the spread of emerging infections is the rapid identification of offending pathogen and timely dissemination of this information. In this session, Middle East respiratory syndrome coronavirus (MERS-CoV) is used as the example to highlight the key role of the laboratory in aiding the public health response.
Characteristics of Enterobacteriaceae carrying blaKPC genes isolated in Australia.

S Partridge¹, A Ginn¹, A Wiklendt¹, P Huntington², J Wong³, J Montgomery⁴, J Iredell¹.

Centre for Infectious Diseases and Microbiology, University of Sydney, NSW¹
Royal North Shore Hospital, Sydney, NSW²
Pathology, Department of Microbiology, Footscray, Victoria³
Austin Health, Melbourne, Victoria⁴

The blaKPC carbapenemase gene was first identified in the eastern USA and is now endemic there, in Israel, China and Greece, with sporadic reports in other parts of the USA, Europe and South America. There are few reports of this gene in Australia since an initial presentation at the ASA meeting in 2010. Here we have partially characterised these first two reported isolates and five other Klebsiella pneumoniae and one Escherichia coli carrying blaKPC isolated in Australia. Multilocus sequence typing identified five isolates as ST258, associated with blaKPC genes in a number of countries, one as ST512, a single locus variant of ST258 and one as a new sequence type (ST1048) and the E. coli as ST131, commonly associated with blaCTX-M-15. All except the ST512 isolate (blaKPC1) carried blaKPC2 and these genes were found in the expected immediate genetic context between ISKpn7 and ISKpn6 and associated with the Tn4401α isoform that gives highest expression. Outer membrane defects appear to be required for expression of carbapenem resistance by isolates carrying blaKPC genes. All ST258 isolates had a mutation in the ompK35 porin gene that would be expected to prevent expression. The ST512 isolate had the same mutation, plus an additional mutation in the ompK36 porin gene, while the ST1048 isolate had apparently functional OmpK35 and OmpK36. S1 nuclease digestion and pulsed-field gel-electrophoresis revealed at one to five plasmids in these isolates and IncFIKp, IncX3 and CoE-type plasmids, previously seen in isolates carrying blaKPC genes, were identified in most by PCR. Conjugative plasmids carrying blaKPC were transferred from most isolates to rifampicin resistant E. coli DH5αRf. In the original isolates from Sydney, from a patient returning from Greece, blaKPC2 and the blaSHV-12 ESBL gene were found on a ~53 kb IncX3 plasmid that appears identical to a plasmid from a Greek patient returning to France. At least some of the other plasmids appear different from those previously found to carry blaKPC genes and complete sequencing of these and other plasmids from these isolates is planned to shed further light on how this important gene is spreading.

Oral presentation at the Australian Society for Antimicrobials Annual Meeting, Sydney, Australia, 21st-23rd February 2013.
Attacca – accurate automated annotation of sequences related to antibiotic resistance.

G Tsafnat¹, S Partridge²
Centre for Health Informatics, University of New South Wales, NSW¹
Centre for Infectious Diseases and Microbiology, University of Sydney, NSW²

Background. Multidrug resistance in Gram-negative bacteria is an increasing global problem and many multiresistance plasmids and regions have now been sequenced. Unfortunately, such sequences are often poorly annotated, as current analysis software focuses on identifying genes and putative functions of encoded proteins. Many resistance gene functions are well known, so consistency and identifying boundaries of associated mobile genetic elements are more important in this domain. We have extended Attacca, which was developed for gene cassette arrays in integrons, to accurately annotate other types of mobile elements and associated resistance genes.

Methods. Resistance genes and relevant mobile elements (from existing websites, where possible) were added a database of “features”. Known rules derived from the literature were used to create computational grammars that can annotate complex resistance regions. Annotations were compared with manually annotated sequences to refine the grammars.

Results. Attacca is able to accurately annotate resistance genes, insertion sequences and transposons, in addition to gene cassettes and integrons, and identify fragments of these features, insertions of one mobile element inside another and direct repeats indicative of insertions.

Conclusions. Attacca provides accurate and consistent annotation of complex sequences relating to antibiotic resistance in Gram-negative bacteria, facilitating detailed comparative analysis to understand the evolution of these regions and their spread. The extended Attacca will be incorporated into the Repository of Antibiotic-resistance Cassettes (www2.chi.unsw.edu.au/rac).

Poster Walk and Poster at the 53rd Interscience Conference on Antimicrobial Agents and Chemotherapy, Denver, 10th-13th September 2013.
Variations in contexts of $bla_{CTX-M-1}$ group genes

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South China Agricultural University, Guangzhou, China$^2$
Centre for Infectious Diseases and Microbiology, The University of Sydney, NSW$^1$

Background. CTX-M enzymes, the dominant extended-spectrum beta-lactamases (ESBL) worldwide, belong to 6 groups. $bla_{CTX-M-3}$, an ancestor of at least some $bla_{CTX-M-1}$ group genes, has been captured from the Kluvyera ascorbata chromosome by $ISEcp1$ and transferred to plasmids, with subsequent mutations generating minor variants, some conferring increased resistance. $ISEcp1$ also carries a promoter expressing captured genes. Unlike other $bla_{CTX-M}$ groups, different "spacer" lengths (45, 48, 80 or 127 bp) have been found between the right end of $ISEcp1$ and $bla_{CTX-M-1}$ group genes. Initially, reported spacers seemed to be characteristic for particular genes, but this is changing. We have examined available context information and isolates to try and understand more about the relationships and evolution of $bla_{CTX-M-1}$ group genes.

Methods. Sequences of $bla_{CTX-M-1}$ group genes that include context information were identified by BLAST searches and obtained from GenBank, with additional information from literature searches. Isolates with a $bla_{CTX-M-1}$ group gene were obtained from various collections. PCR and sequencing were used to identify $bla_{CTX-M}$ genes, spacers and genetic contexts. MICs for cefotaxime were measured for selected isolates by microdilution.

Results. Analysis of available information and/or isolates identified examples of the same gene with different spacers and different genes with the same spacer, e.g. $bla_{CTX-M-3}$, $bla_{CTX-M-15}$ (1 nt difference from $bla_{CTX-M-3}$) and $bla_{CTX-M-55}$ (1 additional nt difference) with a 127, 48 or 45 bp spacer. Similarities in wider genetic contexts suggest the same mutation has occurred on more than one occasion. Consistent with published data, an isolate with $bla_{CTX-M-3}$ and a 48 bp spacer had a substantially high cefotaxime MIC compared with a similar isolate with $bla_{CTX-M-3}$ and a 127 bp spacer, suggesting that reduction in spacer length might be advantageous. In the 80 bp spacer associated with genes closely related to $bla_{CTX-M-4}$, the 48 bp closest to the gene match the typical $bla_{CTX-M-3}$ group spacer sequence, while the first 32 bp match part of a region between $ISEcp1$ and the orfRA14 gene, which could be due to recombination or an $ISEcp1$-mediated event.

Conclusions. Mutations in $bla_{CTX-M-1}$ group genes that give rise to increased ceftazidime resistance appear to have occurred more than once. The different spacers and contexts of $bla_{CTX-M-1}$ group genes may be a combination of movement of different "transposition units", possibly $ISEcp1$-mediated deletions, and recombination.

Poster at the 53rd Interscience Conference on Antimicrobial Agents and Chemotherapy, Denver, 10th-13th September 2013.
The natural distribution of β-lactams resistance in Enterobacteriaceae

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Abstract. Aim. Investigate the natural MIC distribution of β-lactam resistance mechanisms in E. coli. Methods. 200 E. coli clinical isolates were obtained from Sydney and classified on the basis of β-lactamases genes detected by PCR: control group, including 30 isolates, negative for all target resistant genes and sensitive for all antibiotics tested; and blaTEM-1b, blaCMY-2-like, blaCMY-2-like+blaTEM-1b, blaCTX-M and blaIMP-4-like groups; including 33, 23, 18, 46 and 50 isolates, respectively. As β-lactams resistance is usually linked to a multi resistance phenotype, the isolates were screened for other resistance genes and major plasmid types. Finally, MICs for six different β-lactams (cefotaxime, ceftazidime, ticarcillin, imipenem, meropenem and ertapenem) were determined by broth microdilution. The antibiotic MIC distributions for each genotype were compared with the data published by The European Committee on Antimicrobial Susceptibility Testing (EUCAST) (www.eucast.org). Results and Discussion. All groups (control and resistant) show normal MIC distributions for all the antibiotics tested, indicating a clear predictive relationship between genotype and phenotype within isolates participating in the mobile gene pool. Isolates carrying blaTEM-1b gene preceded by a strong (“Pa/b”) promoter were more resistant to ticarcillin than blaTEM-1b preceded by the usual (“P3”) promoter and although these were normally distributed and the stronger promoter was linked to resistance to the clinically important antibiotic Timentin, there was significant overlap. The international standard EUCAST cut-off values were for ertapenem and cefotaxime, include all the blaTEM-1b positive isolates, thus misclassifying them as wild-type. This is also true for ertapenem in 17%, 45% and 73% of isolates carrying blaCMY-2-like, blaCMY-2-like+blaTEM-1b, blaCTX-M genes, respectively. The internationally adopted cut-off values for these important antibiotics are inadequate as epidemiological markers for antimicrobial resistance survey programs.
Limited diversity in the gene pool allows prediction of third-generation cephalosporin and aminoglycoside resistance in *Escherichia coli* and *Klebsiella pneumonia*

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**BACKGROUND:** Early appropriate antibiotic treatment reduces mortality in severe sepsis, but current methods to identify antibiotic resistance still generally rely on bacterial culture. Modern diagnostics promise rapid gene detection, but the apparent diversity of relevant resistance genes in *Enterobacteriaceae* is a problem. Local surveys and analysis of publicly available data sets suggested that the resistance gene pool is dominated by a relatively small subset of genes, with a very high positive predictive value for phenotype.

**AIM:** To test whether it is possible to identify a small subset of plasmid-borne resistance genes that act as reliable predictive markers for resistance (and susceptibility) to third-generation cephalosporins, as well as associated resistance to aminoglycosides, in *E. coli* and *K. pneumoniae* isolates from Sydney.

**METHODS:** 152 *Escherichia coli* and 115 *Klebsiella pneumoniae* consecutive isolates with a cefotaxime, ceftriaxone and/or ceftazidime minimum inhibitory concentration (MIC) of ≥2 μg/mL were collected from seven major hospitals in Sydney in 2008-2009. All isolates were screened for genes conferring resistance to third-generation cephalosporin and aminoglycoside antibiotics by multiplex PCR/reverse line blot hybridisation (mPCR/RLB).

**RESULTS AND DISCUSSION:** Nearly all isolates with a MIC in excess of European Committee on Antimicrobial Susceptibility Testing (EUCAST) resistance breakpoints contained one or more representatives of only seven gene types capable of explaining resistance to third-generation cephalosporins, and this included 96% of those with a MIC ≥2 μg/mL to cefotaxime, ceftriaxone and/or ceftazidime. Similarly, 97% of associated gentamicin-non-susceptibility (MIC ≥8 μg/mL) could be explained by three gene types. In a country like Australia, with a background prevalence of resistance to third-generation cephalosporins of 5-10%, this equates to a negative predictive value of >99.5% for non-susceptibility and is therefore suitable for diagnostic application. This is an important proof-of-principle that should be tested in other geographic locations.
Genetic diversity and antibiotic resistance in *Escherichia coli* from environmental surface water in Dhaka City, Bangladesh

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**Aims of the study.** Emergence of antibiotic resistant bacteria is a major public health concern around the world and bacteria resistant to antibiotics have been detected in a variety of environmental settings. Our aim is to understand the prevalence of antibiotic resistant *Escherichia coli* in environmental water in Bangladesh and characterize their genetic features.

**Methods.** Identification of *E. coli* and their resistant phenotypes were determined by using BD phoenix automated microbiology system. Genetic diversity among isolates were determined by multi locus sequence typing, plasmids were analysed by PCR-based replicon typing and S1 nuclease digestion and pulsed-field gel electrophoresis, antibiotic resistant genes were identified by multiplex PCR-reverse line blot hybridization assay and conventional PCR.

**Results and Discussion.** In this study we characterised 48 *E. coli* isolated from rivers and lakes in and around Dhaka City, the capital of Bangladesh. Forty-eight isolates represented 34 different multi-locus sequence types (ST). Six of these STs were new and included two novel alleles, although most of the identified STs have been previously reported from human isolates. The majority (40/48) carried plasmids, but PCR-based replicon typing identified the plasmid type in only 32 of these, in which IncF and IncI replicon types were most common. Most *E. coli* isolates (n=30) carried multiple antibiotic resistance genes. Two thirds (n=19) carried the ubiquitous extended spectrum β-lactamase gene *bla*\(_{CTX-M-15}\), with much less associated gentamicin resistance than reported elsewhere. Therefore, surface water in Bangladesh appears to be an important reservoir of antibiotic resistance in *E. coli* and may play a major role in dissemination of resistant genes.
Characterization of multi-drug resistant *Klebsiella pneumoniae* from Australia carrying the metallo-β-lactamase gene *bla*<sub>NDM-1</sub>

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The global spread of bacteria carrying the *bla*<sub>NDM-1</sub> carbapenemase gene is of significant public health concern, but to date there are few published reports of this gene in Australia or New Zealand. We have characterised four *Klebsiella pneumoniae* carrying *bla*<sub>NDM-1</sub> isolated in Australia between August 2010 and June 2012. *bla*<sub>NDM-1</sub> was found in the expected genetic context, between a partial copy of ISaba125, which provides a promoter for expression, and a bleomycin resistance gene. Multilocus sequence typing identified one isolate carrying *bla*<sub>NDM-1</sub> as a new sequence type (ST1068), two isolates as ST147 and one as ST11, the latter two being types that are associated with global spread of several important resistance genes. In addition to carbapenem resistance, all isolates displayed high level resistance to gentamicin and amikacin and two had *armA* and one had *rmtC*, while no known 16S rRNA methylase gene was identified in the fourth. *bla*<sub>CTX-M-15</sub>, encoding the globally dominant extended-spectrum β-lactamase, was also identified in all isolates. S1 nuclease digestion and pulsed-field gel-electrophoresis revealed at least one plasmid of ~105-280-kb in all isolates and conjugative plasmids carrying *bla*<sub>NDM-1</sub> plus *armA* or *rmtC* were transferred from three isolates to rifampicin resistant *Escherichia coli* UB5201Rf. One plasmid belonged to incompatibility type IncA/C and another was a variant IncHI1-type, both previously associated with *bla*<sub>NDM-1</sub>. The third had an IncFII<sub>Y</sub>-type replicon (designated allele 4) previously associated with *Yersina pestis* and we believe this is the first time such a replicon has been associated with *bla*<sub>NDM-1</sub>. Transconjugants also all exhibited resistance to all tested carbapenems and β-lactams and high level resistance to gentamicin and amikacin. Examination of *ompK* porin genes and transfer of one plasmid to strains lacking OmpK35 or OmpK36 suggests that porin loss may be less important for achieving clinically relevant levels of resistance for isolates expressing NDM-1 than for other carbapenemases. At least some of the plasmids here appear different from those previously found to carry *bla*<sub>NDM-1</sub> and completely sequencing them will shed further light on how this important gene is spreading.
Global population structure and evolution of *Bordetella pertussis* and their relationship with vaccination

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Bordetella pertussis causes pertussis (whooping cough), a respiratory disease which is most severe for unvaccinated infants. Vaccination against pertussis was introduced in the 1950s, and in the 1990s a resurgence of pertussis was observed worldwide. In particular in 2010 to 2012 large outbreaks were observed in the USA, the UK, the Netherlands and Australia, with significant mortality in infants. Possible causes for the resurgence of pertussis include the switch from whole cell vaccines to less effective acellular vaccines (ACVs), waning immunity and pathogen adaptation. Pathogen adaptation is suggested by antigenic divergence between vaccine strains and circulating strains, and clonal expansions after the introduction of vaccination. Most studies on adaptation of B. pertussis have focused on a limited number of genes or a geographically constrained strain collection. Here we use a more comprehensive approach to study adaptation, by applying comparative genomics to a large worldwide collection of B. pertussis strains. The global phylogeny showed two deep branches; the largest of these contained 98% (336/343) of all strains, and its appearance correlated temporally with the first descriptions of pertussis outbreaks in Europe. We found little evidence of recent geographical clustering of the strains within this lineage, suggesting rapid strain flow between countries. The presence of the second branch suggests that there were at least two introductions of pertussis into the global population from an un-sampled reservoir. We used several approaches to identify genes and gene categories under selection, including SNP density and homoplasy. These approaches consistently suggested that virulence-associated genes and genes coding for surface-exposed proteins were involved in adaptation. Many of the putative adaptive loci we identified have a physiological role, and further studies of these loci may reveal less obvious ways in which the B. pertussis and the host interact. In particular, our analysis revealed putative adaptive mutations in two genes involved in sulphate metabolism. Sulphate can be used to regulate virulence associated genes in vitro, and our results suggest that sulphate may also be an important cue during natural infection.

PLoS Biology 2013 (under review)
The global emergence of a highly-fluoroquinolone resistant *Salmonella enterica* serotype Kentucky ST198 strain

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While the spread of *Salmonella enterica* serotype Kentucky ST198-X1 across Africa and the Middle-East has been described recently, the presence of this strain in humans, food, various animal species (livestock, pets, and wildlife) and in environment is suspected in other countries of different continents. Here, we report results of an in-depth molecular epidemiological study on a global human and non-human collection of S. Kentucky (n=70). We performed XbaI-pulsed field gel electrophoresis and multilocus sequence typing, assessed mutations in the quinolone resistance-determining regions, detected β-lactam resistance mechanisms, and screened the presence of the *Salmonella* genomic island 1 (SGI1). In this study, we highlight the rapid and extensive worldwide dissemination of the ciprofloxacin-resistant S. Kentucky ST198-X1 strain since the mid-2000s in an increasingly large number of contaminated sources, including the environment. This strain has accumulated an increasing number of chromosomal and plasmid resistance determinants and has been identified in the Indian subcontinent, Southeast Asia and Europe since 2010. The second substitution at position 87 in GyrA (replacing the amino acid Asp) appeared
helpful for epidemiological studies to track the origin of contamination. This global study provided evidence leading to the conclusion that high-level resistance to ciprofloxacin in S. Kentucky is a simple microbiological trait that facilitates the identification of the epidemic clone of interest, ST198-X1-SGI1. Taking this into account is essential in order to detect and monitor it easily and to take rapid measures in livestock to ensure control of this infection.

*Frontiers in Microbiology 2013 (under review)*
Proof-of-concept study for successful inter-laboratory comparison of MLVA results

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Multiple-locus variable-number of tandem repeats analysis (MLVA) is widely used for typing of pathogens. Methods based on determining DNA fragment size by the use of capillary electrophoresis have an inherent problem as a non-insignificant offset between measured and real lengths commonly occurs. This discrepancy depends on a variety of factors in the laboratory setup for fragment analysis. To obtain comparable results between laboratories using different setups, some sort of calibration is necessary. A simple approach is to use a set of calibration isolates with known allele sizes. This is a proof-of-concept study showing that using such a calibration set of isolates makes inter-laboratory comparison possible. In this study 20 international reference laboratories were provided with 33 calibration isolates and were asked to characterise 15 test isolates by a 5-locus Salmonella Typhimurium MLVA. When using a calibration isolates, 99.4% of the MLVA alleles of the test strains were assigned correct alleles compared to 64.8% without any compensation. We therefore recommend this concept for obtaining comparable MLVA results.

Eurosurveillance 2013; 18(35):pii=20566
Phenotypically occult multidrug-resistant *Mycobacterium tuberculosis* – dilemmas in diagnosis and treatment

Ho J, Jelfs P, Sintchenko V.

**Objectives:** The clinical significance of the emergence of *Mycobacterium tuberculosis* (MTB) isolates that contain *rpoB* mutations but are phenotypically susceptible to rifampicin remains uncertain. The aim of this study was to determine the prevalence of MTB cases that demonstrate this discordant rifampicin (RIF) resistance patterns and to establish whether these patients have poorer treatment outcomes with RIF based regimes.

**Methods:** *rpoB* sequencing was performed on all MTB isolates demonstrating phenotypic resistance to one or more first-line anti-tuberculosis agents (excluding RIF). RIF minimum inhibitory concentrations (MICs) were determined for *rpoB* mutation positive isolates and clinical charts were reviewed to identify treatment outcomes in these patients.

**Results:** Of the 214 phenotypically drug (excluding RIF) resistant isolates tested, five contained *rpoB* mutations. These isolates demonstrated elevated RIF MICs (RIF G\textsuperscript{R} P\textsuperscript{S}), despite testing susceptible using phenotypic broth based methods. Four of them were isoniazid resistant (INH-R) and one pyrazinamide resistant. One patient experienced a relapse of tuberculosis (TB) two years after completion of a RIF containing regime. These findings are consistent with a recent study which reported treatment failure with RIF based regimes in patients with INH-R MTB and genotypic RIF resistance.

**Conclusions:** While MTB RIF G\textsuperscript{R} P\textsuperscript{S} strains remain relatively uncommon they can be associated with low level RIF resistance and poorer treatment outcomes with RIF based regimes. These recently recognised forms of multidrug-resistant TB should be adequately detected and managed.

*Journal of Antimicrobial Chemotherapy* 2013 (Accepted 13 June 2013).
Transmission of *Mycobacterium tuberculosis* from an Asian elephant (*Elephas maximus*) to a chimpanzee (*Pan troglodytes*) and humans in an Australian zoo

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*Mycobacterium tuberculosis* is primarily a pathogen of humans. Infections have been reported in animal species and it is emerging as a significant disease of elephants in the care of humans. With the close association between humans and animals, transmission can occur. In November 2010, a clinically healthy Asian elephant in an Australian zoo was found to be shedding *M. tuberculosis*; in September 2011, a sick chimpanzee at the same zoo was diagnosed with tuberculosis caused by an indistinguishable strain of *M. tuberculosis*. Investigations included staff and animal screening. Four staff had tuberculin skin test conversions associated with spending at least 10 hours within the elephant enclosure; none had disease. Six chimpanzees had suspected infection. A pathway of transmission between the animals could not be confirmed. Tuberculosis in an elephant can be transmissible to people in close contact and to other animals more remotely. The mechanism for transmission from elephants requires further investigation.

*Epidemiology and Infection* 2013; 141:1488-1497
Software for selecting the most informative sets of genomic loci for multi-target microbial typing

O'Sullivan MV, Sintchenko V, Gilbert GL.

**Background:** High-throughput sequencing can identify numerous potential genomic targets for microbial strain typing, but identification of the most informative combinations requires the use of computational screening tools. This paper describes novel software – Automated Selection of Typing Target Subsets (AuSeTTS) - that allows intelligent selection of optimal targets for pathogen strain typing. The objective of this software is to maximise both discriminatory power, using Simpson’s index of diversity ($D$), and concordance with existing typing methods, using the adjusted Wallace coefficient ($AW$). The program interrogates molecular typing results for panels of isolates, based on large target sets, and iteratively examines each target, one-by-one, to determine the most informative subset.

**Results:** AuSeTTS was evaluated using three target sets: 51 binary targets (13 toxin genes, 16 phage-related loci and 22 SCCmec elements), used for multilocus typing of 153 methicillin-resistant *Staphylococcus aureus* (MRSA) isolates; 17 MLVA loci in 502 *Streptococcus pneumoniae* isolates from the MLVA database (www.mlva.eu) and 12 MLST loci for 98 *Cryptococcus* spp. isolates. The maximum $D$ for MRSA, 0.984, was achieved with a subset of 20 targets and a $D$ value of 0.954 with 7 targets. Twelve targets predicted MLST with a maximum $AW$ of 0.9994. All 17 *S. pneumoniae* MLVA targets were required to achieve maximum $D$ of 0.997, but 4 targets reached $D$ of 0.990. Twelve targets predicted pneumococcal serotype with a maximum $AW$ of 0.899 and 9 predicted MLST with maximum $AW$ of 0.963. Eight of the 12 MLST loci were sufficient to achieve the maximum $D$ of 0.963 for *Cryptococcus* spp.

**Conclusion:** Computerised analysis with AuSeTTS allows rapid selection of the most discriminatory targets for incorporation into binary typing schemes. Output of the program is presented in both tabular and graphical formats and the software is available for free download from [http://www.cidmpublichealth.org/pages/ausetts.html](http://www.cidmpublichealth.org/pages/ausetts.html).

*BMC Bioinformatics* 2013; 14:148
Quantitative estimation of MRSA strain-typing system stability using Kaplan-Meier survival analysis.

O’Sullivan MV, Zhou F, Sintchenko V, Gilbert GL.

Knowledge concerning stability is important in the development and assessment of microbial molecular typing systems and is critical for the interpretation of their results. Typing system stability is usually measured as the fraction of isolates that change type after several in vivo passages, but this does not necessarily reflect in vivo stability. The aim of this study was to utilize survival analysis to provide an informative quantitative measure of in vivo stability and to compare the stabilities of various techniques employed in typing methicillin-resistant Staphylococcus aureus (MRSA). We identified 100 MRSA pairs (isolated from the same patient >1 month apart) and typed them using multilocus sequence typing (MLST), phage-derived open reading frame (PDOR) typing, toxin gene profiling (TGP), staphylococcal cassette chromosome mec (SCCmec) subtyping, pulsed-field gel electrophoresis (PFGE), and spa sequence typing. Discordant isolate pairs, belonging to different MLST clonal complexes, were excluded, leaving 81 pairs for analysis. The stabilities of these methods were examined using Kaplan-Meier survival analysis, and discriminatory power was measured by Simpson’s index of diversity. The probability percentages that the type remained unchanged at 6 months for spa sequence typing, TGP, multilocus variable number of tandem repeats analysis (MLVA), SCCmec subtyping, PDOR typing, and PFGE were 95, 95, 88, 82, 71, and 58, respectively, while the Simpson’s indices of diversity were 0.48, 0.47, 0.70, 0.72, 0.89, and 0.88, respectively. Survival analysis using sequential clinical isolates adds an important quantitative dimension to the measurement of stability of a microbial typing system. Of the methods compared here, PDORF typing provides high discriminatory power, comparable with that of PFGE, and a level of stability suitable for MRSA surveillance and outbreak investigations.

Journal of Clinical Microbiology 2013; 51(1):112-116
Prospective genotyping of hospital-acquired MRSA using a novel, highly discriminatory binary typing system

O’Sullivan MV, Zhou F, Sintchenko V, Gilbert GL

In settings of high methicillin-resistant Staphylococcus aureus (MRSA) prevalence, detection of nosocomial transmission events can be difficult without strain typing. Prospective typing of all MRSA isolates could potentially identify transmission in a timely fashion, making infection control responses to outbreaks more effective. We describe the development and evaluation of a novel 19-target binary typing system for MRSA using the multiplex-PCR/reverse line blot hybridization platform. Pulse-field gel electrophoresis (PFGE), spa typing, and phage-derived open reading frame (PDORF) typing were performed for comparison. The system was utilized to identify transmission events in three general surgical wards over a 12-month period. Initial MRSA isolates from 273 patients were differentiated into 55 unique binary types. One or more potential contacts colonized with the same MRSA strain were identified in 69 of 87 cases (79%) in which definite or possible nosocomial MRSA acquisition had occurred. The discriminatory power of the typing system was similar to that of PFGE (Simpson's index of diversity \( D = 0.994 \), versus 0.987) and higher than that of spa typing \( (D = 0.926) \). Strain typing reduced the total number of potential MRSA-colonized source contacts from 859 to 212 and revealed temporal clustering of transmission events. Prospective MRSA typing using this novel binary typing method can rapidly identify nosocomial transmission events, even in high-prevalence settings, which allows timely infection control interventions. The system is rapid, inexpensive, discriminatory, and suitable for routine, high-throughput use in the hospital microbiology laboratory.

*Journal of Clinical Microbiology 2012, 50(11):3513-3519*
Francisella tularensis subspecies holarctica, Tasmania, Australia, 2011


We report the case of a woman who developed ulceroglandular tularemia following the bite of a ringtail possum (Pseudocheirus peregrinus) in the forests of Tasmania, Australia. Francisella tularensis subspecies holarctica was detected for the first time in the Southern Hemisphere and was identified on the basis of 16S rRNA, recA gene sequence analyses and PCR of the region of difference (RD1).

Emerging Infectious Diseases 2012;18(9):1484-1486.
Impact of a web-based personally controlled health management system on influenza vaccination and health services utilization rates: A randomised controlled trial

Lau AYS, Sintchenko V, Crimmins J, Magrabi F, Gallego F, Coiera E.

Objective: To assess the impact of a web-based personally controlled health management system (PCHMS) on the uptake of seasonal influenza vaccine and primary care service utilisation among university students and staff.

Materials and Methods: A PCHMS called Healthy.me was developed and evaluated in a 2010 CONSORT-compliant two-group (6-month waitlist vs. PCHMS) parallel randomised controlled trial (RCT) (allocation ratio 1:1). The PCHMS integrates an personal health record (PHR) with consumer care pathways, social forums and messaging links with a health service provider.

Results: 742 university students and staff met inclusion criteria and were randomised to 6-month waitlist (n=372) or PCHMS (n=370). Among the 470 participants eligible for primary analysis, participants obtaining an influenza vaccine during the study were 137% higher for the PCHMS group, relative to waitlist (relative risk (RR)=2.4, 95% CI:1.23 to 4.60). The PCHMS group also had 65% higher rate of visiting the University Health Service (UHS) than wait-list (RR=1.6, 95% CI 1.18 to 2.30). A dose-response effect was detected, where greater use of PCHMS was associated with higher proportions of participants receiving an influenza vaccine (P=.001) and visiting UHS during the study (P=0.003).

Discussion: Use of PCHMS has a significant effect on influenza vaccination rates (Φ=.123) and visits to the university primary care service (Φ=0.137).

Conclusions: While evidence for the impact of PHRs on health behaviours is weak, this study demonstrated that integrating a PHR with a suite of tools to facilitate the management of care has the potential to significantly influence consumer behaviours.

Selected by an international editorial board for publication in the 2012 AMIA Informatics in Review Report
Improving resolution of public health surveillance for human *Salmonella enterica* serovar Typhimurium infection: 3 years of prospective multiple-locus variable-number tandem-repeat analysis (MLVA)


**Background:** Prospective typing of *Salmonella enterica* serovar Typhimurium (STM) by multiple-locus variable-number tandem-repeat analysis (MLVA) can assist in identifying clusters of STM cases that might otherwise have gone unrecognised, as well as sources of sporadic and outbreak cases. This paper describes the dynamics of human STM infection in a prospective study of STM MLVA typing for public health surveillance.

**Methods:** During a three-year period between August 2007 and September 2010 all confirmed STM isolates were fingerprinted using MLVA as part of the New South Wales (NSW) state public health surveillance program.

**Results:** A total of 4,920 STM isolates were typed and a subset of 4,377 human isolates was included in the analysis. The STM spectrum was dominated by a small number of phage types, including DT170 (44.6% of all isolates), DT135 (13.9%), DT9 (10.8%), DT44 (4.5%) and DT126 (4.5%). There was a difference in the discriminatory power of MLVA types within endemic phage types: Simpson’s index of diversity ranged from 0.109 and 0.113 for DTs 9 and 135 to 0.172 and 0.269 for DTs 170 and 44, respectively. 66 distinct STM clusters were observed ranging in size from 5 to 180 cases and in duration from 4 weeks to 25 weeks. 43 clusters had novel MLVA types and 23 represented recurrences of previously recorded MLVA types. The diversity of the STM population remained relatively constant over time. The gradual increase in the number of STM cases during the study was not related to significant changes in the number of clusters or their size. 667 different MLVA types or patterns were observed.

**Conclusions:** Prospective MLVA typing of STM allows the detection of community outbreaks and demonstrates the sustained level of STM diversity that accompanies the increasing incidence of human STM infections. The monitoring of novel and persistent MLVA types offers a new benchmark for STM surveillance.

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East African Indian strains of *Mycobacterium tuberculosis* overtook Beijing family as a prevalent cause of tuberculosis in New South Wales, Australia

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**Background:** Mycobacterial interspersed repetitive unit-variable tandem repeat analysis (MIRU-VNTR) has been applied to examine population dynamics and clustering rates of *Mycobacterium tuberculosis* (MTB). The aim of this study was to investigate the dynamics of MTB epidemiology since 2006-2008 and the relative impact of common lineages of MTB.

**Methods:** Total 930 culture confirmed tuberculosis cases identified in 2010, 2011 and 2012 in New South Wales (NSW), Australia were analyzed. MTB isolates were prospectively genotyped by 24 loci MIRU-VNTR method and lineages were assigned using miruvntrplus.org. The associations between MTB lineages, patient demographics, sites of infection and drug resistance were explored.

**Results:** While the proportion of Beijing lineage isolates (28.2%) has not significantly changed since previous report (Gallego et al. 2009), the frequency of East African Indian (EAI) and Central Asian lineages (Delhi/CAS) has increased up to 28.7% and 12.7%, respectively. Cases due to Beijing lineage were more likely to be associated with respiratory disease and drug resistance (p<0.05) whereas EAI strains and Delhi/CAS were associated with non-respiratory TB (p<0.001 and p<0.05, respectively). Age of patients was significantly associated with lineage (p<0.0001). In particular, the age of those who affected with Delhi/CAS (mean age 37) was significantly less than of those affected by MTB Beijing (mean age 42, p<0.05) or MTB EAI (mean age 44, p<0.01). Further the age of those affected by Harleem lineage (mean age 59, p<0.05) was significantly higher than people affected by any other lineages detected in NSW. Recent evidence from China suggested that MTB Beijing with 223325173533 MIRU-VNTR allele can be highly transmissible and related to multi-drug resistance (Hu et al. 2011). We found 85 cases of MTB with these MIRU-VNTR alleles in our patient population.

**Conclusion:** East African Indian strains of *Mycobacterium tuberculosis* recently overtook Beijing family as a prevalent cause of tuberculosis in New South Wales, Australia.

*Presented at the International Union World TB conference, 30 Oct-3 Nov 2013, Paris, France*
No innovation without evaluation: Added value of prospective multiple-locus variable-number tandem-repeat analysis (MLVA) for surveillance of *Salmonella enterica* serovar Typhimurium

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**Objective:** Subtyping of *Salmonella enterica* serovar Typhimurium (STM) by multiple-locus variable-number tandem-repeat analysis (MLVA) can assist in identifying hidden clusters of community infections, as well as sources of endemic and outbreak cases. This public health surveillance study examined the temporal and spatial population dynamics of STM infection using prospective STM MLVA typing.

**Methods:** All STM referred to the New South Wales Enteric Reference Laboratory during the 5-year period between January 2008 and December 2012, were fingerprinted using MLVA-5 and phage typed. Clusters of ≥5 isolates with the same MLVA pattern collected over 4 weeks were followed up epidemiologically and their temporal and spatial densities measured.

**Results:** A total of 8,936 human isolates were analysed. The STM spectrum was dominated by PT170 (44.6% of all isolates) and PT135 (13.9%). 1562 MLVA patterns were observed and 14 ‘endemic’ patterns were responsible for 45-50% of cases. However, the ratio between novel and endemic patterns remained constant. The Simpson’s index of diversity was 0.109 for PT135 and 0.172 for PT170. 88 STM clusters were observed ranging in size from 5 to 262 cases and in duration from 4 to 25 weeks; 43 clusters had novel MLVA types and 23 represented recurrences of previously recorded MLVA types. Re-occurring clusters were larger than initial clusters and less spatially dense (P<0.001). 32 clusters were investigated. The relapse rate for clusters that did or did not trigger any public health actions was similar (34%). However, the former reoccurred on average 9 weeks later than clusters that were not investigated epidemiologically (P<0.001). Gene loss and acquisition in the emerging STM 2-7-6-11-0212 clone of PT170 will be discussed.

**Conclusions:** Prospective STM MLVA typing improves the resolution and timeliness of public health surveillance. Sustained levels of STM diversity were accompanied by an increasing incidence of human infections and the endemicity of PT170 clones.

*Presented at the 10th International Meeting on Microbial Epidemiological Markers (IMMEM-10), 2-5 Oct 2013, Paris, France*
Added value of environmental sampling in nosocomial MRSA outbreak investigations with a novel rapid high-resolution typing system

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Objective: The role of environmental sampling in investigations of nosocomial outbreaks of MRSA remains controversial. This study investigated the utility of environmental sampling when combined with highly discriminatory typing using a novel 19-target binary typing system in a setting of high MRSA endemicity in a large tertiary University Hospital in Sydney, Australia.

Methods: A point prevalence survey of MRSA carriage was conducted on 3 adjacent surgical wards in response to a high rate of clinical infections. Subsequently, selected environmental locations and staff were also screened. All MRSA isolates were typed using a 19-target binary typing system on a multiplex PCR/reverse line blot assay platform, which is rapid, high-throughput, inexpensive and has similar discriminatory power to pulse field gel electrophoresis.

Results: There were 76 patients in the wards at the time of the survey, of whom 10 were already known to be infected or colonized with MRSA. Fourteen patients were found to be colonized with MRSA for the first time during the survey. Thirty-seven returned negative screens and 15 were not screened because they refused or were unavailable at the time. Of the 52 who were negative or not screened, 3 became colonized or infected with MRSA during the next 3 months. Twelve of 144 (8%) environmental samples and 1 of 21 (6%) staff nasal swabs were positive for MRSA. Seventeen binary types were found amongst the 37 isolates (27 from patients; 10 from staff or environment) available for typing; 5 of these 17 types were represented by multiple isolates (range 2-9) while 12 were singletons. Geographic clustering was evident within types, indicating links between patient and environmental isolates.

Conclusion: This rapid, highly discriminatory and inexpensive binary typing system for MRSA redefines the role of environmental sampling in establishing links between environmental contamination with MRSA and patient colonization.

Presented at the 10th International Meeting on Microbial Epidemiological Markers (IMMEM-10), 2-5 Oct 2013, Paris, France
New insights into molecular epidemiology of *Mycobacterium tuberculosis* in the most populous state of Australia

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**Rationale:** Mycobacterial interspersed repetitive unit-variable tandem repeat analysis (MIRU-VNTR) has been applied to examine population dynamics and clustering rates of *Mycobacterium tuberculosis* (MTB). This study aims to investigate local transmission chains to guide public health intervention in a setting where vast majority of MTB strains are imported from the Asia Pacific Regions.

**Methods:** Total 1128 culture confirmed tuberculosis cases identified in 2010, 2011 and 2012 in New South Wales (NSW), Australia were analyzed. MTB isolates were prospectively genotyped by 24 loci MIRU-VNTR method and lineages were assigned using miruvntrplus.org. The associations between MTB lineages, patient demographics, sites of infection and drug resistance were explored using descriptive statistics.

**Results:** While the proportion of Beijing lineage isolates (27.6%) has not significantly changed since previous report (Gallego et al. 2009), the frequency of East African Indian (EAI) and Central Asian lineages (Delhi/CAS) has increased up to 28.5% and 13.8%, respectively. Cases due to Beijing lineage were more likely to be associated with respiratory disease and drug resistance (p<0.05) whereas EAI strains and Delhi/CAS were associated with non-respiratory TB (p<0.001 and p<0.05, respectively). Age of patients was significantly associated with lineage (p<0.0001). In particular, the age of those who affected with Delhi/CAS (mean age 37) was significantly less than of those affected by MTB Beijing (mean age 42, p<0.05) or MTB EAI (mean age 44, p<0.01). Further the age of those affected by Haarlem lineage (mean age 59, p<0.05) was significantly higher than people affected by any other lineages detected in NSW. Recent evidence from China suggested that MTB Beijing with 223325173533 MIRU-VNTR allele can be highly transmissible and related to multi-drug resistance (Hu et al. 2011). We found 93 cases of MTB with these MIRU-VNTR alleles in our patient population. 10 MTB isolates were monoresistant to any first line tuberculosis drugs and 1 isolate was multi-drug resistant.

**Conclusion:** East African Indian strains of *Mycobacterium tuberculosis* recently overtook Beijing family as a prevalent cause of tuberculosis in New South Wales, Australia. Despite this change in molecular epidemiology of MTB the rates of recent transmission in NSW remain low.
Detection of *Bordetella holmesii* in respiratory specimens from patients with suspected pertussis in NSW

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**Background.** *Bordetella holmesii* is a recently identified human pathogen closely related to *Bordetella pertussis*, the causative agent of whooping cough. Initial case descriptions indicated that it was an opportunistic pathogen that caused invasive disease in immunocompromised patients. However, from 2012 onwards there have been numerous reports from different parts of the world suggesting that it is also a significant respiratory pathogen often misdiagnosed as *B. pertussis* by routine PCR-based screening.

**Methods.** A highly sensitive *B. holmesii*-specific real-time PCR assay was developed to retrospectively screen a convenience sample of 518 (from a total of 2252) *B. pertussis*-positive samples referred to the Centre for Infectious Diseases and Microbiology – Public Health between October 2008 and June 2012. This period spanned a large community pertussis outbreak throughout NSW that erupted between mid 2008 and early 2010.

**Results.** *B. holmesii* was detected in 36 samples and further species-specific PCR assays determined that 35 samples were positive for *B. holmesii* and negative for *B. pertussis*, while one was positive for both, indicating a mixed infection. *B. holmesii* was only detected between May 2009 and December 2010, with the highest incidence rates in October and November 2010, where it was detected in 9/35 and 6/17 samples, respectively. This was during a period of low pertussis detection, but *B. holmesii* was also detected during the preceding months of high pertussis activity, indicating that it can co-circulate with *B. pertussis*.

**Conclusions** *B. holmesii* was misidentified as *B. pertussis* in 7.7 % of clinical samples from patients with respiratory illness, indicating that *B. holmesii* has sporadically been a significant contributor to the burden of pertussis-syndrome in NSW. However, prospective screening and assessment of epidemiological data will be needed to assess the potential severity and transmissibility of *B. holmesii* respiratory disease.
Effectiveness of hospital-wide Methicillin-resistant *Staphylococcus aureus* (MRSA) infection control policies differs by ward specialty

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Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major cause of preventable nosocomial infections and is endemic in hospitals worldwide. The effectiveness of infection control policies varies significantly across hospital settings. The impact of the hospital context towards the rate of nosocomial MRSA infections and the success of infection control is understudied. We conducted a modelling study to evaluate several infection control policies in surgical, intensive care, and medical ward specialties, each with distinct ward conditions and policies, of a tertiary public hospital in Sydney, Australia. We reconfirm hand hygiene as the most successful policy and find it to be necessary for the success of other policies. Active screening for MRSA, patient isolation in single-bed rooms, and additional staffing were found to be less effective. Across these ward specialties, MRSA transmission risk varied by 13% and reductions in the prevalence and nosocomial incidence rate of MRSA due to infection control policies varied by up to 45%. Different levels of infection control were required to reduce and control nosocomial MRSA infections for each ward specialty. Infection control policies and policy targets should be specific for the ward and context of the hospital. The model we developed is generic and can be calibrated to represent different ward settings and pathogens transmitted between patients indirectly through health care workers. This can aid the timely and cost effective design of synergistic and context specific infection control policies.

Update of the Australian *Scedosporium* studies with an emphasis on the *S. aurantiacum* MLST scheme and genome project

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*Scedosporium*, are the second commonest fungi after *Aspergillus*, species in the airways of cystic fibrosis (CF) patients in Australia and Europe. The role of fungal colonisation in lung infection and their impact on lung function however is uncertain. We conducted a multicentre longitudinal study of fungal colonisation in children and adults with CF in Australia, with a focus on *Scedosporium*, examining fungal, and clinical factors influencing lung function and CF lung disease. We also developed an MLST scheme for genotyping of *Scedosporium aurantiacum*, the recovery of which is associated with chronic lung disease and CF, and present data on the genome of *S. aurantiacum*.

A total of 188 children and 88 adults from 2 CF clinics were seen at 3-monthly intervals for 2 years (total duration of study 3 years). Airway samples were cultured on standard media and media selective for non-*Aspergillus* moulds. Clinical and microbiological data were recorded at each visit. Amongst children, 111 (59.4%) were colonised with fungi and 68 (36.3%) had *Aspergillus* and/or *Scedosporium* isolated; 6 of 21 *Scedosporium*-colonised patients were colonised only with *Scedosporium*. In adults, 46 (52.7%) were colonised with fungi with 37 (42%) having *Aspergillus* and/or *Scedosporium* isolated (11 *Scedosporium*-colonised, 2 only with *Scedosporium*. Overall, 46.2% of *Scedosporium* spp. were *S. prolificans*, 34.6% *S. aurantiacum* and 19.2%, *S.apiospermum/P. boydii*. 
*S. aurantiacum* is not only an medically important *Scedosporium* species, but has been found in relatively high numbers in the Australian urban environment. To gain insight into the population genetic structure of *S. aurantiacum* a global network including 12 laboratories from Australia, Austria, Germany, Malaysia, Spain, and the UK was formed to develop a multilocus sequence typing (MLST) scheme. Initially 24 genetic loci were screened for polymorphisms on a tester strain set. The six most polymorphic loci finally selected for the MLST scheme were: actin (*ACT*), elongation factor-1α (*EF1α*), calmodulin (*CAL*), RNA polymerase subunit II (*RPB2*), manganese superoxide dismutase (*SOD2*), and β-tubulin (*TUB*). Of 127 clinical, veterinary and environmental isolates, between 6-18 variable sites per genetic locus were revealed, resulting in 10-16 alleles per genetic locus. By MLST analysis an unusual high genetic diversity was observed in the *S. aurantiacum* population, as reflected by 111 unique sequence types. Network analysis revealed a clear separation between Australian and global strains. Phylogenetic analysis showed 6 major clusters, indicating some correlation with geographic origin and infection status (colonization versus infection). There was no clustering of Australian strains by geographic location. In addition, there was also no clustering according to isolate source: clinical, veterinary or environmental. Linkage disequilibrium analysis revealed evidence of recombination within the *S. aurantiacum* population. The high diversity especially among the Australian strains suggests that *S. aurantiacum* may have originated within the Australian continent and subsequently dispersed to other regions, as shown by the close phylogenetic relationships between some of the Australian sequence types and those found elsewhere. MLST data are accessible at mlst.mycologylab.org.

Using the *Galleria mellonella* larvae virulence model two high (WM 06.482 (clinical, Australia) and WM 09.24 (environmental, Australian)) and two (WM 08.202=FMR8630=CBS116910 (clinical, Spain, type culture) and WM 10.136 environmental, Austria) low virulent strains were chosen for whole genome analysis. With the HiSeq 2000 platform between 143,611 and 173,301 raw reads were generated resulting in 104,000 – 129,000 reads. The initial assembly using Velvet generated between 2700 - 4000 contigs. In absence of a *Scedosporium reference* genome the genomes were aligned against the fully annotated genome of *Trichoderma virens* using progressive Mauve for preliminary annotation. For the 4 genomes 31% of genomes have been annotated identifying around 5300 ORFs. The remaining 10,000 possible ORF identified could not be annotated which reflects the fact that the original assembly using Velvet need to be refined. After correct assembly and annotation, SNP analysis will be performed to enable the identification of virulence associated traits.
Understanding the causes of virulence in Scedosporium fungi

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Scedosporium aurantiacum is a fungal pathogen of humans and other animals that is highly prevalent in urban Sydney. The variability of virulence between isolates is putatively a result of genetic differences between them. In an effort to establish the genetic factors underpinning virulence we have generated whole genome sequence for 2 high and 2 low virulence S. aurantiacum strains.

We performed de novo assembly and whole genome multiple alignments that included a well annotated relative. Utilising PyCogent and a new tool we have developed, we predict one-to-one orthologs via alignment based projection from the annotated relative.

Our analyses reveal that the high and low virulence strains are more closely related within a group than between groups. It is possible this relationship reflects a single gain/loss of virulence since the common ancestor of the different phenotypes. However, it is also possible that the greater genetic similarity within phenotype is just a consequence of our small sample size. In other words, the within phenotype genetic similarities may not causally relate to virulence and the evolution of virulence may not be unique.
DNA barcoding of human pathogenic fungi

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With the constant increase in invasive fungal infections, the insufficiency of the current identification techniques (morphology/physiology), the limited available therapies and the emergence of resistant fungal strains there is an urgent need to improve fungal identification to enable a substantial improvement in clinical diseases outcome. Molecular based identification allows for an early identification of the fungal disease agent directly from clinical specimens or pure culture. The Internal Transcribed Spacer (ITS) regions have been used extensively in medical mycology for fungal ID. However, there are no ITS sequences of human pathogenic fungi deposited at BOLD. In 2010 a new ISHAM working group was established, (1) to set up a medical barcode database as part of BOLD by incorporating the different existing fungal group specific databases, (2) to extend the number of quality controlled ITS sequences to cover all medical important fungi, and (3) to achieve a special status as quality controlled reference sequences for those sequences within Ganbank. Currently sequence based ID is based on a cut-off of 98-99% similarity with the type culture of the species in question. Population based studies have shown that the sequence variation in clinical samples is much higher. Fungi have species dependent variable rates of polymorphisms in their ITS1/2 regions. Intra-species variation varies from 0-8.35% (C. parapsilosis 0% and C. tropicalis having 8.35%). Our findings lead to a redefinition of the recommended cut off values to 92% sequence similarity for the ITS1/2 region depending on the fungal species under investigation. As a result of a global collaboration the quality-controlled sequences of more then 2000 fungal strains are now available at www.mycologylab.org. The identified variation raises the question if the ITS region is the most appropriate locus for fungal barcoding. Whole genome sequence comparisons are currently underway to find either alternative genetic loci, better reflecting phylogenetic relationships among fungi, enabling a higher discrimination between fungal species and resulting in a more accurate ID.
Impact of the Changes on Fungal Nomenclature to Ascomycetous Yeasts

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Advances in molecular biology lead to enormous improvements in fungal taxonomy. Multigene phylogenies have been instrumental in the revision of the classification of ascosporic (telemorph) yeasts resulting in a natural system based on lines of decent. This has not yet been done for the anascoporic genus *Candida*, where it is increasingly disappointing to use the same generic name for yeast that have their nearest teleomorphic relatives classified in different families, discrediting all members of the genus *Candida* as potential human pathogens and concealing already known biological information. Generic names should communicate key-knowledge about related species groups. The problem is compounded by the new nomenclature rules reflecting the principle of one fungus = one name. To achieve this principle the current meaning of the name *Candida* = missing of ascospores could be replaced by information on metabolism, lifestyle and phylogenetic relationships if such criteria were integrated in a phylogenetic genus circumscription. Reclassification of *Candida* species by multigene phylogeny can be easily done in well-circumscribed sizable phylogenetic clades allow for the assignment of certain *Candida* species to existing teleomorphic genera with high confidence by multigene phylogeny. *Candida* species that form well-circumscribed sizable multigene phylogenetic clades without any teleomorphic members can become the base for new genera. Phylogenetically isolated species forming long branches in multigene phylogenies, could either be reclassified in small new genera or assigned to the most closely related teleomorph genus. Both options are unsatisfactory as they would result either in many small genera or the addition of distantly related species to homogeneous genera. As such, they should be maintained in the genus *Candida* until neighboring species are described to allow them being integrated in multigene analysis and the resulting groups have gained real biological meaning. When adapting the new nomenclature rules two principles should be followed: 1) Name stability should be honored to the largest possible extend, and 2) Great care should be taken not to create unnecessary names.
Antifungal susceptibility of the emerging pathogen *Cryptococcus gattii* molecular type VGIII

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*Cryptococcus gattii* is a basidiomycetous yeast causing invasive fungal infection and is subdivided into four molecular types, VGI to VGIV, that seem to be genetically isolated cryptic species. Although VGIII has emerged recently in several regions worldwide including Australia, little is known about its epidemiology or response to antifungal treatment.

**Aim:** To determine the susceptibility of this emerging pathogen to the commonly used antifungal drugs.

**Methods:** One-hundred seventeen clinical, veterinarian and environmental *C. gattii* VGIII isolates from eight countries were tested against amphotericin B, 5-fluorocytosine and the azoles posaconazole, voriconazole, itraconazole and fluconazole, using Sensititre® Yeastone®. Minimum inhibitory concentrations (MICs) were the lowest drug concentrations that produced either 100% (amphotericin B) or ≥50% inhibition of growth compared to that of the control.

**Results and Discussion:** The MIC90 and susceptibility ranges obtained were 0.5 (0.12-2) µg/ml for amphotericin B, 4 (0.5-8) µg/ml for 5-fluorocytosine, 0.12 (0.015-0.25) µg/ml for posaconazole, 0.12 (≤0.008-1) µg/ml for voriconazole, 0.12 (≤0.015-0.12) µg/ml for itraconazole and 16 (1-128) µg/ml for fluconazole. Flucytosine and azoles had excellent *in vitro* activity against all tested isolates. However, the high ranges to amphotericin B and fluconazole suggest that the use of these drugs may lead to tolerance or resistance of the pathogen over time.
MALDI-TOF MS Enables the Rapid Identification of the Major Molecular Types within the Cryptococcus neoformans/C. gattii Species Complex

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\end{itemize}

\textbf{Background:} The Cryptococcus neoformans/C. gattii species complex comprises two sibling species that are divided into eight major molecular types, C. neoformans VNI to VNIL and C. gattii VGI to VGIV. These genotypes differ in host range, epidemiology, virulence, antifungal susceptibility and geographic distribution. The currently used phenotypic and molecular identification methods for the species/molecular types are time consuming and expensive. As Matrix-Assisted Laser Desorption Ionization-Time-of-Flight Mass Spectrometry (MALDI-TOF MS) offers an effective alternative for the rapid identification of microorganisms, the objective of this study was to examine its potential for the identification of C. neoformans and C. gattii strains at the intra- and inter-species level.

\textbf{Methodology:} Protein extracts obtained via the formic acid extraction method of 164 C. neoformans/C. gattii isolates, including four inter-species hybrids, were studied.

\textbf{Results:} The obtained mass spectra correctly identified 100% of all studied isolates, grouped each isolate according to the currently recognized species, C. neoformans and C. gattii, and detected potential hybrids. In addition, all isolates were clearly separated according to their major molecular type, generating greater spectral differences among the C. neoformans molecular types than the C. gattii molecular types, most likely reflecting a closer phylogenetic relationship between the latter. The number of colonies used and the incubation length did not affect the results. No spectra were obtained from intact yeast cells. An extended validated spectral library containing spectra of all eight major molecular types was established.

\textbf{Conclusions:} MALDI-TOF MS is a rapid identification tool for the correct recognition of the two currently recognized human pathogenic Cryptococcus species and offers a simple method for the separation of the eight major molecular types and the detection of hybrid strains within this species complex in the clinical laboratory. The obtained mass spectra provide further evidence that the major molecular types warrant variety or even species status.
Association between virulence and the major molecular types of the emerging pathogen Cryptococcus gattii

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Cryptococcosis is a life-threatening disease caused mostly by Cryptococcus neoformans, although the number of cases due to C. gattii has increased recently, affecting more frequently immunocompetent hosts. C. gattii strains are sub-divided into four major molecular types, VGI to VGIV, which differ in their host range, epidemiology, antifungal susceptibility and geographic distribution. From studies on the Vancouver outbreak strains, it is known that the sub-genotype VGIIa is highly virulent compared to the sub-genotype VGIIb. However, not much is known about the virulence of VGI, VGIII and VGIV strains. In order to evaluate the virulence of all genotypes of this emerging pathogen, 5 female Balb/C mice were inoculated intranasally with 10⁸ yeast cells from 8 VGI, 10 VGIIa, 12 VGIIb, 17 VGIII and 8 VGIV strains. The mice were checked daily and weigh twice weekly to identify signs of disease and weight loss. By comparing the number of survival days after inoculation between the studied strains, the VGIV strains showed the highest virulence followed by the VGIIa, VGI, some VGIII and some VGIIb strains. The VGIII strains showed a wide range of virulence. After 60 days of inoculation, the mice inoculated with some of the low virulent VGIIb strains, including the strain CDCR272, as well as the majority of the VGIII strains did not present signs of disease or weight loss. Granulomas were observed in the lungs independent of the infecting strain. India ink stains made directly from lung tissue showed that both cellular and capsular size of all strains increased drastically in comparison with the cellular and capsular size of the same strains before inoculation (p<0.0001), emphasizing the importance of the capsule as a major cryptococcal virulence factor. Culture of heart blood showed the presence of cryptococcal cells in the blood system. Posterior analysis was carried out to determine tissue burden, brain invasion and histological findings. The obtained results correlated with those subsequently obtained with the Galleria mellonella larvae model. The results obtained so far indicate that all the C. gattii major molecular types show a range of virulence among the strains, with some sub-types of them showing a higher virulence than others, indicating the necessity to sub-type isolates in order to chose an appropriate public health response in an outbreak setting. Overall the molecular type VGIV showed the highest virulence amongst C. gattii.
Analysis of *S. aurantiacum* strains exhibiting different virulence using a phenotype microarray

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Genotyping of Australian *Scedosporium* isolates, an emerging human pathogenic fungus, has revealed strong prevalence of a new species *Scedosporium aurantiacum*. In this work, a high throughput Phenotype Microarray (PM) analysis using 96 assorted substrates (sugars, amino acids, hexose-acids and carboxylic acids) was carried out for a series of *S. aurantiacum* strains exhibiting different virulence.

The strains tested were two Australian *S. aurantiacum* isolates of high virulence (WM 06.482 (clinical, Australia) and WM 09.24 (environmental, Australian)) and two isolates showing low virulence (WM 08.202=FMR8630=CBS116910 (clinical, Spain, type culture) and WM 10.136 environmental, Austria). A difference was observed in substrate utilisation pattern for low and high virulence strains where certain carbohydrate sources such as b-Gentibiose, Sucrose and D-Salicin were utilised only by low virulence strains. Similarly some sugar derivatives such as D-Turanose induced respiration only in high virulence strains. Different respiration kinetics was detected under different stress conditions (high salt concentration, lithium chloride and nalidixic acid) at two different temperatures (28°C and 37°C) where respiration was induced in low virulence strains only at high temperature as compared to the high virulence strains which reported high respiration rates at both the temperatures. This indicates that the respiration-based phenotype assay can reveal differences between the strains that showed no correlation between the degree of virulence (low/high) and a specific genotype in the *Galleria mellonella* larvae virulence model.

In summary, information collected from the phenotype microarrays can provide leads for the determination of virulence factors. Integration of the data into metabolic pathway maps and upcoming whole-genome sequences will facilitate studies into physiological and genetic basis of virulence.
The ISHAM ITS database for Human/Animal Pathogenic Fungi - pros and cons of the ITS region as universal fungal DNA barcoding marker

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Correct and fast identification of the causative agents of human mycoses is of great importance for the adequate choice of early and efficient anti-fungal treatments. However, traditional morphological and biochemical based identification of human pathogenic fungi is time-consuming, laborious and needs specially trained personal. To overcome those limits the Internal Transcribed Spacer (ITS) regions of the ribosomal DNA gene cluster have been used for molecular identification of human pathogenic fungi long before it had been selected as the official fungal DNA barcode in 2012. Besides the large number of ITS sequences that have been deposited in GenBank the identification of human pathogenic fungi is often ambiguous and the obtained species names are not always reliable, because of the vast number of incorrect sequences deposited, the limited taxonomic range covered and the limited strain diversity represented. To surmount this problem, a number of quality control ITS databases have been created to ensure the proper identification of fungi. As the result of a global collaboration between 13 medical mycology reference laboratories within the ISHAM working group for “DNA barcoding of human/animal pathogenic fungi" a quality control database has been established, that currently contains ITS sequences of approximately 2000 strains representing 380 human pathogenic species, and is accessible via the www at
http://www.mycologylab.org/BioloMICSID.aspx. These ITS sequences will also be part of the new GenBank reference sequence database. In general the ITS1/2 region has a high probability to successfully identify an agent of human mycosis to the species level, however, the intra-species variation did range from 0-8.5% depending on the species under investigation, raising the question: “Is the ITS region the most appropriate locus for fungal barcoding?” Based on these findings alternative genetic loci are currently under investigation to either be used as secondary DNA barcode in species where the resolution of the ITS is not sufficient or even totally replace it with a more powerful DNA barcoding marker.


**In vitro interactions of *Pseudomonas aeruginosa* and *Scedosporium* spp.**

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*Pseudomonas aeruginosa* and filamentous fungi colonize airways of cystic fibrosis (CF) patients. *P. aeruginosa* has direct/indirect inhibitory effects on fungal growth *in vitro*. Here we assessed *P. aeruginosa* inhibitory effects on *Scedosporium* growth.

Twenty-five *P. aeruginosa* strains (isolate PA14, 9 mucoid and 15 non-mucoid isolates) and 1 strain each of *S. aurantiacum* and *S. prolificans* were studied. Strains were co-cultured on Saboraud’s dextrose agar. The zone of inhibition index (Zx) was calculated. The fluorescent stain FUN-1 was used to qualitate degree of hyphal damage after co-culturing with selected *P. aeruginosa* strains. Growth inhibition of *Scedosporium* in liquid media was studied using the XTT metabolic assay.

Nineteen (76%) and 20 (80%) *P. aeruginosa* strains demonstrated Zx values of <1 for *S. aurantiacum* and *S. prolificans*, respectively. 5/9 (55.5%) mucoid strains had a Zx <1 for both *Scedosporium* species with 14/16 (87.5%) and 15/16 (93.7%) non-mucoid strains showing similar Zx for *S. aurantiacum* and *S. prolificans*, respectively. Overall, similar mean inhibition (Zx=0.76) was observed for both *Scedosporium* species. However, mean Zx was 0.89-0.88 for mucoid strains and 0.68-0.69 for non-mucoid strains respectively. On FUN-1 staining, *S. aurantiacum* and *S. prolificans* co-cultured with live bacteria appeared stunted in growth, damaged or dead. Co-cultivation with dead bacteria revealed actively growing fungi. XTT experiments showed a 55-60% decrease in metabolic activity at 24 hours for both *Scedosporium* species. When co-cultivated with strain PA14, the OD readings were 0.87 for *S. aurantiacum*, and 0.95 for *S. prolificans* (vs. ODs of >2 without PA14 present). Similar results were obtained using a mucoid *Pseudomonas* clinical strain SP-01.

We found that *P. aeruginosa* inhibited the growth of *S. aurantiacum* and *S. prolificans* on solid media, in liquid media and by fluorescent microscopy staining. Moderate differences in degrees of inhibition were seen with mucoid vs. non-mucoid strains.
Are bed bugs in Australia resistant to commonly used insecticides?

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Insecticide resistance in bed bugs has been nominated as a major factor in the pest’s resurgence. Recent studies using field and laboratory strains of *Cimex lectularius* and *Cimex hemipterus* across Europe, Africa, Asia and North America have variously demonstrated resistance to the pyrethroids, carbamates and, to a lesser extent, the organophosphates.

Resistance has been suspected in Australia, with anecdotal reports of treatment failures due to poor product performance. Early efficacy investigations on a range of formulated products also found indications of resistance in an Australian derived strain of *C. lectularius*. To confirm if resistance was present, four compounds (permethrin, deltamethrin, bendiocarb and pirimiphos-methyl) encompassing the major groups of insecticides then registered for bed bug control in Australia were selected for bioassay along with one compound (imidacloprid) to which modern strains should not have received any exposure at that time. LD₅₀ values (at 24 hours) were then determined via topical application of the technical grade compounds serially diluted in acetone against a suspected resistant strain (collected from and designated the ‘Sydney’ strain) and a susceptible laboratory strain imported from Bayer CropScience AG, Germany (the ‘Monheim’ strain).

All compounds tested against the Monheim strain demonstrated high levels of insecticidal activity. However, for the Sydney strain only pirimiphos-methyl and imidacloprid showed high levels of efficacy. Bendiocarb, permethrin and deltamethrin all failed to return greater than 60% mortality at the maximum applied rate of 100μg/μL. The resistance factor (calculated as: Sydney LD₅₀ / Monheim LD₅₀) for each compound was: permethrin = 1.4 million, deltamethrin = 430,000, bendiocarb = 240, pirimiphos-methyl = 2.8, imidacloprid = 2.7. Thus, resistance is present to the pyrethroids and carbamates, but not the organophosphates or neonicotinoids (with the differences reflected against those compounds likely due instead to a minor resistance-related fitness cost or physiological difference between the strains). This research has significant implications for current and future insecticide management when attempting to control bed bugs. Further studies are ongoing to determine the mechanism(s) of resistance.
The biology, distribution and genetics of *Culex molestus* in Australia?

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The *Culex pipiens* subgroup of mosquitoes includes some of the most important vector species involved in mosquito-borne disease transmission internationally and four species within this subgroup are found in Australia. One of these species, *Culex molestus*, is thought to have been introduced into Australia in the 1940s. Closely associated with subterranean urban habitats, this mosquito has the potential to cause serious nuisance biting impacts but also may cause significant public health risks through the transmission of endemic arboviruses. Exotic pathogens, such as West Nile virus, may also pose a potential threat to biosecurity of Australia. Our review of the literature has confirmed that the current Australian distribution of *Cx. molestus* is limited to areas south of latitude -28.17ºS. However, given that the mosquito is established in habitats south of the corresponding zone in the northern hemisphere, there is potential for *Cx. molestus* to spread north into QLD and NT. Molecular analysis of the mosquito indicated that Australian *Cx. molestus* shared stronger genetic similarity with specimens from Asia than specimens from Europe or North America. Laboratory and field studies have shown that the mosquito is uniquely adapted to urban environments through the expression of autogeny (ability to lay their first batch of eggs without a blood meal) and stenogamy (ability to mate in confined spaces). *Culex molestus* is active throughout the year and the current trend towards increased water storage in urban areas of Australia has raised concerns of increased nuisance-biting and public health risks in the future. However, the results of our studies indicate that there may be biological and ecological barriers that may lessen the importance of this mosquito in urban mosquito-borne disease cycles. A delay in blood feeding resulting from their obligatory autogeny, combined with limited access to potential reservoir hosts, may reduce the likelihood of them playing a significant role in pathogen transmission.
Understanding the ecological importance of mosquitoes to insectivorous bats and the implications for mosquito-borne disease management in coastal Australia

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Management of mosquito-borne disease risk in coastal Australia faces many challenges. Urbanisation is increasing the size and proximity of the community to productive mosquito habitats. Coastal wetlands are also the focus of conservation and rehabilitation efforts. Mosquitoes associated with these wetlands, in particular the saltmarsh mosquito, Aedes vigilax, are abundant, widely dispersing and key vectors of Ross River and Barmah Forest viruses. These mosquitoes may also represent an abundant prey resource for threatened and endangered insectivorous bat species and local authorities are reluctant to approve broadscale mosquito control programs due to concerns regarding indirect impacts on local bat populations. A combination of diet analysis, radio-tracking and prey abundance studies were undertaken. Analysing prey DNA within guano collected from 52 individuals representing five local bat species demonstrated that bats consumed a diverse range of prey dominated by lepidopterans. Consumption of Ae. vigilax was restricted to two species, Vespadelus pumilus and V. vulturnus. Radiotracking of 13 V. vulturnus individuals during periods of relatively large and small population abundances of Ae. vigilax, together with monitoring of prey abundance, revealed that foraging ranges of bats shifted in response to mosquito abundance (and no other prey). These findings suggest that there are species-specific relationships between bats and mosquitoes and that there may be site-specific strategies required to balance mosquito management and bat conservation.
A loop-mediated isothermal amplification (LAMP) assay for *Strongyloides stercoralis* in stool that uses a visual detection method with SYTO-82 fluorescent dye

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An assay to detect *Strongyloides stercoralis* in stool specimens was developed using the loop-mediated isothermal amplification (LAMP) method. Primers were based on the 28S ribosomal subunit gene. The reaction conditions were optimized and SYTO-82 fluorescent dye was used to allow real-time and visual detection of the product. The product identity was confirmed with restriction enzyme digestion, cloning, and sequence analysis. The assay was specific when tested against DNA from bacteria, fungi and parasites and 30 normal stool samples. Analytical sensitivity was to <10 copies of target sequence in a plasmid and up to a $10^{-2}$ dilution of DNA extracted from a *Strongyloides ratti* larva spiked into stool. Sensitivity was increased when further dilutions were made in water, indicative of reduced reaction inhibition. Twenty-seven of 28 stool samples microscopy and PCR positive for *S. stercoralis* were positive with the LAMP method. Based on these findings, the assay warrants further clinical validation.

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Microsporidial myositis caused by *Annaliia algerae*: an emerging opportunistic infection

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The insect microsporidian, *Annaliia algerae* was first described in 2004 as a cause of fatal myositis in an immune suppressed person from Pennsylvania. Two cases have subsequently been reported and we outline 2 additional cases, including the only survivor. We reviewed all 5 case histories with respect to clinical characteristics, diagnosis and management; and summarized organism life cycle and epidemiology. In all cases, immune suppressive medications were used for rheumatoid arthritis or solid organ transplantation. Four of the 5 were from Australia. All diagnoses were confirmed by skeletal muscle biopsy, however, peripheral nerves and other tissues may be infected. The surviving patient received albendazole, had a reduction of immune suppression and measures to prevent complications. Although insects are the natural hosts for *A. algerae*, human contact with water contaminated by spores may be a mode of transmission. *A. algerae* has emerged as a cause of myositis, particularly in coastal Australia.

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**Current status of matrix-assisted laser desorption ionisation-time of flight mass spectrometry in the clinical microbiology laboratory.**

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The integration of matrix-assisted laser desorption ionisation-time of flight mass spectrometry (MALDI-TOF MS) into many clinical microbiology laboratories has revolutionised routine pathogen identification. MALDI-TOF MS complements and has good potential to replace existing phenotypic identification methods. Results are available in a more clinically relevant timeframe, particularly in bacteraemic septic shock. Novel applications include strain typing and the detection of antimicrobial resistance, but these are not widely used. This review discusses the technical aspects, current applications, and limitations of MALDI-TOF MS.
Hendra virus: a one health tale of flying foxes, horses and humans

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Hendra virus, a member of the family Paramyxoviridae, was first recognized following a devastating outbreak in Queensland, Australia, in 1994. The naturally acquired symptomatic infection, characterized by a rapidly progressive illness involving the respiratory system and/or CNS, has so far only been recognized in horses and humans. However, there is potential for other species to be infected, with significant consequences for animal and human health. Prevention of infection involves efforts to interrupt the bat-to-horse and horse-to-human transmission interfaces. Education and infection-control efforts remain the key to reducing risk of transmission, particularly as no effective antiviral treatment is currently available. The recent release of an equine Hendra G glycoprotein subunit vaccine is an exciting advance that offers the opportunity to curb the recent increase in equine transmission events occurring in endemic coastal regions of Australia and thereby reduce the risk of infection in humans.
Viral pneumonitis is increased in obese patients during the first wave of pandemic A(H1N1) 2009 virus.


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INTRODUCTION:

There is conflicting data as to whether obesity is an independent risk factor for mortality in severe pandemic (H1N1) 2009 influenza (A(H1N1)pdm09). It is postulated that excess inflammation and cytokine production in obese patients following severe influenza infection leads to viral pneumonitis and/or acute respiratory distress syndrome.

METHODS:

Demographic, laboratory and clinical data prospectively collected from obese and non-obese patients admitted to nine adult Australian intensive care units (ICU) during the first A(H1N1)pdm09 wave, supplemented with retrospectively collected data, were compared.

RESULTS:

Of 173 patients, 100 (57.8%), 73 (42.2%) and 23 (13.3%) had body mass index (BMI) <30 kg/m(2), ≥30 kg/m(2) (obese) and ≥40 kg/m(2) (morbidly obese) respectively. Compared to non-obese patients, obese patients were younger (mean age 43.4 vs. 48.4 years, p=0.035) and more likely to develop pneumonitis (61% vs. 44%, p=0.029). Extracorporeal membrane oxygenation use was greater in morbidly obese compared to non-obese patients (17.4% vs. 4.7%, p=0.04). Higher mortality rates were observed in non-obese compared to obese patients, but not after adjusting for severity of disease. C-reactive protein (CRP) levels and hospital length of stay (LOS) were similar. Amongst ICU survivors, obese patients had longer ICU LOS (median 11.9 vs. 6.8 days, p=0.017). Similar trends were observed when only patients infected with A(H1N1)pdm09 were examined.

CONCLUSIONS:

Among patients admitted to ICU during the first wave of A(H1N1)pdm09, obese and morbidly obese patients with severe infection were more likely to develop pneumonitis compared to non-obese patients, but mortality rates were not increased. CRP is not an accurate marker of pneumonitis.
**CIDM-PH Research reports & works in progress**

**Viral epidemiology & disease surveillance – Other projects & works in progress**

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**Varicella zoster virus quantitation in blood from symptomatic and asymptomatic individuals.**

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Primary infection with varicella zoster virus (VZV) occurs in immunocompromised and immunocompetent individuals. Clinical and asymptomatic reactivation with shedding of infectious virus and viremia may occur. The prevalence of VZV viremia is unknown. The aim of this study was to detect VZV viremia and quantify VZV DNA using quantitative polymerase chain reaction (qPCR) in blood from different populations. A qPCR-based method using EvaGreen® was used to quantify VZV DNA in 491 samples, including whole blood, plasma and buffy-coat, from patients hospitalized with varicella-associated disease (Group 1, n=10) and three groups with no VZV disease: individuals with a first clinical diagnosis of central nervous system demyelination (Group 2, n=213) with their age and sex-matched controls (Group 3, n=218); and HIV-infected individuals (Group 4, n=50). VZV-specific IgG antibody titres were measured in Group 3. The proportion positive for viremia and mean detectable VZV DNA load (copies/ml) were: Group 1: 100% (10/10) and 4.6 × 10(6) ± 1.4 × 10(7); Group 2: 4% (9/213) and 1.5 × 10(3) ± 1.8 × 10(4); Group 3: 8% (17/218) and 1.1 × 10(3) ± 7.8 × 10(3); Group 4: 12% (6/50) and 7.7 × 10(1) ± 2.8 × 10(2). VZV DNA load and IgG titres were not significantly correlated (Group 3 only). VZV load in Group 1 was significantly elevated compared to Groups 2-4 (P<0.001); the latter were not significantly different from each other (P=0.05). VZV genotypes from clades 1-5 were identified in Group 1. VZV DNA was detected but at low frequency and viral load in both immunocompetent and immunocompromised individuals asymptomatic for VZV infection, compared to individuals with active VZV infection.
Human rhinovirus C in adult haematopoietic stem cell transplant recipients with respiratory illness.

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**BACKGROUND:** A previously unidentified species of human rhinovirus, HRV-C, was described in 2006 in association with lower respiratory tract infection (LRTI). Features of infection in immunosuppressed adults are poorly characterised.

**OBJECTIVES:** This study aims to determine the epidemiology of HRV-C in haematopoietic stem cell transplant (HSCT) recipients in a single centre.

**STUDY DESIGN:** A prospective cohort study of all HSCT recipients admitted to Westmead Hospital, Westmead, Australia from 1 July 2005 to 30 September 2007 was undertaken. Nose/throat samples were collected from all patients at the time of admission and patients developing pre-defined symptoms and/or signs of respiratory infection during the admission. Samples were processed and tested for rhinoviruses and 14 other respiratory viruses using nucleic acid-based methods, immunofluorescence and culture. HRV genotyping was performed by sequencing a region of the rhinovirus 5’ untranslated region (UTR). Clinical data on each episode were collected prospectively.

**RESULTS:** HRVs were identified in 24 episodes: 8% of 299 episodes of clinically-defined respiratory infections and 39% of 61 episodes in which respiratory viruses were detected. HRV-C was most frequent (HRV-C: nine, HRV-A: eight and HRV-B: two). Seven episodes of HRV-C, five with pneumonia, occurred within 100 days of HSCT. Co-pathogens were frequent.

**CONCLUSIONS:** The newly described HRV-C was the most common rhinovirus group detected in HSCT recipients with respiratory infection, with co-pathogens being frequent. Further research is required to understand the activity and pathogenicity of this virus in HSCT recipients.
Teams of clinicians and academics (a) involved in day-to-day disease control and policy development, and (b) representing laboratory and public health

R & D teams based in WS LHD and NSW Pathology settings accredited for healthcare delivery and clinical training

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<tr>
<td>Molecular epidemiology and high resolution surveillance of Salmonella enterica serovar Typhimurium in Australia</td>
<td>Lan R Sintchenko V Tanaka M Octavia S</td>
<td>Project</td>
<td>USYD</td>
<td>2013-2015</td>
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<tr>
<td>Reducing the prevalence of TB in a highly endemic setting by community-wide active case finding: &quot;turning off the tap&quot;</td>
<td>Marks G Nguyen L Dinh S Nhung NV</td>
<td>Project</td>
<td>USYD</td>
<td>2013-2015</td>
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<td></td>
<td>Sintchenko V Fox G Wood J</td>
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<tr>
<td>Mechanisms and markers of tuberculosis transmission within Australia</td>
<td>Sintchenko V Marais B Gilbert GL</td>
<td>Project</td>
<td>USYD</td>
<td>2013-2015</td>
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<tr>
<td>Centre for Research Excellence in Tuberculosis Control: From discovery to public health policy</td>
<td>Britton W Gilbert GL et al.</td>
<td>CRE</td>
<td>USYD</td>
<td>2013-2017</td>
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### OTHER

<table>
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<tr>
<th>POPULATION HEALTH AND HEALTH SERVICES RESEARCH SUPPORT PROGRAM</th>
<th>INVESTIGATORS</th>
<th>GRANT TYPE</th>
<th>INSTITUTION</th>
<th>PERIOD</th>
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## CIDM-PH Existing Grants in 2013

<table>
<thead>
<tr>
<th>Title</th>
<th>Investigators</th>
<th>Grant Type</th>
<th>Funding Body</th>
<th>Institution</th>
<th>Period</th>
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<tbody>
<tr>
<td><strong>National Health and Medical Research Council (NHMRC) Grants: Centre of Research Excellence</strong></td>
<td>Iredell Sorrell Gilbert Kerridge Booy Dwyer Sintchenko Jones Bennet</td>
<td>Centre for Research Excellence 1001021</td>
<td>NHMRC</td>
<td>University of Sydney</td>
<td>2010–14</td>
</tr>
<tr>
<td>Multidisciplinary, translational science to prevent &amp; control infectious diseases in high risk settings</td>
<td>Coiera Glasziou Liaw Sintchenko Ruciman Magrabi</td>
<td>Centre for Research Excellence 1032664</td>
<td>NHMRC</td>
<td>University of NSW</td>
<td>2011-14</td>
</tr>
<tr>
<td>Centre for Research Excellence in E-health</td>
<td>MacIntyre McIntyre Booy Leask Wood Jones Menzies Kaldor Beutels Dwyer</td>
<td>Centre for Research Excellence 1031963</td>
<td>NHMRC</td>
<td>University of NSW</td>
<td>2012-16</td>
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<tr>
<td>Immunisation in understudied and special risk populations</td>
<td>Meyer Robert Ellis Chen</td>
<td>Project Grant 1010452</td>
<td>NHMRC</td>
<td>University of Sydney</td>
<td>2011-13</td>
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<tr>
<td>Microevolution &amp; transmission of MRSA in a hospital setting</td>
<td>Gilbert Sintchenko O’Sullivan Iredell</td>
<td>Project Grant 1031952</td>
<td>NHMRC</td>
<td>University of Sydney</td>
<td>2012-14</td>
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<td>DNA Barcoding of pathogenic fungi as basis for the development of novel standardised diagnostic tools</td>
<td>Meyer Robert Ellis Chen</td>
<td>Project Grant 1031943</td>
<td>NHMRC</td>
<td>University of Sydney</td>
<td>2012-14</td>
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<tr>
<td>Topic</td>
<td>Investigator(s)</td>
<td>Grant Type</td>
<td>Funding Body</td>
<td>Institution</td>
<td>Year(s)</td>
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<td>Non-NHMRC/ARC Grants ACGR</td>
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<tr>
<td>NSW Health Capacity Building Infrastructure Grant - Round 3</td>
<td>Gilbert Iredell Sorrell Dwyer Sintchenko Meyer Russell</td>
<td>Infrastructure Grant</td>
<td>NSW Ministry of Health</td>
<td>Western Sydney Local Health District</td>
<td>2010-13</td>
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<td>International Grants</td>
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<tr>
<td>Prediction of possible endemic areas for <em>Cryptococcus neoformans/C. gattii</em> in Colombia: ecological modelling of risk areas</td>
<td>Escandon Castaneda Meyer</td>
<td>Project Grant</td>
<td>Colciencia Colombia</td>
<td>Instituto Nacional de Salud, Bogotá, Colombia</td>
<td>2012-14</td>
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<tr>
<td>Functional genomic analyses of emerging <em>Cryptococcus</em> subtypes in North America</td>
<td>Keim Engelthaler Lockhart Meyer Thompson</td>
<td>Project Grant</td>
<td>NIH</td>
<td>USA</td>
<td>2012-14</td>
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<tr>
<td>Grants (not part of Aust Competitive grants register, but peer-reviewed)</td>
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<tr>
<td>Sydney Emerging Infections and Biosecurity Project</td>
<td>Sorrell</td>
<td>Establishment</td>
<td>Syd Med School</td>
<td>SEIB (USYD)</td>
<td>2011-15</td>
</tr>
</tbody>
</table>


24. Comparing the use of, and considering the need for, lumbar puncture in children with influenza or other respiratory virus infections. Khandaker G, Heron L, Rashid H, Li-Kim-Moy J, Lester-Smith D, Kesson A,


34. Laboratory surveillance of invasive pneumococcal disease in New South Wales, Australia, before and after introduction of 7-valent conjugate vaccine: reduced disease, but not antibiotic resistance rates. Oftadeh S, Gidding HF, Gilbert GL. Epidemiol Infect. 2013 Sep;141(9):1797-806


61. Vancomycin AUC/MIC ratio and 30-day mortality in patients with Staphylococcus aureus bacteremia. 
   Holmes NE, Turnidge JD, Munckhof WJ, Robinson JO, Korman TM, O'Sullivan MV, Anderson TL, Roberts SA, 

   MLVA working group, Møller Nielsen E. Euro Surveill. 2013 Aug 29;18(35):20566

63. Tuberculosis: an old world disease providing new world challenges in NSW. Marks GB, Christensen A, 
   abstract available.

64. The use of mycobacterial interspersed repetitive unit typing and whole genome sequencing to inform 

65. Tuberculosis, public health and gathering new evidence to guide control efforts. Christensen A, Lowbridge 
   abstract available.

66. Software for selecting the most informative sets of genomic loci for multi-target microbial typing.O'Sullivan 

67. Transmission of Mycobacterium tuberculosis from an Asian elephant (Elephas maximus) to a chimpanzee 
   (Pan troglodytes) and humans in an Australian zoo.Stephens N, Vogelnest L, Lowbridge C, Christensen A, 
   Marks GB, Sintchenko V, McAnulty J. Epidemiol Infect. 2013 Jul;141(7):1488-97. doi: 
   10.1017/S095026881300068X. Epub 2013 Mar 28.

68. Quantitative estimation of the stability of methicillin-resistant Staphylococcus aureus strain-typing systems 

69. The "how" of polymerase chain reaction testing for Bordetella pertussis depends on the "why".McIntyre PB, 
   available.

70. Marieke J Bart, Simon R Harris, Abdolreza Advani, Yoshichika Arakawa, Daniela Bottero, Valérie Bouchez, 
   Pamela K Cassidy, Chuen-Sheue Chiou, Tine Dalby, Norman K. Fry, Maria Emilia Gaillard, Marjolein van 
   Gent', Nicole Guiso, Hans O Hallander, Eric T. Harvill, Qiushui He, Han GJ van der Heide, Kees Heuvelman, 
   Daniela F Hozbor, Kazunari Kamachi, Gennady I Karataev, Ruiting Lan, Anna Lutyńska, Ram P Maharjan, 
   Jussi Mertsola, Tsatsu Miyamura, Sophia Octavia, Michael A. Quail, Vitali Sintchenko, Paola Stefanelli, M Lucia 
   Tondella, Raymond SW Tsang, Yinghua Xu, Shu-Man Yao,Shumin Zhang, Julian Parkhill, Frits R. Mooi. Global 
   population structure and evolution of Bordetella pertussis and their relationship with vaccination. 

71. Jonas T Larsson1, Mia Torpdahl1, MLVA working group2, Eva Møller Nielsen and MLVA workinggroup: Anna 
   Aspán, Sophie Bertrand, Chien-Shun Chiou, Celsey Goodman, Max Heck, Lester Hiley, Katie Hopkins, Geoff 
   Hogg, Eija Hyttia-Trees, Hidemasa Izumiya, Cecilia Jernberg, Simon Le Hello, Bjørn-Arne Lindstedt, 
   BurkhardMalorny, Deirdre Prendergast, Catherine Ragimbeau, Vitali Sintchenko, Anthony Smith, Gitte 
   Sørensen, Erhardt Tietze. Proof-of-concept study for successful inter-laboratory comparison of MLVA results 

72. Ho J, Jelfs P, Sintchenko V. Phenotypically occult multidrug-resistant Mycobacterium tuberculosis – 
   dilemmas in diagnosis and treatment. Journal of Antimicrobial Chemotherapy 2013 (Accepted 13 June 
   2013).


Books & Monographs


8. Outhred AC, Kok J, **Dwyer DE.** Laboratory diagnosis of CNS infections. Chapter 4, pp87-128. in "Neuroviral Infections: General Principles and DNA Viruses". Editors: Singh SK, Ruzek D. CRC Press (Taylor and Francis Group), Boca Raton, FL, USA 2013.

CIDM-Public Health holds a number of seminars, workshops, and symposia each year. Programs and selected PowerPoint presentations are available at: www.cidmpublichealth.org

31st May 2013
Whole Genome Sequencing in Clinical and Public Health Microbiology Workshop
Speakers:
Professor Eddie Holmes, University of Sydney and Fogarty International Center, National Institute of Health, USA
Dr Grant Hill-Cawthorne, SEIB and the University of Cambridge, UK
Dr Tanya Golubchik, Departments of Medicine and Statistics, University of Oxford, UK
A/Prof Ruiting Lan, University of New South Wales
Dr Sebastian van Hal, Royal Prince Alfred Hospital, Sydney
Westmead Education & Conference Centre, Westmead Hospital

2nd September 2013
Bioinformatics for Microbiologists: Analysis of Complex Recombinatorial DNA Sequences in Microbial Genomes Seminar
Speakers:
Dr Sally Partridge, Centre for Infectious Diseases and Microbiology, University of Sydney
Dr Guy Tsafnat, Centre for Health Informatics, University of New South Wales
Westmead Education & Conference Centre, Westmead Hospital

22nd November 2013
Healthcare Associated Infections – More New Tricks for Old Dogs Symposium
Speakers:
Prof Jon Iredell, Centre for Infectious Diseases & Microbiology, Westmead
Prof Nicholas Graves, Faculty of Heath, Queensland University of Technology, Brisbane
Prof Lyn Gilbert, Centre for Infectious Diseases & Microbiology, Westmead
Dr Vicky Sheppeard, Director, Communicable Diseases, Health Protection NSW
Westmead Education & Conference Centre, Westmead Hospital
Broad Street Pump Newsletters

The following Broad Street Pump newsletters were distributed during 2013, and are available on the CIDM-Public Health website www.cidmpublichealth.org

February 2013 Issue

❖ A World United Against Infectious Diseases: Cross-Sectoral Solutions
  Michael Ward & Siobhan Mor
❖ SEIB and One Health
  Tania C Sorrell, Lyn Gilbert, Ben Marais, Michael Ward

April 2013 Issue

❖ Next Generation Sequencing in the Clinical Microbiology Laboratory
  Adrian Ong
❖ Applications of Whole Genome Sequencing: Tuberculosis
  Grant A. Hill-Cawthorne

August 2013

❖ The Tuberculosis Centre of Research Excellence
  Gabriella Scandurra
❖ The Fight Against Tuberculosis in Vietnam
  Greg J. Fox
❖ Tuberculosis Research in China
  Magda Ellis

October 2013

❖ When it comes to mosquito-borne disease prevention, awareness and surveillance is critical,
  Cameron E Webb
❖ More than mozzies: The health risks associated with Australian arthropods
  Cameron E Webb & Stephen Doggett
❖ The reality of new technologies in arbovirus and mosquito surveillance in NSW
  Cheryl S Toi
❖ Are we providing the right advice on personal protection measures against endemic and exotic mosquito-borne diseases
  Cameron E Webb
❖ Can botanical products assist the control of dengue outbreaks in Malaysia?
  Liyana Mokhtar
CIDM-PH 2013 REPORTS & PROMOTION

2013 CIDM-PH Reports:

Please contact Lou Orszulak for a copy of any of the following CIDM-PH 2013 reports:

- 2009-2013 R3 CIDM-PH CBIG Final Report
- 2013 CIDM-PH Annual Report
- 2013 CIDM-PH Colloquium Report

CIDM-PH Brochure:

A copy of the CIDM-PH Brochure can be found at the CIDM-PH Website: [www.cidmpublichealth.org](http://www.cidmpublichealth.org)
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