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CIDM-PH Welcome & Overview

CIDM-Public Health: collaborating in research and development to prevent and control communicable diseases in New South Wales, Australia and our region.

Professor Lyn Gilbert, Director, CIDM-Public Health

Senior investigators (SI) in CIDM-PH have been very active in the past 3 years, in developing collaborations and initiating or contributing to new research consortia to further research, capacity building and knowledge translation in communicable disease research and improvements in surveillance, diagnosis and management, with invaluable support from the NSW Health Capacity Building Infrastructure Grant.

New initiatives to which CIDM-PH SIs have contributed include:

**Sydney Emerging Infections and Biosecurity Institute (SEIB)** at the University of Sydney led by CIDM-PH SI, Professor Tania Sorrell. SEIB brings together infectious diseases and related researchers in several faculties in the University of Sydney, including Medicine and Health Sciences, Veterinary Science, Science, Agriculture, Law, Engineering and Information Technology, Arts & Social Sciences. SEIB has already established multidisciplinary collaborations based on “One Health”, recognising the interdependence of humans, animals and the environment health, especially in the South East Asia/Pacific region, where SEIB is establishing or building on existing research collaborations. CIDM-PH and SEIB have held several joint symposia and workshops with SEIB in the past 2 years.

CIDM-PH SIs have also been involved as chief investigators in several successful NHMRC-funded Centres of Research Excellence (CREs):

**Professor Jon Iredell leads the CRE in Critical Infections, which began in 2011.** It is collaboration between infectious diseases physicians, including CIDM-PH SIs and intensivists, among others. Research topics include: innovative approaches to diagnosis and management of sepsis; epidemiology of serious diseases of unknown cause such as encephalitis; mechanisms of antibiotic resistance; and legal and ethical barriers to research in high acuity settings.

**The CRE in Immunisation in Vulnerable Populations (2011),** is a collaboration with the University of UNSW and the National Centre for Immunisation Research and Surveillance (NCIRS) led by Professor Raina MacIntyre. CIDM-PH SI, Professor Dominic Dwyer is a chief investigator (CI). This collaboration builds on our longstanding collaboration with NCIRS.

**The CRE in eHealth and Health Informatics (2012),** based at the Australian Institute of Health Innovation, UNSW, is led by Professor Enrico Coriera, with whom CIDM-PH has had a longstanding collaboration through SI Associate Professor Vitali Sintchenko, who is a CI on this CRE.

**The CRE in Tuberculosis (2012),** based at the University of Sydney and led by Professor Warwick Britton has strong representation among CIs from both SEIB and CIDM-PH, including Associate Professor Vitali Sintchenko and Professor Lyn Gilbert.

CIDM-PH SIs, associate investigators and students have, of course, also continued research into many aspects of diagnostic, applied and molecular microbiology and the epidemiology, prevention and control of infectious diseases, with continued success in NHMRC project grant applications (including at least 5 new ones awarded in the latest round).
Bugs, Bats, Bacteria & Birds: A overview of Medical Entomology

Cameron E Webb

Department of Medical Entomology
University of Sydney & Westmead Hospital

The impact of medically important arthropods on Australia is constantly changing. Current and emerging public health risks associated with biting and stinging insects, ticks and mites are the focus of the Department to improved public health outcomes for the local community.

The management of mosquitoes and mosquito-borne disease in Australia is of growing concern. The inland flooding of previous years in south-eastern Australia has seen the re-emergence of the potential fatal Murray Valley encephalitis virus (MVEV), highlighting the need to remain vigilant. 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. Cameron Webb, Stephen Doggett and Richard Russell were called upon to provide expert advice to the Environmental Health Branch of NSW Health in the development of strategic plans to respond to future outbreaks of MVEV in NSW.

In June 2012, the Department celebrated the career of Prof Richard Russell with a one-day Festschrift titled “Medical Entomology in Australia: Past, Present & Future Concerns. With over 40 years of experience in Medical Entomology, Prof Russell has published over 200 scientific papers and is internationally recognised as one of the world’s leading authorities in the field. Prof Russell established the Department at Westmead Hospital in 1987 and his legacy will continue as the breadth and diversity of research projects continues to grow. The event attracted some of Australia’s leading researchers in the field of medical entomology to Westmead Hospital, including many interstate and international attendees.

The Department continues to provide expert advice on bed bug management both locally and internationally. Stephen Doggett has been invited to share this expertise on bed bug management at a wide range of local and international conferences and workshops in the past year. Stephen Doggett was also the lead author on a comprehensive review of bed bug literature that appeared in the Journal of Clinical Microbiology Reviews.

The Department continues to supply disinfected maggots for Maggot Debridement Therapy (MDT) to health care facilities across the country and requests for this service have risen steadily over the past five years. As well as providing the disinfected maggots for wound treatment, particularly diabetic leg ulcers, the Department has also had requests to supply the veterinary industry. Feedback from clients has been unanimously positive. In addition, the Department supplies insects to the Department of Agriculture, Fisheries & Forestry (previously Australian Quarantine and Inspection Service (AQIS) to assist in surveillance and control of exotic insect pests.
Russell continues as a Deputy Chair of the National Arbovirus and Malaria Advisory Committee to the Commonwealth Department of Health and Ageing, and as a member of the Technical Advisory Group for the Eradication of *Aedes albopictus* to the Queensland Department of Health.

Two post-graduate research students are currently associated with the department, Nur Faeza Abu Kassim (University of Sydney) is currently investigating the biology and ecology of the exotic mosquito *Culex molestus* and is expected to complete her studies in 2013. Leroy Gonsalves (Australian Catholic University) successfully completed his PhD, an investigation into the ecological interactions between mosquitoes and insectivorous bats, in early 2012.

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 local and international workshops, conferences and meetings as well as providing lectures to undergraduate and postgraduate courses at the University of Sydney.
**Culex molestus** in Australia: Does this underground mosquito pose a mosquito-borne disease risk in Australia?

Nur F Abu Kassim, Cameron E Webb & Richard C Russell

Department of Medical Entomology
University of Sydney & Westmead Hospital

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 and is closely associated with subterranean urban habitats. Our review of the literature has confirmed that the current Australian distribution of the species is limited to areas south of latitude -28.17ºS. However, given that the species is established in habitats south of the corresponding zone in the northern hemisphere, there is potential for *Culex molestus* to spread north into QLD and NT.

The 2012 outbreak of West Nile virus in North America has once again highlighted the potential importance of the *Culex pipiens* group of mosquitoes in mosquito-borne disease transmission cycles in the urban environment. Our laboratory and field studies have shown that the species 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). The species 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.

Notwithstanding the risk associated with exotic arboviruses such as West Nile virus, locally important arboviruses such as Kunjin virus and Murray Valley encephalitis virus pose a potential risk in SE Australia. However, the results of our studies indicate that there may be biological and ecological barriers that may lessen the importance of this species 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. Gaps in our knowledge remain regarding the vector-competence of this species for local and exotic arboviruses as well as the dispersal patterns of this species in urban habitats.
Mosquito-borne disease is a growing concern in Australia. Notwithstanding the risks associated with exotic mosquitoes and pathogens, urbanisation brings people closer to both productive mosquito habitats and potential reservoir hosts of a range of arboviruses. Management of current and future mosquito-borne disease risks will rely on timely and effective surveillance strategies. New technologies are currently under review with a view to incorporating them into the NSW Arbovirus Surveillance & Mosquito Monitoring Program as well as improve our understanding of local mosquito-borne disease risk factors.

The NSW Arbovirus Surveillance & Mosquito Monitoring Program is an annual program that provides an early warning system of arbovirus activity based on monitoring mosquito populations and the environmental drivers of mosquito activity and the presence of arboviruses (e.g. Ross River virus, Barmah Forest virus, Murray Valley encephalitis virus and Kunjin virus) in field collected mosquito specimens throughout coastal and inland areas. The program currently relies on cell culture and fixed-cell Enzyme-Linked Immunosorbent Assay, using both generic and viral specific monoclonal antibodies, for arbovirus detection and identification.

Recently, a unique arbovirus surveillance system has been developed to detect arboviruses expectorated in mosquito saliva during sugar feeding. Carbon dioxide-baited updraft box traps collect mosquitoes that are then allowed to feed on honey-soaked nucleic acid preservation cards that are then analyzed using real-time reverse transcription-PCR. Field trials of this system have successfully detected the presence of Ross River and Barmah Forest viruses. The implementation of nucleic acid testing will increase the sensitivity and timely detection of elevated arbovirus activity, decrease costs and save on labour time. This system holds great potential for use in NSW and we will be developing laboratory procedures to incorporate this new technology into the current surveillance program.

Molecular studies are also planned to investigate the genetic similarities and host-feeding preferences of key vector species in NSW. Published studies have raised questions regarding the genetic similarities of taxonomically indistinguishable mosquitoes. With these questions come concerns regarding geographic variability in vector competence and associated public health risk factors. Similarly, many gaps in our knowledge of host-feeding preferences of pest mosquito species exist. An understanding of how enzootic and epidemic vectors of local mosquito-borne pathogens interact with reservoir hosts will greatly assist both surveillance and management of public health risks. Analysis of mosquito blood meals from urban and peri-urban areas of NSW are planned to elucidate some of these relationships.
Communicating knowledge to motivate change in prevention and control of healthcare-associated infections.

Professor Lyn Gilbert

The personal, medical and financial costs of healthcare associated infections (HAIs) continue to increase, in parallel with the growing complexity of medical and surgical therapies and an aging hospital population, with high rates of chronic disease. HAIs – especially those due to antibiotic resistant bacteria - have become a major public health problem as the distinctions between inpatient, community and nursing home-based care are blurred. Despite increasingly vulnerable patients, an estimated 50%-70% of HAIs could be prevented by consistent application of simple, well-established infection prevention and control (IPC) measures (hand hygiene, aseptic insertion and care of invasive devises, appropriate use of antibiotics etc.). However, for a variety of often poorly understood reasons, healthcare workers (HCWs) often fail to comply with evidence-based IPC policies and procedures. Novel approaches are needed to define the practical and psychological barriers to compliance and motivate HCWs, especially doctors, to take IPC (which encompasses the whole hospital population and environment) as seriously as they do individual patient care.

Our current research is based on two major NHMRC-funded projects. One aims to improve HAI surveillance by a) individual review of all HA Staphylococcus aureus (and other) bacteraemias, to identify preventable factors and provide rapid feedback about individual cases to the relevant units, and periodic aggregate data analysis (rates, risk factors, peer-group comparisons etc.), to the hospital community; b) development and implementation of routine methicillin resistant S. aureus (MRSA) strain typing, sensitive enough to allow identification of individual transmission events and rapid enough to motivate staff to identify and correct breeches of IPC. Routine strain typing is underway and regular reporting has been partially implemented and well received in some high acuity wards, with tangible (albeit early) benefits. An automatic electronic alert system is under development.

In parallel with MRSA surveillance, we are collaborating with colleagues at the Centre for Health Communication, UTS, in a project in which videos of staff carrying out routine tasks (taken with their consent) are played back to them to allow them to reflect on and discuss ways to improve interactions with each other, with patients and the environment, to improve efficiency and reduce the risks of pathogen transmission.

Our hypothesis is that a better understanding of the epidemiology and consequences of HAIs and the effects of individual actions and work practices in causing or preventing them, will motivate HCWs to take personal responsibility for, and apply the principles of IPC, thoughtfully, rather than blindly following (or failing to follow) protocols devised by others.

In a related project, we have developed a strain typing system for Clostridium difficile which allows rapid identification of ribotypes and subtypes. This has allowed us to monitor C. difficile infections at Westmead and contribute to the relatively sparse local data about the community and hospital epidemiology, in NSW, of this important and topical HAI, which has caused serious, widespread hospital outbreaks of diarrhoeal disease in the northern hemisphere, over the past 5-10 years.
CIDM-PH Research Reports & Works in Progress

Bacterial epidemiology & disease surveillance

Development and implementation of a rapid strain typing system to control MRSA outbreaks

Matthew O’Sullivan, Fei Zhou, Rosemarie Sadsad, Frances Jenkins, Vitali Sintchenko and Lyn Gilbert

Background and Aims:
Strain typing is an important part of public health and hospital infection control surveillance for many organisms. Traditionally, strain typing has been utilized in a retrospective fashion, to verify outbreaks that were suspected based on temporospatial clustering of cases. Increasingly, however, prospective typing is being utilised where all isolates are genotyped in a timely fashion, and observation of a cluster of genotypes is the stimulus for further investigation. This approach is particularly useful for common infections where temporospatial clusters may not be easily discernible against the background high prevalence. Prospective typing is used routinely to great advantage for Salmonella and tuberculosis surveillance. The aim of this project was to develop a rapid, highly discriminatory stain typing system for MRSA, and to explore its utility when used prospectively to identify nosocomial transmission events in a setting of high prevalence.

Methods:
Multiplex PCR-reverse line blot assay binary typing was used since it is rapid, high throughput, inexpensive, robust, reproducible and flexible enough to allow interrogation of virtually any genetic target. A panel of 19 targets (including Panton-Valentine leukocidin) were identified by specially developed software (AuSeTTS) as the most discriminatory, and were incorporated into the typing system. The ability of the typing system to identify nosocomial transmission events was initially examined using isolates from surgical wards over a 12 month period. Subsequently it was rolled out in a prospective typing system for all MRSA isolates at Westmead and Blacktown hospitals.

Results:
The typing method was rapid (<24 hour turnaround time), inexpensive (US$2 per isolate for consumables), and highly discriminatory (Simpson’s index of diversity 0.994, versus 0.987 for pulse field gel electrophoresis). 273 surgical patients were identified as being colonized or infected with MRSA over the 12 month period. Typing of these isolates identified 55 MRSA strain types. The number of possible source patients for all 87 hospital-acquired cases was reduced from 859 to 212 with the addition of strain typing.

Conclusions:
Subsequent introduction of the typing method in a prospective, universal fashion has facilitated rapid recognition of nosocomial transmission events and allowed prompt, targeted infection control interventions. This has included identification of an outbreak due to an emergent virulent clone of MRSA in the neonatal intensive care unit.

Rapid prospective typing of microbial pathogens with inexpensive and highly discriminatory typing systems can be used to rapidly and reliably identify outbreaks. This facilitates more efficient use of public health and infection control resources.
Antibiotic resistance in *Mycoplasma genitalium*

Kaitlin Tagg (BSc Hons Student, Macquarie University)

CIDM-PH supervisors: Dr Neisha Jeoffreys & Professor Lyn Gilbert

*Mycoplasma genitalium* is a significant sexually transmitted pathogen causing up to 25% of cases of non-gonococcal urethritis in men, and is strongly associated with cervicitis and pelvic inflammatory disease in women. It is most often treated with azithromycin, although a rise in the number of patients failing treatment over the last 5 years indicates resistance to azithromycin is increasing. The mutations responsible for this resistance are found in the 23S rRNA gene, having been identified in numerous *M. genitalium* populations worldwide. The second-line antibiotic moxifloxacin, is thought to be 100% effective when azithromycin treatment fails. However, recent studies have identified mutations in the *parC* and *gyrA* genes that may confer resistance to moxifloxacin.

This study aimed to identify the incidence of resistance to these antibiotics in *M. genitalium* strains in Sydney by detection of resistance-inducing mutations in the 23S rRNA gene, *parC* and *gyrA*. 186 *M. genitalium* positive DNA extracts collected from patients attending sexual health clinics in Sydney from 2008 to 2011 were tested in this study. Through PCR amplification and DNA sequence alignment, we have identified known resistance-associated mutations in the 23S rRNA gene in 44% of patient samples. Analyses of *parC* and *gyrA* sequences have revealed mutations in 16% of patient samples, collectively. These findings support empirical evidence of azithromycin and moxifloxacin treatment failure reported in Sydney. With this knowledge of resistance, testing and treatment protocols for *M. genitalium* infections may need revising.
Our team of scientists has been working on translating molecular epidemiology into information to improve infectious disease risk assessment and control. One example of research and development has been the molecular epidemiology of pathogens with epidemic potential such as *Bordetella pertussis*. The resurgence of pertussis in vaccinated populations can be, at least in part, explained by genetic changes that increase the fitness of circulating *B. pertussis* isolates. A collaborative project with University of NSW funded by the NHMRC documented that (a) *B. pertussis* in Australia has undergone significant clonal expansion, and (b) a possible association between the presence of *ptxP3* and the introduction of acellular pertussis vaccines. The latter observation is of particular concern as higher levels of PT production have been reported overseas in isolates with *ptxP3* compared with *ptxP1* and this observation has been linked to increased pertussis hospitalizations. Pertussis syndrome can occur as the result of infection with *Bordetella pertussis* or *B. parapertussis* and is usually diagnosed by culture, PCR or serological methods.

Various PCR protocols have been employed that target different regions in the *Bordetella* genomes: repetitive insertion sequences IS481 and IS1001, the pertussis toxin promoter region, the adenylate cyclase gene, filamentous haemagglutinin gene or house keeping recA and 16S rRNA genes. Since IS481 is also present in *B. holmesii*, disease due to this pathogen can be mistakenly attributed to *B. pertussis* if a single IS481 target PCR test is used for laboratory diagnosis. It is important to differentiate *B. holmesii* from *B. pertussis* and *B. parapertussis* on epidemiological grounds. It might be also relevant to differentiate them on clinical grounds as *B. holmesii* appears to have a predisposition to cause invasive disease and its MICs to macrolide antibiotics may be ten times higher than in *B. pertussis*.

Another direction of CIDM-PH activities has been the capacity building in emerging infectious diseases. We played a significant role in the discovery of tularemia in Tasmania. In February 2011 a 44-year-old female was bitten on her right index finger by a wild ringtail possum on the West Coast of Tasmania. Molecular testing in our laboratory suggested that this first case of tularemia in Australia was likely to be caused by *F. tularensis* subspecies *holarctica* biovar *japonica* which differs significantly from previously reported local isolates. The epizootic reservoir of the disease in Australian marsupials warrants further investigation.

The third important direction is the implementation and evaluation of genomics guided public health surveillance. Prospective typing of *Salmonella enterica* serovar Typhimurium (STM) by multiple-locus variable-number tandem-repeat analysis (MLVA) assists in identifying clusters of STM cases that might otherwise have gone unrecognised, as well as sources of sporadic and outbreak cases. 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 and offers a new benchmark for STM surveillance.
Computational Models for Rapid MRSA Outbreak Detection and Infection Control

Rosemarie Sadsad\textsuperscript{1,2}, Vitali Sintchenko\textsuperscript{1,2}, Gwendolyn Gilbert\textsuperscript{1}, Matthew O’Sullivan\textsuperscript{1}, Fei Zhou\textsuperscript{1}, Frances Jenkins\textsuperscript{1}, Geoff McDonnell\textsuperscript{2}

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Investigating outbreaks of methicillin resistant \textit{Staphylococcus aureus} (MRSA) in hospitals is difficult using epidemiologic information alone. It is particularly challenging in settings where MRSA is highly clonal and endemic. To increase the resolution of MRSA surveillance for outbreak detection, we have developed an automated rule-based MRSA outbreak detection system that uses MRSA genotype information and data on patient movement between wards during their stay in Westmead hospital. A novel high-throughput, inexpensive and discriminatory genotyping method, developed and implemented at CIDM-PH, is employed to distinguish clusters of MRSA patients by their MRSA subtype and to identify probable MRSA transmission events. The system utilises patient movement data from the hospital patient information system to determine the likely wards where MRSA transmission may have taken place. Using computer simulated scenarios, we also evaluated several hospital infection control policies for reducing MRSA acquisitions in different ward specialities. Together, this information enables timely MRSA outbreak detection and investigations, which includes identifying sources and secondary cases for MRSA transmission events, and targeted infection control.
The Australian Pathogen Intelligence Community Space (APICS): A Secure Online Portal for Spatiotemporal Infectious Disease Surveillance

Nadine Holmes¹, Jeff Kelley², Umair Cheema², Francisco Piragibe², Peter Jelfs³, Shahin Oftadeh³, Ken McPhie³, Dominic Dwyer¹, Lyn Gilbert¹, Tania Sorrell¹, Vitali Sintchenko¹

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Objectives: eResearch tools and open-source software are gaining recognition and popularity amongst biomedical scientists. The Australian Pathogen Intelligence Community Space (APICS) aims to improve infectious disease surveillance by providing a secure online environment for collaborative research and the sharing of data, knowledge and resources between specialist pathology and microbiology providers, epidemiologists, public health professionals and multidisciplinary research teams.

Methods: Data management and analysis tools have been developed for the secure uploading, trend/cluster analysis, and spatial/temporal visualisation of infectious disease data. The database incorporates comprehensive pathogen profiling information, including molecular and phenotypic sub-typing and antimicrobial resistance information and de-identified clinical and geo-demographic patient data. External population and socioeconomic information from the Australian Bureau of Statistics will also be integrated into the application.

Results: Three test disease modules have been used to build and evaluate APICS. These are based on Mycobacterium tuberculosis genotyping and antibiotic resistance data, serotyping and antibiotic resistance monitoring of Streptococcus pneumoniae isolates associated with invasive pneumococcal disease and influenza sub-typing and antiviral resistance data. Output from these modules, linking infectious disease cases with specific pathogen profiles, will allow us to distinguish epidemic strains from sporadic or emerging variants and monitor spatiotemporal changes in pathogen subtypes and resistance profiles.

Conclusions: Wide-scale adoption of APICS at state and national levels has the potential to create data sharing and analysis networks for the prospective monitoring of pathogen transmission and changes in pathogen/disease patterns. Network information could also be used to pinpoint key areas for future infectious disease research and optimise disease prevention and intervention strategies.
**Bacterial epidemiology & disease surveillance**

**Bordetella holmesii: A newly recognised important contributor to pertussis outbreaks**

Nadine Holmes¹, Neisha Jeoffreys², Lyn Gilbert¹,², Vitali Sintchenko¹,².

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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, more recent reports from different parts of the world suggested it could also be a transmissible respiratory pathogen that can account for up to a third of all PCR-diagnosed cases of clinical pertussis syndrome. *B. pertussis*-PCR targeting the multicopy number insertion sequence IS481 has been widely used because of its high sensitivity, but it cannot distinguish *B. pertussis*, which contain 50-200 copies, from *B. holmesii*, containing 8-10 copies of IS481.

**Methods.** A *B. holmesii*-specific real-time PCR assay was developed to retrospectively screen *B. pertussis*-positive samples referred to the Centre for Infectious Diseases and Microbiology – Public Health between April 2008 and May 2012. This period spanned a large community pertussis outbreak throughout NSW that erupted between mid 2008 and early 2010.

**Results.** Of the 2001 specimens collected during the specified time period, 375 have been screened. *B. holmesii* was detected in 6.3% of these isolates. So far all *B. holmesii*-positive specimens were collected between May 2009 and December 2010. The highest incidence rate of *B. holmesii* detection was in November 2010, where it was present in 50% (6/12) of the samples screened.

**Conclusions.** The detection of *B. holmesii* in clinical samples obtained from patients with respiratory illness suggests that it could have sporadically contributed to the burden of pertussis in NSW. However, further epidemiological studies will be needed to determine the magnitude of *B. holmesii* respiratory disease. Potential approaches for introducing additional diagnostic assays to distinguish between *B. holmesii* and *B. pertussis* will be discussed.
Mycobacterium Tuberculosis Genetic Lineage and Transmissibility: An Assessment of Child Contacts

Kristina L Flego, Pamela Banner, Alexander C Outhred, Ben J Marais, Vitali Sintchenko

Mycobacterium tuberculosis strains can be classified into six genetic lineages. Emerging evidence suggests that lineage is associated with disease phenotype. However, differences in transmissibility associated with lineage have not been rigorously assessed. Australia provides an excellent setting due to limited community exposure, high culture confirmation rates with routine genotyping (using 24-locus MIRU-VNTR), compulsory case notification and well-functioning contact tracing systems. Children exposed to a tuberculosis source case are an important risk group, and in the absence of previous tuberculosis or BCG exposure represent a rare opportunity to assess genotype–specific transmissibility.

This study assessed rates of tuberculin skin test conversion among child contacts of all patients with culture-confirmed pulmonary tuberculosis identified at the Liverpool and Parramatta Chest Clinics from 2007 to 2011. Forty to fifty index cases were identified per study year at each site. Epidemiological data for these cases were retrieved from Chest Clinic medical records, and from databases including the Chest Clinic Surveillance System and NSW Notifiable Conditions Information Management System. Half of the index cases had child contacts. The genetic lineage of the strain cultured from these index cases was determined by the NSW Mycobacterium Reference Laboratory, and approximately one quarter of strains were from the East Asian (Beijing) lineage. A multivariate analysis was performed to determine the association between lineage, rate of transmission, and other epidemiological factors.
Molecular typing of methicillin-resistant *Staphylococcus aureus*

Fei Zhou\(^1\)*, Matthew O’Sullivan\(^1,2\), Frances Jenkins\(^1\), Vitali Sintchenko\(^1,2\), Gwendolyn L. Gilbert\(^1,2\)

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Objective: Methicillin-resistant *Staphylococcus aureus* (MRSA) has a diverse population structure, with variable transmissibility and virulence. Most typing methods cannot distinguish strains within common circulating clones. We developed and validated a novel, discriminatory and rapid molecular typing system (using multiplex PCR-based reverse line blot [mPCR/RLB] assays) for MRSA surveillance.

Methods: We collected the first isolate from each newly identified MRSA-colonised or -infected patient and from each episode of infection in two Sydney hospitals during 15 months (Aug 2011 - Oct 2012). All isolates were tested by mPCR/RLB, using two probes for each of 19 genetic markers. SCC\textit{mec}, \textit{spa} and multilocus sequence typing (MLST) and PFGE were also performed on representative isolates from an MRSA outbreak in a neonatal intensive care unit (NICU). We also compared mPCR/RLB assays with MLST on selective isolates.

Results: Among over 1400 clinical isolates, there were 230 unique RLB types, of which the four commonest accounted for nearly half (45%) of the isolates - 14\%, 11\%, 11\% and 10\%. During the NICU outbreak, 20 neonates were colonized or infected (six) with MRSA. Isolates from 15 infants were indistinguishable by RLB typing; five were colonized with unrelated strains. The outbreak strain was characterized as a Panton-Valentine leucocidin (PVL)-producing ST22, SCC\textit{mec} IVc, \textit{spa} t005 MRSA. mPCR/RLB assays showed good concordance with MLST.

Conclusions: Important clinical and epidemiological correlates reflected in the population ecology of MRSA can be monitored by use of this rapid, discriminatory strain typing system, which can detect transmission events and emergence of hypervirulent strains, and has the potential to improve infection control.
A study of virulence genes (and other characteristics) in *Escherichia coli* isolates causing urinary tract infections (uropathogenic *E. coli*).

**Timothy Kudinha** (PhD student): CIDM-PH Associate Supervisor: Professor Lyn Gilbert

Urinary tract infections (UTIs), which are caused mostly by specialized strains of *Escherichia coli*, termed uropathogenic *E. coli* (UPEC), are among the commonest bacterial infections of humans responsible for considerable morbidity, mortality, and high medical costs worldwide. UPEC possess distinct accessory traits, known as virulence factors (VFs), which the organism uses to invade and injure the host. Most studies on UTI pathogenesis have centered on VF genes, which clearly have immediate practical relevance with respect to virulence.

The increasing prevalence of antibiotic resistance among uropathogens, and the emerging threat of multidrug-resistant *E. coli* sequence type 131 (ST131), adds to the problem of UTI management. Thus, any study that will improve understanding of disease mechanisms and, potentially, of UTI management could have significant social and economic benefits.

A total of 1645 *E. coli* isolates comprising cystitis, pyelonephritis, and healthy fecal control isolates, from children (total, 327 isolates), men (total, 389 isolates) and reproductive-age women (total, 953 isolates), from the Central west region of New South Wales (NSW), Australia, were studied. The isolates were characterized genotypically and phenotypically for several bacterial characteristics (VFs, phylogenetic groups, clonal group ST131, O types, and antimicrobial susceptibility), using a variety of methods, one of which was developed as part of this study.

We developed a highly specific and sensitive multiplex-PCR reverse line blot (mPCR/RLB) for the simultaneous detection of 22 UPEC VF genes. Our results showed a gradient of molecularly inferred virulence, among the three patient groups, from *E. coli* strains causing more invasive UTI syndromes, such as pyelonephritis, through those causing cystitis, to fecal strains. No single VF gene or combination of VF genes was unique to a particular uro-clinical syndrome or patient group, highlighting the fact that UTI is multiply determined. Further, we documented that the multidrug-resistant ST131 has both resistance and virulence advantages over other *E. coli*, which probably explain its impressive emergence worldwide. Our results implicate ST131 as a major antimicrobial-resistant UTI pathogen in this region of Australia, and should be considered a public health threat. Therefore, more studies are needed to determine the distribution of, and risk factors for, acquisition of this clonal group so that effective control measures can be devised.
Rapid Identification of Gram Positive Pathogens from Positive Blood Culture Broth Using a Multiplex Tandem Real-Time Polymerase Chain Reaction Assay

Briony J. Hazelton, Lee C. Thomas, Tuba Unver, Jonathan R. Iredell

Background: The early initiation of targeted antibiotic therapy in patients with bacteraemia and septic shock impacts favourably on outcomes. Rapid methods are therefore increasingly employed for bacterial identification directly from positive blood culture bottles, but with variable success.

Method: We evaluated the performance of the Gram positive-12 multiplex tandem polymerase chain reaction (MT-PCR) assay (AusDiagnostics; Catalogue number: 6202, Version: 07) containing targets for the identification of staphylococci including *Staphylococcus aureus*, streptococci including *Streptococcus pneumoniae*, enterococci including *Enterococcus faecalis* and *Enterococcus faecium* and their common antibiotic resistance genes (*mecA*, *vanA*, *vanB*). A total of 673 aerobic and anaerobic blood culture broths demonstrating Gram positive cocci on microscopy were analysed in parallel with traditional phenotypic methods.

Results: Amplification of the internal control was inhibited in 79/673 (11.7%) of samples, however, MT-PCR was in concordance with phenotypic identification to the genus level in 96.6% (537/556) of remaining monomicrobial specimens and to the species level, where applicable, in 100% (172/172). MT-PCR for 94.7% (36/38) of polymicrobial samples matched traditional phenotypic identification. Methicillin and vancomycin susceptibility determined by MT-PCR in blood culture broths demonstrated complete agreement with phenotypic methods in all 143 *S. aureus* isolates and 8 *E. faecium* isolates, respectively.

Conclusion: Gram positive pathogens and their key antibiotic resistance markers were reliably identified within three hours of a positive blood culture being identified with this assay.
Deciphering the emergence of tularaemia in Australia: Culture-independent analysis of *Francisella* 16S rRNA and recA gene sequences from lymph nodes following possum bites

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**Background:** *Francisella tularensis* subspecies *tularensis* and *holarctica* cause Type A and Type B tularaemia, respectively, with zoonotic reservoirs and human disease reported only in the Northern Hemisphere. Delays in clinical recognition in low incidence countries adversely affect the recovery of these pathogens. This study presents microbiology findings from a recently diagnosed first human case of tularaemia in Tasmania.

**Materials:** Patient developed a PUO followed by an ascending lymphadenopathy after a ring-tail possum bite. Elbow and axilla lymph node tissues were collected 6 weeks after symptom onset. No bacteria were isolated but *F. tularensis* DFA and PCR were both found positive by a CDC LRN partner laboratory in Sydney. Local and multiple sequence alignments (MSA) were conducted using BLASTn, ClustalW and MUSCLE in MEGA v.5.

**Results:** Amplicons of 16S rRNA and recA genes from DFA microscopy-positive samples (GenBank accession numbers JQ277265 and JQ277266) were aligned with reference sequences from the GenBank/EMBL/DDJB database and two cases of *F. novicida* and *F. philomiragia* infections previously reported (Whipp et al, 2003) in the Northern Territory (NT) and Victoria, respectively (Figure). Both 16S rRNA and recA genes’ sequences showed 100% query coverage and E-value=0.00 in BLASTn with 36 submissions of *F. tularensis*. The unrooted bootstrap consensus tree generated from MSA coupled with the neighbour-joining method (overall mean distance equals 0.01) suggested the homology between the causative agent of the tularaemia from Tasmania and *F. tularensis subsp. holarctica*. Subsequent sequencing of the Regions of Difference (Huber et al, 2010) further narrowed this identification to biovar “Japonica”. Isolate 3523 from NT appeared to resemble *F. novicida* RB401 from Thailand.

**Conclusions:** Culture-independent amplification of recA and 16S rRNA genes and Regions of Difference can assist in the identification of *Francisella* species in tissues several weeks after symptom onset and the start of antibiotic therapy. The first case of tularaemia in Australia was likely to be caused by *F. tularensis subsp. holarctica biovar japonica* and differs significantly from previously reported local isolates. The epizootic reservoir of the disease in Australian marsupials warrants further public health investigation.

*Presented at the American Society for Microbiology Annual Meeting, San Francisco, CA June 2012*
High Levels of Macrolide (Azithromycin) Resistant Syphilis in Sydney

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4. The Kirby Institute, University of New South Wales

**Background**

The genetic mutation A2058G in the 23S ribosome of *Treponema pallidum* confers resistance to azithromycin, and is associated with macrolide treatment failure. This mutation has been detected in samples from San Francisco, Dublin and China. This is the first study to report this mutation in Australia.

**Methods**

PCR amplification of the 23S rDNA gene was performed on 319 *T. pallidum* PCR positive DNA samples collected from Sydney clinics between 2004 and 2011. 23S rDNA amplicons were subject to restriction endonuclease digestion. Samples containing the A2058G mutation were identified by agarose gel electrophoresis.

**Results**

Amplification of 23S rDNA was successful in 298 samples, with restriction endonuclease digestion identifying 254 of these (85.2%) as containing the genetic mutation A2058G. Azithromycin resistance in *T. pallidum* increased dramatically from 66% in 2005 to 93% in 2006, then remained consistently high through 2011.

**Discussion**

The high prevalence of azithromycin resistant syphilis in Sydney (85.2%) is similar to that seen in other countries with syphilis epidemics in men who have sex with men, such as the USA (76.5% in 2005) and Ireland (88% in 2004). However, it is interesting to note that the prevalence of resistance in Sydney increased rapidly in 2006, rather than gradually increasing as seen in other sites. Widespread use of macrolides for treatment of other bacterial infections may have contributed to this phenomenon. Further analyses will correlate azithromycin resistance with genotype, HIV status, macrolide use and sexuality. This study illustrates that macrolides should not be used for treatment of syphilis in Sydney.
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.

Shahin Oftadeh, Heather Gidding, Lyn Gilbert

We compared serotype distributions of *Streptococcus pneumoniae* isolates from patients aged <5 and ≥5 years with invasive pneumococcal disease in New South Wales, Australia, and antibiotic susceptibilities of isolates from the <5 years age group only, before (2002–2004) and after (2005–2009) introduction of the 7-valent pneumococcal conjugate vaccine (PCV7). Overall, there were significant decreases in the mean annual number of referred isolates (770 vs. 515) and the proportion belonging to PCV7 serotypes (74% vs. 38%), but non-PCV7 serotypes, particularly 19A, increased (5% vs. 18%). All changes were more marked in the <5 years age group. Susceptibility testing of isolates from the <5 years age group showed variation in resistance between serotypes, but significant overall increases in penicillin non-susceptibility (23% vs. 31%), ceftriaxone resistance (2% vs. 12%) and multidrug resistance (4% vs. 7%) rates; erythromycin resistance fell (32% vs. 25%). Continued surveillance is needed to monitor changes following the introduction of 13-valent PCV in 2012. (from Epidemiol. Infect. 2012; doi:10.1017/S095026881200218X)

Further changes in serotype distribution 2010-11.

Note continued increase in 19A and (from much lower base) in serotype 1, 7F, 6C and 22F in <5 year age-group and 19A, 7F, 3 and 22F in those aged >5 years. Change following the introduction of the 13 valent PCV will be awaited with interest. (see graph next page)
Annual average and cumulative percentages of S. pneumoniae serotypes and serogroups from IPD in <5year old referred to NSW PRL before and after PCV7 and PCV13

Annual average and cumulative percentages of S. pneumoniae serotypes and serogroups from IPD in >=5year old referred to NSW PRL before and after PCV7 and PCV13
Molecular serotype identification for culture negative specimens

Since 2010, the NSW Pneumococcal Reference Laboratory has used a molecular method to identify *S. pneumoniae* serotypes in culture negative specimens. The method was validated using a panel of DNA extracts of well-characterised culture positive (and some negative) throat swabs in which one or more different serotypes had been identified. We then used it to test samples from an outbreak of serotype 1 infections in the Northern Territory. Subsequently it has been used to identify serotypes in >60 pneumococcal PCR-positive/culture negative specimens (mainly of pleural fluid or CSF) referred from throughout Australia and the method has been modified to improve its sensitivity.

Molecular epidemiology of *S. pneumoniae* serotypes 19A and 19F

The aim of this study was to identify the predominant clonal complexes (CC) or multilocus sequence types (MLST) involved in the expansion of serotype 19A following the introduction of PCV7 and their relationship to those belonging to 19F, which had been one of the major (and most antibiotic resistant) prevaccine childhood IPD serotypes.

The target CCs investigated initially were CC199 (mainly penicillin intermediate, and predominant among prevaccine 19A isolates) and CC320 (penicillin and multidrug resistant and common among prevaccine 19F isolates). Allele-specific primers were developed to rapidly identify single nucleotide polymorphisms (SNPs) in two house-keeping genes that are characteristic of these CCs (CC199: *aroE8-gki14* and CC320: *aroE4-gki19*). 42% of 19F and 49% of 19A isolates belonged to one of the target CCs. They were evenly represented among 19F isolates but CC199 was much more common among 19A. All but one CC320 isolates were penicillin resistant but CC199 isolates were either susceptible or, particularly in serotype 19A, had intermediate resistance. (see table next page).
<table>
<thead>
<tr>
<th>Serotype 19F: 126 (34%)</th>
<th>Pen S</th>
<th>Pen I</th>
<th>Pen R</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC199: 26 (20%)</td>
<td>75 (60%)</td>
<td>16 (13%)</td>
<td>35 (27%)</td>
</tr>
<tr>
<td>CC320: 28 (22%)</td>
<td>24 (92%)</td>
<td>2 (7%)</td>
<td>0</td>
</tr>
<tr>
<td>Other: 72 (58%)</td>
<td>50 (69%)</td>
<td>14 (20%)</td>
<td>8 (12%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Serotype 19A: 243 (66%)</th>
<th>Pen S</th>
<th>Pen I</th>
<th>Pen R</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC199: 99 (41%)</td>
<td>89 (37%)</td>
<td>125 (52%)</td>
<td>26 (11%)</td>
</tr>
<tr>
<td>CC320: 20 (8%)</td>
<td>58 (60%)</td>
<td>39 (40%)</td>
<td>0</td>
</tr>
<tr>
<td>Other: 123 (51%)</td>
<td>31 (25%)</td>
<td>86 (70%)</td>
<td>20</td>
</tr>
</tbody>
</table>

Total: 369 | 164 (45%) | 141 (38%) | 61 (17%) |
**CIDM-PH Research Reports & Works in Progress**

**Bacterial epidemiology & disease surveillance**

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**Improving Public Health Surveillance of Salmonellosis by Prospective Genotyping of *Salmonella Typhimurium.***

Shopna BAG\(^1\), Peter HOWARD\(^1\), Qinning WANG\(^1\), Jennie MUSTO\(^3\), Neil FRANKLIN\(^3\), Tania SORRELL\(^1,2\), Vitali SINTCHENKO\(^1,2\)

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**Introduction:** In NSW, the majority of notifiable foodborne diseases are *Salmonella enterica*. 4903 (59%) of these were identified as *S. Typhimurium* (STM) in 2011. Timely surveillance and investigation of outbreaks, requires strong linkage between epidemiological data and reliable subtyping of STM. Multiple locus variable-number tandem repeat analysis (MLVA) is a rapid method of typing with the promise of high discrimination. The aim of this project was to describe the role of STM MLVA typing in the surveillance and investigation of food-borne outbreaks.

**Methods:** Retrospective analysis of STM MLVA typing results obtained between January 2009 and June 2011 was conducted. STM clusters of isolates with identical MLVA patterns were identified and linked to the NSW outbreak reports and NSW OzFoodNet records.

**Results:** Within STM 170, MLVA subtyping identified 110 patterns. 30 food-borne outbreaks, associated with 9 clustered MLVA patterns, were investigated by public health units. Public health investigation of outbreaks seemed to decrease the size, duration and delay the onset of subsequent clusters, within a MLVA profile.

**Conclusions:** *Salmonella Typhimurium* MLVA typing improves the discrimination of common phage types and can be employed with temporo-spatial mapping for prospective public surveillance and timely outbreak detection. Future research with prolonged MLVA surveillance data is required, to understand the natural progression of MLVA fluctuation.

**Key Message:** MLVA typing of STM improves the detection of clusters, enabling early public health investigation and institution of control measures.
Invasive fungal infections (IFIs) are demanding of high health resources because of the risk groups affected (these typically need high-end medical care, technology and drugs) and the high attributable morbidity and mortality (the latter ranges from 10-70%).

The Sorrell and Chen groups work on the epidemiology and pathogenesis of invasive fungal infections in order to develop new diagnostics, identify individuals at high risk of infection, develop and implement preventative and therapeutic management strategies that improve health outcomes (including a collaboration in economic modelling), and generate diagnostic and therapeutic algorithms at local, national and international level. Multicentre, multi-state projects led from CIDM PH include the following: critically ill patients in an ICU (invasive candidiasis), both children and adults with cystic fibrosis (filamentous fungi, eg Aspergillus and Scedosporium spp), patients with haematological malignancies and haematopoietic stem cell transplantation (Candida, Aspergillus, Mucorales, Fusarium) and patients with AIDS/other risk factors for cryptococcosis (C. neoformans and C. gattii).

As an example, we have recently completed data collection and clean-up for an NHMRC-funded project to develop a risk prediction model for invasive candidiasis in ICU patients. More than 6000 patients from 7 hospitals in three Australian states were enrolled. Preliminary analysis indicates that clinical factors, antimicrobial therapy and colonisation status are independent risk factors for IC. Work is now in progress to devise a simple model to predict the risk of IC and inform antifungal therapy for those at highest risk. This project has generated a follow-up project on economic modelling of antifungal therapeutic strategies for IC in the ICU setting, which was funded by the NHMRC to start in 2012. Diagnostic tests for cryptococcosis that are applicable in resource poor countries have been evaluated and a simple test for monitoring the response of cryptococcal meningitis to antifungal therapy is under development. Chayanika Biswas is completing a PhD on identifying the mode of action of a new potential antifungal compound, miltefosine, using DNA libraries generated from the model yeast, S. cerevisiae. Co-supervised by Chen, Sorrell and Meyer, Shilpa Hemant is undertaking a longitudinal clinical study to determine the incidence of, and impact on lung function, of fungal colonisation of the respiratory tract of patients with cystic fibrosis. She is also studying the interactions between Pseudomonas and Scedosporium in vitro.

The Meyer group is internationally known for their role in a global network on the phylogeny of pathogenic fungi, and their work on the influence of molecular type on the pathogenesis of the serious invasive fungal infection, cryptococcosis. The Meyer lab is generating the data on fungi of medical importance as part of
the Atlas of Living Australia and the Quarantine Barcoding of Life project (aimed at rapid identification of emerging fungal and non-fungal pathogens and outbreaks. The have published recently on the use of MALDI-TOF (mass spectrometry/phenotypic analysis) in the diagnostic lab for the very rapid identification of yeasts and cryptococci and on a method applicable in resource-poor settings (rolling circle amplification) for rapid speciation of cryptococcal genotypes. Importantly this lab has contributed to the development of a global quality-controlled data-base for pathogenic fungal identification, based on the Internal transcribed spacer (ITS) region of the mitochondrial gene region. (http://www.mycologylab.org/BioloMICSID.aspx). The definition of a fungal species is still under debate as clinical definitions are much less stringent than molecular (species previously identified by conventional means may exhibit only 91.5% similarity by molecular testing). There is a major, multicentre effort underway to develop “barcodes” for pathogenic fungi as a basis for standardisation of nomenclature and automated molecular identification (and hence standardised diagnostics). Molecular typing of cryptococci has revealed differences in virulence in experimental models and in susceptibility to standard antifungal agents. These findings have implications for therapy and efforts at preventing acquisition of Cryptococci from the environment.

Much of the work described has received competitive funding from the NHMRC and other sources and is being/has been presented at national and international meetings and published in rigorously peer reviewed international journals.
Hospital acquired *Pneumocystis jirovecii* pneumonia (PJP)

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*Pneumocystis jirovecii* is an opportunistic fungus that causes severe pneumonia in immunocompromised patients. *P. jirovecii* pneumonia (PJP) is an important infection-related complication, whose mode of transmission remains uncertain. In order to investigate hospital acquired PJP and to support epidemiological data, we have established a multilocus sequence typing (MLST) scheme for the genotyping of *P. jirovecii*, based on sequence analysis of four genetic loci: the internal transcribed spacer 1 and 2 (ITS1/2) regions including the 5.8S rRNA gene of the nuclear rRNA gene cluster, the β-tubulin (β-Tub), dihydropteroate synthase (DHPS) and mitochondrial large subunit rRNA (mtLSU) genes. In total, we have studied 53 samples of *P. jirovecii*, 44 associated with nosocomial PJP cases, mainly in kidney transplant recipients, and 9 isolated cases as controls. Nine genotypes have been identified with three closely related genotypes for the isolates causing outbreaks in Sydney and Brisbane. Outbreak isolates are identical to each other. The obtained MLST data together with contact tracing data supported the notion of patient-to-patient transmission. The generated MLST data are incorporated into our in house *P. jirovecii* database, which is globally accessible at [http://mlst.mycologylab.org](http://mlst.mycologylab.org). Using the online MLST database and given the high reproducibility of sequencing based MLST analysis it is now possible to discriminate subsequent PJP cases and generate an increases epidemiological knowledge of the spread of *P. jirovecii* in Australia and globally.
Correlation of virulence and specific hybrids in the *C. neoformans/C. gattii* species complex

**Mojgan Aminnejad** (PhD student), Shuyao Duan, Supervisors: Tania Sorrell & Wieland Meyer

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The basidiomycetous yeasts *Cryptococcus neoformans* and *Cryptococcus gattii* are important human pathogens infecting immunocompromised and immunocompetent individuals respectively. *C. neoformans* is comprised of two varieties: *C. neoformans* var. *grubii* (serotype A; molecular types VNI and VNII) and *C. neoformans* var. *neoformans* (serotype D; molecular type VNIV) as well as hybrids of both varieties (serotype AD; molecular type VNIII). *C. gattii* belongs to serotype B or C (molecular types VGI, VGII, VGIII and VGIIV). Recently, in addition to VNIII AD hybrids, new AD hybrids with the combination pattern of VNII/VNIV have been discovered. Also, several BD and AB inter-species hybrids and intra-varietal serotype A hybrids between VNII & VNB and VNI & VNII have been described. The aim of this study was to characterize the pathogenicity of 17 newly found hybrid strains belonging to different origins, molecular type and serotype/mating type allele pattern [VNI/VNII (αAA), VNII/VNIV (αAD), and VNI/VGII & VNI/VGII (αABA)] within the *C. neoformans/C. gattii* species complex. To assess the effects of hybridization on pathogenicity, the virulence of the hybrid strains was investigated in a *Galleria mellonella* (the greater wax moth) model and survival of infected larvae were monitored. All groups of hybrids killed *G. mellonella* larvae at 37°C when infected with 106 CFU/larvae but the rate of killing depended on the strains as a significant variation of mortality rate was observed among larvae infected with different groups of strains. All clinical VN/VG hybrids yielded 100% mortality within the study period (14 days post-infection) and VNI/VGII strains showed more virulence than VNI/VG strain. All clinical VNI/VNII strains showed 100% mortality before day 14 except for WM 2975 (which may had technical problems during inoculation) and the veterinary isolate WM 714, which showed 20% and 85% mortality respectively. Clinical and environmental strains presenting the VNII/VNIV (αAD) hybrids caused 0%-60% mortality whereas VNIII (αADA) control strains, WM 628 and CBS 132, showed 100% mortality within the study period. This suggests that the presence of the Aα mating type allele in AD hybrid strains is correlated with virulence. High virulent strains, H99 (Aα) and CDC R265 (Bα), and low virulent strains, JEC20 (Da) and CDC R272 (Bα), were used as control strains. All hybrid strains were more virulent than JEC20 (Da) and VN/VG hybrids were also more virulent than CDC R272. To our knowledge this is the first study in which different groups of hybrids were compared in terms of pathogenicity in an experimental *G. mellonella* model. The results showed that hybrid strains presenting the Aα mating type locus are highly virulent in the *G. mellonella* model. These results are consistent with the previously reported virulence data of AαDa hybrid strains in an experimental murine model. Hybrid strains comprise an important component of *C. neoformans/C. gattii* species complex. They are recovered from clinical isolates and as results showed are virulent in experimental models except for VNII/VNIV (αAD) hybrids.
The Epidemiology Of Nosocomial Candidemia In Nonneutropenic, Nonintensive Care-based Patients

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Background:
Candidemia (C) is underappreciated outside ICU and oncology settings and risk factors are not well described but affect empiric treatment. We describe C in this population and analyze risk factors for C against patients with bacteremia (B) and those with neither.

Methods:
Retrospective case-case-control study (Jan 2003-Dec 2008). Non-neutropenic, non-ICU patients with nosocomial C were matched by age, medical/surgical specialty, year of admission and length of stay against patients with B (1:1) and patients with neither B nor C (1:2).

Results:
There were 37 patients with C, 37 with B and 74 with neither. By univariate analysis risk factors common to patients with B or C included non tunnelled central venous catheterisation, urinary catheterisation, corticosteroid exposure, hypoalbuminemia and neutrophilia. Factors unique to C were prior invasive gastrointestinal procedure (odds ratio (OR) 5.83; 95% confidence interval (CI) 2.09,16.21; p=0.001), use of broad-spectrum antibiotics (OR 3.21; 95%CI 1.41,7.33;p=0.006), red cell transfusion (OR 3.53; 95%CI 1.43,8.68;p=0.006), and enteral feeding (OR 3.08; 95%CI 1.04,9.08; p=0.042). By multivariate analysis neutrophilia was a common risk factor, whilst unique factors to C were broad spectrum antibiotic exposure within the previous 7 days (OR 10.26; 95%CI 2.95,35.7; p<0.05) and elevated albumin (protective) (OR 0.89; 95%CI 0.82,0.96; p=0.004). Mortality was 38% in the candidemia group, 24% in the bacteremia group and 1% in controls. C. albicans was most frequent(35%), then C parapsilosis (27%).

Conclusions:
Classic risk factors for candidemia were identified in non-ICU, non-neutropenic patients. Empiric antifungals should be considered in hypoalbuminemic gastrointestinal surgery patients receiving broad-spectrum antibiotics.
Introduction: Invasive candidiasis (IC) has a high mortality in critically ill patients. We assessed the impact of *Candida* colonization on development of IC as part of a multicenter national study aimed at developing a risk predictive model for IC.

Methods: Throat and perineal swabs and urine were collected from patients 72 h post-ICU admission and then twice weekly until discharge or death, from seven large Australian hospitals, between 15/6/2007 and 1/1/2011. Swabs and 10µL urine were cultured on *Candida* Chromogenic agar (Chromagar, France). Organisms were speciated by colour, and semi-quantitative estimates of colonization density were made.

Results: Of 6,021 patients enrolled, 73 (1.2%) developed IC. 3,613 (60%) patients were colonized 72 h post-ICU admission and this percentage did not change in the first ~12 days post-ICU admission. Colonization preceded development of IC in 58 (79.5%) patients who developed IC. The risk of IC was independent of the actual site colonized, the number of sites, the density of colonization and the colonizing species. Other than improved speciation, molecular surveillance (MT-PCR, n=83 isolates) did not significantly improve detection of colonization status or the time to detection, compared with culture. The average time from ICU admission to the development of IC and the average time from colonization to infection was 12 d.

Conclusions: *Candida* colonization predicted the development of IC. Sensitivity was highest when no additional colonization indices were applied. The high NPV (99%) of *Candida* colonization can reduce unnecessary antifungal prophylaxis. We propose that screening the throat, perineum and urine at 72 h post-ICU admission (in combination with clinical risk factors) should capture all patients at high risk of developing IC.
Monitoring Cryptococcal Meningitis In A Developing Country -

Evaluation of staining techniques to determine viability of Cryptococcus in low-resource settings

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Affiliations: \textsuperscript{1}Centre for Infectious Diseases and Microbiology Laboratory Services, Westmead Hospital, Sydney; \textsuperscript{2}Westmead Clinical School, University of Sydney; \textsuperscript{3}Sydney Emerging Infections and Biosecurity Institute, University of Sydney and Westmead Millennium Institute, Sydney; \textsuperscript{4}Centre for Infectious Diseases and Microbiology, Westmead Millennium Institute, Sydney

Background: Cryptococcosis is a life-threatening fungal infection causing meningitis in immune-compromised and immune-competent individuals. Viability of organisms in CSF after 2 weeks of therapy has implications for prognosis and treatment and is traditionally determined by culture-based methods, which are slower and less widely available than microscopy. Trypan blue staining and flow cytometry are both methods which may be adapted to give rapid indication of viability of Cryptococcus seen in CSF microscopy.

Methods: Cryptococcus neoformans was grown on Sabouraud’s Dextrose Agar (SDA) and adjusted to a concentration of 1 McFarland standard in phosphate-buffered saline (1.4-3.2\times 10^6 organisms/mL). Different proportions of live and heat-killed organisms were mixed and examined by light microscopy with Trypan blue staining and by flow cytometry to determine viability, correlated with quantitative cultures.

Results: Proportions of 100% live; 50% live, 50% dead; and 10% live, 90% dead were examined by the above methods with results within 10% of those obtained by quantitative cultures.

Conclusions: Trypan blue staining and flow cytometry show promise for determining viability of Cryptococcus when compared with cultures and should be examined in clinical CSF samples.
Isolation and identification of *Scedosporium* spp. in cystic fibrosis patients

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The epidemiology and clinical relevance of fungal colonization/infection of the airways in cystic fibrosis (CF) is incompletely defined. In a prospective study we investigated the prevalence of *Scedosporium* spp. (a genus of particular interest) in CF patients, to be related to clinical risk factors and lung function in the future. Respiratory samples (n=755) from 177 children and 81 adults with CF were cultured on Sabouraud’s and DRBC (Dichloran Rosebengal Chloramphenicol) agars. Restriction fragment length polymorphism (RFLP) analysis of the ITS1/2 region was used to identify *Scedosporium* species. *Scedosporium* colonization was evident in 11.1% adults and 11.8% children by culture, 4.9% adults and 6.2% children were colonized with *P. prolificans* whilst *Pseudallescheria boydii* complex were recovered in 7.4% adults and 6.2% children. Co-colonization with *Aspergillus fumigatus* occurred in 8.6% of adults and 5.5% of children. Of 55 isolates, 31 (56%) were recovered only on DRBC agar. Based on ITS-RFLP analysis of 54 isolates, 35.1% were *S. aurantiacum*, 20.3% were *P. boydii/S. apiospermum* and 44.4%, *S. prolificans*. Estimated frequency of *Scedosporium* colonization was ≈11%. DRBC is necessary for isolation of this fungus and ITS-RFLP accurately identifies *Scedosporium* species and distinguishes *S. aurantiacum* from other species of the *P. boydii* complex.
**Galleria mellonella as a model system to study fungal pathogens**

Shuyao Duan and Wieland Meyer

Molecular Mycology Research Laboratory, Westmead Millennium Institute, Westmead Hospital, Westmead, NSW, Sydney, Australia, Centre for Infectious Diseases and Microbiology, Westmead Hospital, Westmead, NSW, Sydney, Australia, and Sydney Medical School-Westmead, Westmead Hospital, The University of Sydney, Sydney, Australia,

**Background:** The larvae of *Galleria mellonella* (Greater wax worm) have been used widely to study fungal pathogens and virulence factors. As an invertebrate model, *G. mellonella* shows a number of advantages for fungal virulence studies. First, they are easily to be maintained under various temperatures from 25°C to 37°C. It is suitable to mimic the condition in human and other mammals and also enable to establish temperature-related virulence studies. Second, innate immune systems of both *G. mellonella* and human have high functional similarities. Third, *Galleria* larva has soft cuticle that is facile to apply needle or topical applications. Injection inoculations could deliver the precise amount of fungal cells, which is impossible on mouse by respiratory inoculums. Furthermore, the experimental period for the *Galleria* larvae model is much shorter than in the mouse model. The maintenance costs for *Galleria* larvae is cheaper than for mice. **Methods:** Tall wide mouth glass jars are used for the storage of *Galleria* larvae, which are covered by fine metal mesh and housed at 26°C and 60% humidity. They are fed on: 22% Honey (standard honey from supermarket), 22% Glycerol, 4% water (Mount Franklin spring water), 48% Cereal (Farex® original multigrain cereal—fine grains, 6+months, 125g one bag), 4% Yeast (Lowan® Instant Dried Yeast). The low virulent *Cryptococcus gattii* strain WM 02.39 and high virulent strain WM 02.32 were used for our initial start up experiments. The larvae were in injected with a Hamilton pipet. Post-injection the larvae were kept in Petri dishes in a 37°C incubator and checked twice daily. To measure the yeast capsule the fresh dead body was smashed and stained using Indian ink. **Results:** the high virulent strain WM 02.39 died after 3 days and the virulent strain WM 02.32 after 8 days, mirroring exactly the findings form our mouse model. Histological studies to determine the spread of the infection through the larvae are currently under way using the frozen section technique with O.C.T embedding medium and hematoxylin and eosin stain (H&E) staining or Toluidine blue staining. Our findings show that the Galleria model is a valuable alternative to vertebrate virulence studies for human pathogenic fungi.
Clinical utility of the cryptococcal antigen lateral flow assay in a diagnostic mycology laboratory

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Affiliations: \textsuperscript{1}Centre for Infectious Diseases and Microbiology Laboratory Services, Westmead Hospital, Sydney; \textsuperscript{2}Sydney Emerging Infections and Biosecurity Institute, University of Sydney and Westmead Millennium Institute, Sydney; \textsuperscript{3}Department of Microbiology and Infectious Diseases, St. Vincent's Hospital, Sydney

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Background: Cryptococcus neoformans causes life-threatening meningitis. A new lateral flow immunoassay (LFA) that detects cryptococcal antigen (CRAG) is reportedly more rapid and convenient than latex agglutination (LA), but has not been evaluated in a routine diagnostic laboratory.

Methods: 106 serum, 42 CSF and 20 urine samples from 92 patients with known/suspected cryptococcosis were tested by LA and LFA. Results were correlated with laboratory-confirmed cryptococcosis.

Results: All sera (n=56) from 25 patients with confirmed cryptococcosis were positive by LFA (sensitivity 100%, 95% confidence interval (CI) 93.6-100%); 51/56 were positive by LA (sensitivity 91.1%, 95% CI 80.7-96.1%). Fifty sera from 67 patients without cryptococcosis tested negative in both assays. LA yielded more false negative results (5/56) but this was not statistically significant (p=0.063). Nine CSF samples from patients with cryptococcal meningitis were positive in both assays whilst 17/18 urine samples from patients with cryptococcosis were positive by LFA. The LFA detected CRAG in C. gattii infection (4 patients). Agreement between LFA and LA titres from individual samples was not good, though correlation between log-transformed titres (\(r\)) was 0.84. Turn-around-time was 20 minutes for LFA and 2 h for LA. The cost per qualitative test was 18USD and 91 USD, respectively and per titred sample was 38USD and 144USD, respectively.

Conclusions: Qualitative agreement between LFA and LA assays performed on serum and CSF was good, but agreement between titres was imperfect. Ease of performance of LFA and its applicability for testing urine suggest it has a role in the routine laboratory as a rapid point-of-care test.
The ecological impact of antibiotics

Jon Iredell

Microorganisms are inextricably connected to human health, and a major goal of modern healthcare is to suppress bad microbial interactions while simultaneously promoting (or at least preserving) good ones. Antibiotic therapy may lead to emergence of antibiotic resistance and/or disruption of the normal beneficial microflora but available evidence indicates that not all antibiotics have the same effects on selectable resistance and microbial ecology. Modern genomic and bioinformatic approaches offer great promise for immediate applications and even long term solutions but hint at major ecological threats - some of the technical and intellectual problems we face when dealing with these issues are discussed, with particular reference to transmissible gene pools and human microflora under intense antibiotic selection pressure.
Recombinatory evolution in antibiotic resistance plasmids

Sally Partridge

Much of the antibiotic resistance in the Enterobacteriaceae is due to mobile resistance genes carried on a variety of plasmids that can move between bacterial cells, including those of different species. These resistance genes, along with the mobile genetic elements (insertion sequences, transposons and integrons) involved in their capture and spread, are often found clustered in large complex multi-resistance regions. These regions are inserted in the relatively conserved plasmid “backbones”, which encode functions such as plasmid replication, stability and conjugative transfer. Multi-resistance regions are largely composed of well-defined elements from a limited set that are arranged in different ways as a result of recombinatorial evolution. As they often carry repeats that are longer than sequence read lengths conventional approaches to assembly of ‘deep’ sequencing data are often inadequate. Conventional approaches to annotation generally define genes and the functions of their products on the basis homology to known genes/proteins but ignore the important boundaries of mobile elements and so are also ill-suited to annotating multi-resistance regions. The poor annotation evident in major datasets such as GenBank and potentially unreliable reported assemblies of complex mobile elements in the published literature means that comparative analyses are often inaccurate and genetic relationships and evolutionary signals within the gene pool are obscured. Alternative bioinformatic annotation strategies, using BLASTn searches with a database of defined “features” and novel “grammars”, have therefore been developed and combined with predictive mapping approaches to overcome many of these problems and permit accurate informative analysis.
CIDM-PH Research Reports & Works in Progress

Antibiotic resistance surveillance & rapid diagnostics

Not all antibiotics are created equal
Andrew Ginn

Animal models suggest that certain antibiotics are associated with prolonged proteobacterial blooms in the gut microflora, which may in turn be associated with increased horizontal gene transfer. We present controlled data from humans that are consistent with this and with clinical observations regarding antibiotic effect that have important implications for antibiotic policy.
WHAT’S HAPPENING IN INFLUENZA?

Dominic E. Dwyer
CIDMLS, ICPMR, Pathology West, Westmead Hospital

The 2009 pandemic focused the attention of public health, clinical and laboratory medicine experts on Influenza. Although the influenza A(H1N1)pdm09 virus has now become a ‘seasonal’ influenza strain, questions continue to be asked about its impact. The WHO National Influenza Centre, based in CIDMLS, continues to monitor influenza virus types and subtypes in the winters that followed the 2009 pandemic. It identified that the influenza A(H1N1)pdm09 virus has been largely supplanted by influenza A/H3N2 and influenza B viruses by 2012. Ongoing surveillance for influenza viruses remains important, as there continues to be regional variation in circulating viruses. The WHO NIC also monitors for antiviral drug resistance, and contributes to national and international influenza surveillance through the WHO Collaborating Centre in Melbourne. There is a linkage with the WHO GLAMOUR project, a world-wide analysis of influenza mortality since the 2009 pandemic done in conjunction with investigators at NSW Health and UNSW. Clinical questions continue to be investigated, such as the role of bacterial and viral co-infections in severe influenza, and the role of obesity as a newly recognised factor for severe disease. CIDM-PH investigators collaborate with the INSIGHT network, a NIH funded international observational database of influenza. CIDM-PH investigators work with colleagues at the NCIRS and UNSW in studies of influenza interventions in aged-care facilities, as well as trials of mask use to prevent transmission of influenza and respiratory viruses to health care workers. CIDM-PH investigators are also involved in training in influenza diagnostics, as exemplified by a WHO Laboratory Twinning project with the Republic of the Maldives. Visitors from other countries, eg: Viet Nam, Hong Kong and Indonesia have also undergone laboratory training in diagnostic virology. A number of influenza vaccine studies are under way in collaboration with the NCIRS and the University of Sydney. CIDMLS-PH investigators are also involved in a national surveillance program for severe hospitalized influenza (the FLUCAN Study).
Viral and bacterial coinfection in pandemic (H1N1) 2009 infection

Jen KOK, Jonathan R. IREDELL, Dominic E. DWYER, The INSIGHT Influenza Study Group

Centre for Infectious Diseases and Microbiology Laboratory Services, Institute of Clinical Pathology and Medical Research; Sydney Institute for Emerging Infections and Biosecurity; Centre for Research Excellence in Critical Infections, University of Sydney, Westmead Hospital, Westmead, New South Wales, Australia

The epidemiology and impact of viral and bacterial coinfection in pandemic (H1N1) 2009 infection (hereafter A(H1N1)pdm09) is unclear. Previous influenza pandemics have identified the significant role of bacterial coinfection, but the impact of viral coinfection has been less well studied. In severe A(H1N1)pdm09 infection, bacterial coinfection complicated ~25% of patients admitted to Australian intensive care units (ICU). This data may not reflect outpatient or hospitalized patients not admitted to ICU. The observational INSIGHT FLU 002 outpatient and FLU 003 hospitalization studies aim to assess the epidemiology, clinical and laboratory aspects of patients with A(H1N1)pdm09 to identify risk factors for severe complications and/or fatal outcomes. We compare viral and bacterial coinfection data from Australian ICUs and FLU 002 and 003. Correlation with clinical data may identify risk factors for predicting bacterial and/or viral coinfection. This data may guide empiric antimicrobial choice in patients with severe or complicated A(H1N1)pdm09 infection.
Deep sequencing of hepatitis C virus to detect low-frequency drug resistant mutations

Adrian Teck Leng Ong\textsuperscript{1,2,3}, Rowena Bull\textsuperscript{4}, Fabio Luciani\textsuperscript{4}, Monkol Lek\textsuperscript{5}, Peter White\textsuperscript{4}, Jacob George\textsuperscript{2}, Dominic Dwyer\textsuperscript{1,3}, Mark W. Douglas\textsuperscript{1,2,3}

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\textbf{Aims:} Three platforms used for deep sequencing hepatitis C virus (HCV) were compared; Illumina, Roche 454 and Ion Torrent.

\textbf{Introduction:} HCV is the primary cause of liver transplantation and hepatocellular carcinoma in Australia. Recently approved direct acting antiviral drugs (DAAs), protease inhibitors boceprevir and telaprevir increase rates of sustained virological response (SVR) and are standard of care for treating genotype 1 HCV.

Traditional sequencing only detects quasispecies present at >20\% whilst next-generation sequencing is more sensitive in detecting low frequency resistant variants. Treatment response with DAAs may be reduced in the presence of low frequency drug-resistant virus and we anticipate a future role for drug resistance testing to guide HCV therapy.

\textbf{Methods:} Firstly, full-length HCV genomes were amplified and sequenced from reference plasmids by long range PCR. Next, artificial quasispecies were created by mixing 2 plasmids differing by a single point mutation, at specific ratios of 1:9, 1:99, and 1:999, then sequenced. Lastly, HCV was amplified from plasma of patients with genotype 1 infection, then sequenced. An external service provider performed Illumina and 454 sequencing whilst Ion Torrent sequencing was performed in-house.

\textbf{Results:} The frequency of sequencing errors was determined by comparison with plasmid reference sequences. The accuracy in measuring the relative composition of low prevalence quasispecies was also determined. From human samples the frequency of known drug-resistant mutations was determined and compared across platforms.

\textbf{Conclusion:} Deep sequencing platforms are useful in the detection of low-frequency drug-resistant HCV mutations.
Epidemiology of Barmah Forest virus infections

Linda Hueston (PhD student): Supervisors Professors Lyn Gilbert & Tania Sorrell

Barmah Forest virus (BFV) is a mosquito-borne alphavirus unique to Australia. First isolated in 1974 the virus spent its first two years in a freezer at the John Curtin School before its discoverer Ian Marshall had the opportunity to investigate it. It did not give up its secrets easily and was originally thought to be a bunyavirus until the work of Dalgaro et.al. proved it was “an alphavirus with unusual properties”.

This virus is the second most common cause of arboviral disease in Australia and the experiments in this thesis aim to improve the diagnosis and detection of BFV disease in humans.

In the first part of the project a real time RT-PCR was developed, validated and evaluated for routine diagnostic work. This is the first report of the use of PCR in the diagnosis of BFV disease. By sequencing the PCR product of positive samples investigation into strain variation of this virus can be undertaken. In the second part of the work a series of ELISAs were developed, validated and evaluated for use in routine diagnosis. These tests were then applied to samples collected over a 15 year period. These tests not only allowed for rapid diagnosis of human infection but highlighted an unusually delayed IgG response which lays the foundation for further study into the pathogenesis of BFV.

Finally by studying the clinical presentation and immune response in 94 subjects the study has clearly defined the clinical picture in BFV disease and provided testing strategies to improve clinical diagnosis.
Human rhinovirus C is frequently detected in adult hematopoietic stem cell transplant recipients with respiratory illness

Patricia E. Ferguson, Nicole M. Gilroy, Ian M. Mackay, Theo P. Sloots, Michael D. Nissen, Dominic E. Dwyer and Tania C. Sorrell

**Background:** A previously unidentified species of rhinovirus, HRV-C, was described in 2006 in association with lower respiratory tract infection (LRTI). Features of infection in immunosuppressed adults have not been characterised.

**Objectives:** To understand 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, NSW from July 1, 2005 to September 30, 2007. Nose/throat samples were collected from patients at the time of admission and those developing pre-defined symptoms and/or signs of respiratory infection during the admission. Samples were tested for rhinoviruses and fourteen other respiratory viruses using nucleic acid-based methods, immunofluorescence and culture. HRV genotyping was performed by sequencing a region of the rhinovirus 5’UTR. Clinical data on each episode were collected prospectively.

**Results:** Human rhinoviruses (HRV) were identified in 24 episodes: 8% of 299 episodes of clinically-defined respiratory infections and 39% of 61 in which respiratory viruses were detected. HRV-C was most frequent (HRV-C: 9, HRV-A: 8 and HRV-B: 2). 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 commonest rhinovirus group detected in HSCT recipients with respiratory infection; possible co-pathogens were frequent. Further research is required to understand the activity and pathogenicity of HRV-C in immunosuppressed patient groups.
The true global prevalence of human strongyloidiasis is not known because infection with *Strongyloides stercoralis* is a chronic subclinical infection and it is difficult to establish a laboratory diagnosis. Individuals remain infected for years after the original exposure because this parasite has the ability to reinfest the same host.

Seroepidemiology of *S. stercoralis* in two groups of people living in different socio-economic settings in Dhaka city were compared. Group A were the residents of a slum and group B were established city dwellers. Group A compared to group B were significantly different (p<0.001) in relation to IgG and IgG subclass antibodies against *Strongyloides* antigen. This showed serological evidence of strongyloidiasis in a high risk community in Dhaka city. A second high-risk group of squatters occupying a temporary slum area of Dhaka city were then selected to perform both serology and faecal examination. *Strongyloides* larvae in 147 stool specimens were found in 23% by Harada-mori culture. Total IgG, IgG1 and IgG4 antibodies to *Strongyloides* were found in 61.2%, 31.3% and 36.1% of participants, respectively. No correlations were found between positive serology and detection of *Strongyloides* infection in stool, socio-demographic factors or domestic hygienic practices. However, positive stool cultures showed significant associations with irregular nail trimming, walking bare-footed and irregular hand washing after defecation (p<0.05). Other enteric parasites detected in stools of some participants did not show any correlation with *S. stercoralis* infection or socio demographic factors.

Five different methods were used to extract *Strongyloides* DNA from larvae spiked into human stool and the target DNA was detected by a real-time PCR assay. The PowerSoil (Mo Bio Laboratories, Inc. Carlsbad, CA) kit was found to be the best DNA extraction method when used in conjunction with the real-time PCR assay. Subsequently, DNA was extracted from 160 human stool samples using this kit, and real-time PCR was performed. Specimens with high to moderate larval numbers were all positive by PCR. However, only 15% of samples with low larval numbers were PCR positive.

Primers targeting internal transcribed spacer regions 1 and 2 (ITS1 & ITS2) of *S. stercoralis* produced PCR products from one isolate collected from USA, 12 isolates from Bangladesh and one isolate from Australia. Sequence data from these products showed that five intra-species single nucleotide polymorphisms (SNPs) were in the ITS1 gene and 2 SNPs in 5.8S rRNA gene. No SNPs were found in ITS2 gene. These SNPs could be used as epidemiological markers to study the strain differences.

This study will helped to provide a better understanding of disease status in Bangladesh within different population groups and preliminary data arising from the ITS study forms the basis for molecular subtyping of *S. stercoralis*.
DETECTION OF *STRONGYLOIDES STERCORALIS* IN STOOL USING THE LOOP MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) METHOD.

Matthew Watts, Rogan Lee

Centre for Infectious Diseases and Microbiology: Public Health, Institute of Clinical Pathology and Medical Research, Westmead Hospital, NSW, Australia, 2145.

Western Clinical School, Westmead Hospital, University of Sydney, NSW Australia, 2145.

*Strongyloides stercoralis* is a parasitic nematode that can cause chronic infections. These range in severity from asymptomatic to fatal. *Strongyloides* is prevalent in northern Australia, particularly in indigenous communities. The most effective conventional methods for detection in stool require live larvae. Nucleic acid detection can be performed on non-viable larvae, enabling easier specimen transportation and removing the risk of occupational acquisition. The LAMP method is a cost-effective nucleic acid detection method.

*Aim:* To apply loop-mediated isothermal amplification (LAMP) of nucleic acid for the detection of *S. stercoralis* DNA in stool.

*Methods:* Primers specific to the strongyloides 28S rRNA gene were designed. The LAMP reaction was optimised using *Strongyloides ratti* from culture. Analytical sensitivity was determined using serial dilutions of a PCR product and stool specimens spiked with *S. ratti*. Specificity was tested using 30 normal stool controls and DNA from a range of bacteria, fungi and parasites. Clinical specimens containing *S. stercoralis* were tested.

*Results:* The assay demonstrated good analytical sensitivity and specificity. *S. stercoralis* and spiked *S. ratti* were detected in human stool specimens.

*Discussion:* LAMP assays can be performed using simple equipment and are suited to settings that are resource-limited. These results indicated that LAMP can be used for the diagnosis of *S. stercoralis* in stool. Further work will include comparison of the LAMP method with conventional and PCR tests, in addition to field validation studies.
CIDM-PH Executive Group, Staff, Students & Investigators

Organizational Chart

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Director

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Project Officer

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Peter Collignon
Lyn Gilbert
Deb Hyland
Louisa Jorn
Jeremy McAnulty
David Smith
Tania Sorrell

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Jon Iredell
Wieland Meyer
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Investigators
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Rogan Lee
Jimmy Ng

Staff
Menuk Jayawardena
Lou Orszulak
Rosie Sadsad
Fei Zhou

CIDM-PH Annual Report | 12/12/2012
### CIDM-PH New Grants Awarded in 2012

#### National Health and Medical Research Council (NHMRC) Grants

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# CIDM-PH Grants Current in 2012

## National Health and Medical Research Council (NHMRC) Grants

### Centre of Research Excellence

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### Project Grants

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<td>Sydney</td>
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</tbody>
</table>
### Australian Research Council (ARC) Linkage Grants

<table>
<thead>
<tr>
<th>Economic, social and cross cultural issues in non-pharmaceutical protection of front line responders to pandemic influenza and emerging infections</th>
<th>MacIntyre, <strong>Dwyer</strong> Nga, Ferguson McLaws, Maher Seale, Wood, Newall</th>
<th>Linkage Project Grant LP0990749</th>
<th>NHMRC</th>
<th>University of NSW</th>
<th>2009-2012</th>
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</thead>
<tbody>
<tr>
<td>Ross river virus – identification of virulence determinants in clinical isolates from across Australia</td>
<td>Mahalingam, Rulli, <strong>Russell</strong>, Herrng, Johansen, Hall, Smith, Lindsay, <strong>Gilbert</strong>, Smith</td>
<td>Linkage Project Grant LP0990827</td>
<td>NHMRC</td>
<td>Griffith University</td>
<td>2010-12</td>
</tr>
</tbody>
</table>

### Non – NHMRC/ARC Grants ACGR

| NSW Health Capacity Building Infrastructure Grant - Round 3 | Gilbert, Iredell, **Sorrell**, **Dwyer**, Sintchenko, Meyer, Russell | Infrastructure Grant | NSW Ministry of Health | Western Sydney Local Health District | 2010-13 |

### International Grants

<table>
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<tbody>
<tr>
<td>Supplemental vector intervention required to eliminate lymphatic filariasis in the South Pacific</td>
<td>Dobson, <strong>Russell</strong>, Ritchie, Mercer, Sinkins, Bossin</td>
<td>Project</td>
<td>Bill and Melinda Gates Foundation</td>
<td>University Kentucky, USA</td>
<td>2008-12</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, <strong>Meyer</strong></td>
<td>Project Grant</td>
<td>Colciencias, 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 Cryptococcus subtypes in North America</td>
<td>Keim, Engelthaler Lockhart, <strong>Meyer</strong>, 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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<td>---------------------------------------------------------------</td>
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<tr>
<td><strong>Australian Biosecurity Intelligence Network (ABIN) Project grant</strong></td>
<td>Sintchenko, Sorrell, Gilbert, Dwyer</td>
<td>Capacity building</td>
<td>CSIRO</td>
<td>SEIB (University of Sydney)</td>
<td>2011-2012</td>
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<tr>
<td><strong>Sydney Emerging Infections and Biosecurity Project</strong></td>
<td>Sorrell</td>
<td>Establishment</td>
<td>S Med School Foundation</td>
<td>SEIB (University of Sydney)</td>
<td>2011-15</td>
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<tr>
<td><strong>Rapid identification of “super bugs”</strong></td>
<td>Sorrell</td>
<td>Project/equipment</td>
<td>Raymond Purves Foundation</td>
<td>SEIB (University of Sydney)</td>
<td>2012</td>
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<tr>
<td><strong>Establishing an integrated database for Australian Microbiological Culture Collections</strong></td>
<td>Meyer</td>
<td>Project</td>
<td>Atlas of Living Australia</td>
<td>2010-2012</td>
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</tbody>
</table>
Dominic Dwyer


CIDM-PH Executive Group 2012 Publications

Lyn Gilbert


CIDM-PH Executive Group 2012 Publications

Jon Iredell


CIDM-PH Executive Group 2012 Publications

Wieland Meyer


Vitali Sintchenko


CIDM-PH Executive Group 2012 Publications

Tania Sorrell


CIDM-PH Executive Group 2012 Publications

Cameron Webb


CIDM-PH Education & Training in 2012

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

3-4 May 2012
Healthcare-associated Infection Prevention & Control; new approaches to old problems short course
The aim of the HAIPC short course was to showcase novel approaches to HAIPC, involving epidemiology, ethics, economics and surveillance, which are often neglected in conventional approaches to infection control.

Westmead Education & Conference Centre, Westmead Hospital

29 June 2012
Medical Entomology in Australia: Past, Present & Future Concerns
A one day symposium to honour the retirement of Professor Richard Russell. The program focused on arthropod pests of public health, how they affect the health of the nation, and what challenges and threats these creatures pose to the future.

Westmead Education & Conference Centre, Westmead Hospital

3 September - 26 October 2012
Molecular Diagnostics for Infectious Diseases
An international postgraduate training program including two modules, Molecular testing in diagnostic laboratory and Molecular fingerprinting of pathogens with epidemic potential.

Centre for Infectious Diseases & Microbiology, Westmead Hospital
Reports

- 2012 CIDM-PH Colloquium Report
- 2012 CIDM-PH Annual Report
- 2012 CIDM-PH CBIG Progress Report

Broad Street Pump Newsletters

The following Broad Street Pump newsletters are available on the CIDM-Public Health website
www.cidmpublichealth.org

February 2012 Issue

- SALMONELLA Associate Professor. Vitali Sintchenko
- Plasmids and antibiotic resistance in Salmonella Typhimurium Jason Patterson
- Salmonella Typhimurium genotyping for surveillance Dr. Shopna Bag
- Molecular serotype & phage type identification Dr Qinning Wang
- National Harmonization of MLVA typing for Salmonella Typhimurium Qinning Wang

April 2012 Issue

- Matrix-assisted laser desorption/ionization – time of flight (MALDI-TOF) mass spectrometry – bringing clinical microbiology into the new century? Professor Jon Iredell
- MALDI-TOF MS; on-going evolution of a revolution in clinical microbiology Jen KOK and Sharon C-A CHEN

June 2012 Issue

- Abstracts related to the Health-associated Infection Prevention and Control (HAIPC); new approaches to old problems: Short Course

August 2012

- Abstracts related to the Medical Entomology in Australia: past, present and future concerns Symposium