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The Broad Street Pump

Medical mycology: closing the gap in diagnostics and emerging fungal species?

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The incidence of invasive fungal disease (IFD) has increased in parallel with the expansion of the population at risk for IFD to include patients with cancer, solid organ transplant (SOT) and haematopoietic stem cell transplant (HSCT) recipients, those receiving immunosuppressive therapy, and patients with AIDS, major surgery, premature birth and advanced age. Additionally, the spectrum of pathogens has evolved. *Candida albicans* and *Aspergillus fumigatus* remain the most common yeast and mould pathogens, respectively, but infections caused by other non-*albicans* species, non-*Candida* yeasts and non-*Aspergillus* moulds are increasing in number. Early and accurate diagnosis, including species identification, is essential for optimal patient outcomes.

Traditional microbiological culture-based tests for fungi are slow and insensitive, but they are still important since they are inexpensive and provide isolates on which to perform antifungal susceptibility testing, which has become increasingly relevant due to changing patterns of resistance and species-specific differences. Nonetheless, rapid culture-independent, non-invasive molecular and novel serological tests are increasingly being adopted into routine use.¹



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Identification of fungal cultures

From cultured isolates, the routine application of matrix assisted laser desorption ionization – time of flight (MALDI-TOF) mass spectrometry (MS) has revolutionised the identification of yeasts. In less than 5 years, phenotypic identification methods for yeasts have been replaced by MALDI-TOF MS, allowing a single isolate to be identified in < 5 mins at a cost of < AUD \$0.5 compared to 24-48 h and AUD \$9. Supplementation of the manufacturer's reference database with spectra from well-characterised clinical strains from a laboratory improves the reliable identification of not only less frequently encountered organisms but also common yeast species. Unlike biochemical methods, MALDI-TOF MS is able to identify uncommon yeast species and differentiate between closely related species within a complex (e.g. *Candida parapsilosis*, *Candida metapsilosis* and *Candida orthopsilosis*). Its strength also lies in the fact that it does not misidentify an organism.²

The use of MALDI-TOF MS to identify moulds in culture is less straightforward but supplementation of the reference database with an 'in-house' library from clinical strains greatly enhances the accuracy of the technique, and most genera can be clearly separated with good species separation for many genera.

Molecular-based identification of moulds, particularly those that are slow growing or lack distinguishing morphological characteristics, is growing in popularity as expertise in morphological identification continues to decline. DNA sequencing and comparative sequence analysis of the whole internal transcribed spacer (ITS) region of the rDNA gene cluster (ITS1-5.8S-ITS2) is the gold standard for identification of fungi.³ Its use has both simplified and complicated identifications by revealing the existence of cryptic species within a morphologically described species. Importantly, the utility of the ITS region for identification of some fungi is insufficient for species level resolution and analysis of other genes is recommended. For example, two types of ITS sequences may be present in a single strain of *Fusarium* and analysis of the elongation factor alpha subunit (*EF-1a*) gene is recommended for species level identification.¹

Culture independent methods

We have been utilising culture independent tests to aid in the rapid diagnosis of IFD for > 12 years. For the early diagnosis of invasive aspergillosis (IA), when used as a screening test, the high negative predictive values of our 'in-house' *Aspergillus* PCR assay or the FDA-approved Platelia® *Aspergillus* EIA (Bio-Rad) to detect the galactomannan antigen, are utilised most effectively to exclude IA in high-risk patients.^{4,5} In our hands, our panfungal PCR assay followed by DNA sequencing is complimentary to culture, and is most valuable for identifying fungi in culture negative, histologically proven tissue biopsy specimens, where species identification helps guide antifungal therapy.⁶ In our experience,

the clinical utility of applying a broad-range PCR to non-sterile samples, particularly bronchoalveolar lavage fluid, is poor due to the amplification of commensal fungi, particularly *Candida* species.¹

A novel *Aspergillus* lateral flow assay (LFA) has recently become available, offering a 'point of care' test for the rapid diagnosis of IA, with results available within 15 minutes. A limited number of retrospective diagnostic accuracy studies in Europe have reported promising sensitivity (80%) and specificity (98%),⁷ however no large prospective studies have been performed. We are involved in a proposed multi-centre prospective study of the *Aspergillus* LFA for evaluation in Australian haematology populations in 2015-2016, to determine its' test performance in the local setting and position in the Australian diagnostic algorithms for IA.

Evolving epidemiology of yeast and mould infections

Since local epidemiological patterns of fungal infections are important to inform antifungal treatment and other management, a number of national initiatives are continuing to address patterns of infection. During 2004-2007, the Australian Candidaemia Study (performed under the auspices of the Australia and New Zealand Mycoses Interest Group) identified *C. albicans* in the majority of episodes (47%), with *C. glabrata* accounting for far fewer (16%) episodes. Many cases were acquired from outpatient health-care related encounters. Fluconazole-resistance amongst *C. albicans* was rare.⁸ A recent follow up candidaemia study (still ongoing) is being performed since the echinocandins are now used widely and may have impacted on the epidemiology. Preliminary findings suggest that there is a substantial shift to *C. glabrata* (see **Changing epidemiology of candidaemia in Australia: the Australian Candidaemia Study 10 years on**, in this issue) with potential implications for empirical antifungal therapy.

Another retrospective multicentre study of the incidence of non-*Aspergillus* mould infections in Australian tertiary hospitals from 2004-2012 reported 162 episodes of non-*Aspergillus* IFD due to 29 etiologic species complexes.⁹ Mucormycetes were the leading cause of IFD (45.7%), but unlike reports in the literature, *Scedosporium* spp. (33.3%) rather than *Fusarium* spp. (8%) were the second commonest non-*Aspergillus* pathogen reported in Australia. The range of 'at risk' populations identified was outside those traditionally deemed at high-risk for IFD (haematological malignancy, SOT recipients and diabetics). Emerging at risk populations included those with chronic lung disease, rheumatologic conditions or those sustaining trauma (21%). Of note, 15% of patients had no comorbidities or immunocompromise, highlighting the need to develop definitions of IFD in non-immunocompromised hosts.



Figure 1: Macroscopic appearance of *Microsphaeropsis arundinis* on sabouraud dextrose agar

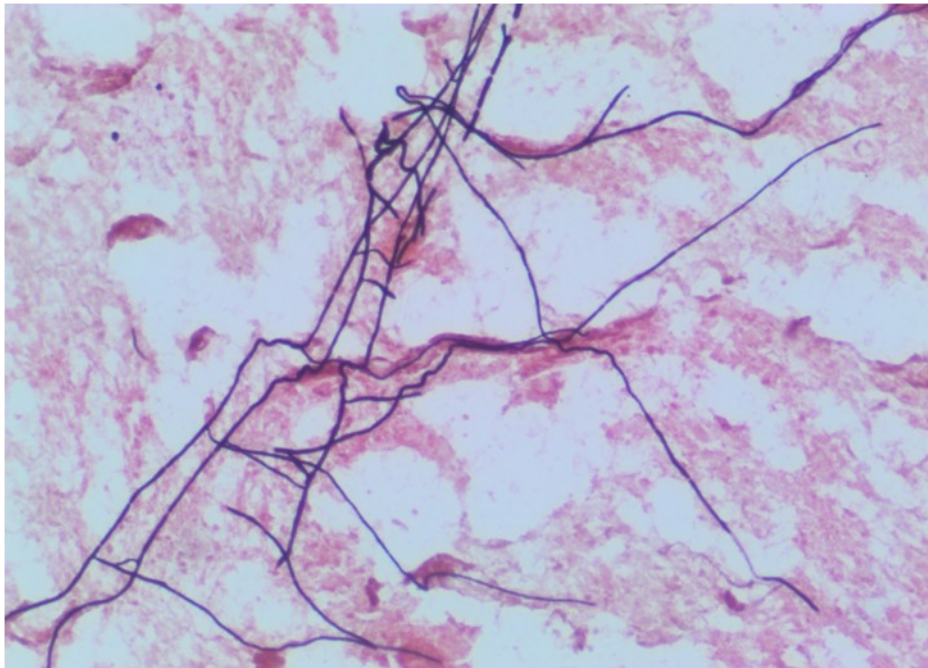


Figure 2: Growth of *Verruconis gallopava* in blood culture

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IFD due to dematiaceous fungi also appears to be emerging for example, infections caused by *Microspheeropsis arundinis* which is ubiquitous in soil and fresh water, and which has a well-known association with 'elephant grass' (*Arundo donax*). Indeed, the first human cases of *M. arundinis* were reported in 2004 involving two patients, from Sydney, with diabetes and chronic renal disease receiving long-term corticosteroids.¹⁰ In the same year, an infection due to *M. arundinis* was also reported in a cat from Sydney with a deep tissue infection.¹¹ Morphological identification of *M. arundinis* is problematic due to its poor sporulation, and has likely contributed to its' under reporting in the literature (Figure 1). Our laboratory sequenced the whole ITS region and D1/D2 region of the 28S rDNA gene cluster from these isolates and submitted them to GenBank. Since that time, we have identified at least a further four renal transplant recipients with skin and soft tissue infection and an additional 6 cats all with subcutaneous phaeohyphomycosis caused by *M. arundinis*.¹²

Verruconis gallopava (previously *Ochroconis gallopava*) is another emerging dematiaceous mould pathogen; unlike many other genera of "dark fungi", which tend to present as localised skin and subcutaneous infections, *V. gallopava* has a propensity to cause disseminated infection in at risk individuals. Most recently, the fungus was isolated from blood cultures of a patient (Figure 2) who subsequently was found to have a large (3.4 cm diameter) left ventricular mass, with skin lesions and dental tissue showing invasion by the fungus (Jennings *et al.*, unpublished). A number of patients in Australia with similar disseminated illness have been reported.⁹

Conclusion

Medical Mycology in Australia continues to be colourful in both the clinical spectrum of pathogens and in diagnostic and therapeutic challenges. Better molecular-based and other non-culture diagnostic approaches are the key to optimising patient outcomes.

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Changing epidemiology of candidaemia in Australia: the Australian Candidaemia Study 10 years on

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The Australian Candidaemia Study (ACS) was a prospective, population-based laboratory surveillance for candidaemia in Australia undertaken over a three year period between 2001 and 2004¹. A decade on, the Second Australian Candidaemia Study (ACSII) is underway. The ACSII is an active surveillance study that will also build on the findings of the original ACS with respect to outcome determinants for candidaemia. One of the major aims of the ACSII is to determine contemporary species distribution and antifungal susceptibilities for *Candida* in Australia, and compare these to the earlier study and current global trends for candidaemia.

Candidaemia in context

The yeast *Candida* is a leading cause of bloodstream infections, with high mortality, but patient outcomes are known to be substantially improved with prompt, appropriate antifungal therapy². In order to achieve this, local knowledge of *Candida* species distribution and antifungal resistance is crucial. *C. albicans* is the most common species causing candidaemia, and fluconazole has traditionally been the antifungal therapy of choice for treatment of candidaemia. Globally over the past decade however, increased use of fluconazole has led to the emergence of resistant strains of *C. albicans*². In addition there has been a shift in the epidemiology of *Candida* towards more non-*C. albicans* strains². In particular there is concern regarding increased isolation of *C. glabrata*, which is often resistant to fluconazole and other azole antifungals³.

In response to the growing incidence of azole resistance of *Candida* isolates, a new class of antifungals, the echinocandins, have been introduced into clinical practice⁴. Echinocandins inhibit the synthesis of 1,3-beta-D-glucan, which is a key molecule in the fungal cell wall. The echinocandin class of antifungals was introduced globally in 2001, with caspofungin⁴. At the time of the ACS the use of echinocandins in Australia was uncommon. A key aim of the present ACSII is to collect data to inform contemporary antifungal treatment in Australia.

Second Australian Candidaemia Study (ACSII) design

The ACSII is a one year prospective nationwide study comprised of a laboratory-based active surveillance component (Part A) and a matched case control component (Part B). The ACSII commenced in mid-2014 and involves around 30 public and private microbiol-

ogy laboratories in all Australian states and territories. Approval for the study has been obtained from the human ethics review committees of institutions providing clinical data. Adults, children and neonates with ≥ 1 blood culture yielding yeast are eligible for enrolment in Part A of the study, while Part B of the study is restricted to adults. Data collected for Part A of the study include sex, age, mortality at 7 and 30 days from positive blood culture, isolate genera and species, and isolate minimum inhibitory concentrations (MICs) for nine antifungals. The nine antifungals are amphotericin B, fluconazole, itraconazole, voriconazole, posaconazole, 5-flucytosine, and the echinocandins caspofungin, anidulafungin and micafungin. Isolate identification and antifungal susceptibility is confirmed by one of two reference laboratories. Data collected for Part B of the ACSII include healthcare setting, risk factors, concomitant conditions, portal of entry, clinical signs of sepsis, complications of candidaemia, results of diagnostic studies, and antifungal therapy. Interim analysis has been undertaken on data collected to date for Part A of the study. Some of the key findings to date with respect to *Candida* species distribution and antifungal susceptibility are presented here.

ACSII species distribution

To date, over less than a one year period the ACSII has recorded 256 episodes of candidaemia yielding 262 isolates. In comparison, over a three year period the ACS recorded 1,068 candidaemias yielding 1,094 isolates.

Of the 262 *Candida* isolates to date, identification has been confirmed by a reference lab for 151, in most cases using matrix assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS). Of these, major species isolated are *C. albicans* (40%), *C. glabrata* complex (31%) and *C. parapsilosis* complex (16%). Compared with the original ACS, isolation of *C. albicans* has decreased by 7% and *C. parapsilosis* by 4%, while *C. glabrata* complex has increased by 15%. Within the *C. glabrata* complex isolates, 96% have been identified as *C. glabrata* sensu stricto, and 4% as *C. bracariensis*. Within the *C. parapsilosis* complex, 67% have been identified as *C. parapsilosis* sensu stricto and 17% each as *C. metapsilosis* and *C. orthopsilosis*. This latter distribution is noticeably different from the distribution of *C. parapsilosis* complex isolates in the original ACS, where the majority of isolates were *C. parapsilosis* sensu stricto.

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Among the confirmed ACSII isolates minor species occurring are *C. tropicalis* (5%), *C. krusei* (3%) and *C. lusitanae* (2%), *C. lipolytica* has been isolated on two occasions, and *C. dubliniensis* and *C. quercitrusa* each on a single occasion. The distribution of isolation of minor species is similar between the present ACSII and the previous ACS.

ACSII antifungal susceptibility

In vitro susceptibility of ACSII isolates has been assessed at the reference laboratories using Sensititre YeastOne YO10. Susceptibility to fluconazole and voriconazole has been interpreted according to Clinical and Laboratory Standards Institute (CLSI) breakpoints. Of the 151 isolates confirmed to date, only two isolates (both *C. glabrata*) have been determined as resistant to fluconazole, and a single isolate (*C. tropicalis*) has been determined as resistant to voriconazole. With respect to the echinocandins, only a single isolate (*C. glabrata*) has been determined as resistant to caspofungin according to epidemiological cut-offs. No isolates to date have been determined as resistant to either anidulafungin or micafungin.

Conclusions to date

Ten years on from completion of the ACS, preliminary results from the ACSII suggest changes in the epidemiology of candidaemia in Australia. Of these, the most significant appears to be a proportional increase in the frequency of isolation of *C. glabrata* compared with *C. albicans*, which is in line with global trends. Thankfully, at present azole resistance across all *Candida* species appears uncommon, and resistance to echinocandins is likewise rare. Given preliminary findings of the ACSII to date, it is suggested that empirical echinocandin therapy is appropriate in high-risk candidaemic patients. Treatment with fluconazole

remains appropriate for non-*C. glabrata* candidaemia. As further data becomes available from both Part A and Part B of the ACSII, it will be interesting to analyse the effect of the changing epidemiology of candidaemia in Australia with reference to contemporary risk factors, complications and clinical outcomes.

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Growth inhibition of *Scedosporium* and *Lomentospora* spp. by *Pseudomonas aeruginosa*

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Important sites of bacterial-fungal interactions include the lung and oral cavity, typically in association with chronic infection¹. Lung disease in patients with cystic fibrosis (CF) is one such clinical setting. Chronic *Pseudomonas aeruginosa* infection in CF leads to more rapid decline in lung function and increased mortality²⁻⁵. Filamentous fungi are often isolated from CF airways with *Aspergillus fumigatus* the commonest pathogen (50-65%) followed by *Scedosporium* spp. (8.5-17.4%)⁵⁻⁸. However, associations between fungal colonisation and declining lung function have been contradictory^{7,9,10}. Using an agar plate assay we examined the effect of 25 *P. aeruginosa* isolates on growth of *Lomentospora prolificans* (formerly *Scedosporium prolificans*) and *Scedosporium aurantiacum*.

Methodology

The *P. aeruginosa* strains tested were PA14, a non-mucoid wild-type laboratory "reference" strain (a gift from Dr. Liisa Kautto, Macquarie University), 15 phenotypically non-mucoid, and nine mucoid, clinical *P. aeruginosa* isolates. Two fungal isolates were studied: *L. prolificans* strain WM.14.140 and *S. aurantiacum* WM.11.78, both from the sputum of CF patients.

L. prolificans or *S. aurantiacum* suspensions ($\sim 10^5$ CFU/ml) were spread on to a Sabouraud Dextrose Agar (SDA) plate and sterile filter paper disks (Whatman no. 1), 15-mm in diameter, were placed in the center. Fifty microliters of *P. aeruginosa* suspension ($\sim 10^8$ CFU/ml) were impregnated onto the disks, the plates incubated at 35°C and inspected at 48h for zones of inhibition of fungal growth. The ratio of the diameter of the disk to the total diameter of disk plus inhibition zone (Iz) was measured as an index of inhibitory activity, i.e. an Iz value of 1.0 indicated no inhibitory activity. Experiments were performed thrice and the median inhibitory activity (mlz) values from these replicates calculated. Experiments were also performed using killed pseudomonal cells (by exposure to 100% methanol for 2h).

Results

Seventeen of the 25 (68%) *P. aeruginosa* isolates inhibited the growth of both fungi. Zones were clearly defined at 48 h (Figure 1a) with diameters varying with pseudomonal isolate. Killed *Pseudomonas* (Figure 1b) produced no inhibition zones. Among the *P. aeruginosa* strains tested, 14/16 non-mucoid strains (87.5%) had a mlz of <1.0 against both fungal species, versus 3/9 mucoid strains (33.4%) (P=0.01). Overall there was a significant difference in distribution of mlzs by *P. aeruginosa* phenotype (P<0.001). For *S. aurantiacum*, the median mlz value was 0.65 for non-mucoid strains vs. a median mlz of 1.0 for mucoid strains (P=0.015). For *L. prolificans*, the corresponding median mlz values were 0.66 and 1.0, respectively (P=0.020).

Conclusions

This simple robust assay was reproducible and semi-quantified differences between the effects of mucoid and non-mucoid *P. aeruginosa* on fungal growth. Previously the assay had only been used to qualitatively document organism-organism inhibition^{11,12}.

That non-mucoid strains inhibited growth to a significantly greater degree than their mucoid counterparts (P≤0.028 for both) is notable. The clinical relevance of this finding is uncertain. Most CF patients are colonized with non-mucoid strains initially, which are replaced by the mucoid variant over years. We hypothesize that colonization by non-mucoid strains precedes fungal colonization and protects against the latter by inhibiting fungal growth. The appearance of filamentous fungi may be a marker of mucoid conversion with clinical deterioration⁴. The study of mechanisms underlying this inhibition is essential to the understanding of the pathobiology of airway infections in CF.

Growth inhibition of *Scedosporium* and *Lomentospora* spp. by *Pseudomonas aeruginosa*

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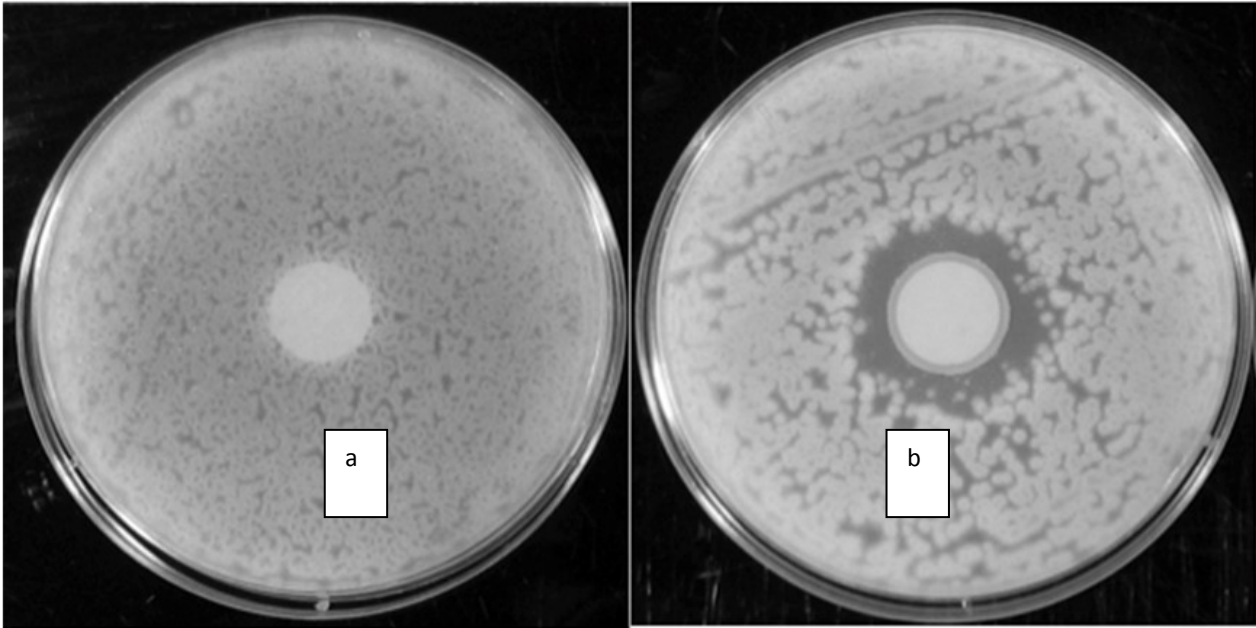


Figure 1 Effect of a suspension of killed cells of *Pseudomonas aeruginosa* strain CIDMLS-PA-28 on the growth of *Scedosporium aurantiacum* WM.11.78 on Sabouraud dextrose agar (a) in contrast to the inhibitory effect of viable *P. aeruginosa* cells of the same strain on *Scedosporium* growth as demonstrated by a zone of inhibition (b).

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Facing the challenge of growing numbers of fungal infections – sequence based fungal identification using the ISHAM-ITS reference database

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The number of human and animal fungal infections is continuously increasing worldwide. Of an estimated 2 million fungal species, ~600 cause human and animal disease affecting billions of people annually. While most fungal infections are relatively minor, millions contract fungal disease that kill at least as many people as tuberculosis or malaria. The population at risk for life-threatening invasive fungal infections (mortality 40%-100%) is growing due to modern medical interventions, increasing number of immunocompromised patients and use of immunosuppressive therapies. Invasive fungal infections account for ~10% of all hospital-acquired infections with attendant mortalities of 50 to up to 100%, despite modern therapeutic approaches¹. The impact of systemic mycoses is often misjudged. Conservative estimates show that they account for 1-1.5 million human deaths annually world-wide¹, with the top 10 infections being: aspergillosis, candidiasis, cryptococcosis, mucormycosis, *Pneumocystis pneumonia*, blastomycosis, coccidiomycosis, histoplasmosis, paracoccidiomycosis and penicillioses. Mycoses are common opportunistic infections in HIV/AIDS patients, causing an estimated 10 million oesophageal candidiasis and 1 million cryptococcal meningitis cases, with 600,000 deaths annually (20-50% mortality)². Fungal infections can lead to serious public health burdens and increased risk of biodiversity loss among animal species, e.g. *Geomyces destructans* in bats, *Batrachochytrium dendrobatidis* in frogs and *Aspergillus sydowii* in coral reefs³. In addition, animals can represent sentinel species for environmental fungal pathogens, which include the more important human agents and have an important role in monitoring the risk of environmentally acquired human infections⁴. Increasing human travel and climate change trends have also led to the appearance of novel fungal threats to human, animal, and ecosystem health, such as emergence of clusters of *Cryptococcus gattii* and *Fusarium* infections in humans³.

The resulting economic burden is extreme, especially from invasive fungal infections, chronic lung infections, allergic fungal diseases, mucosal, skin, hair and nail infections, which require long term hospitalisation and/or costly antifungal therapy over months, years or even lifelong. US based estimates indicate a cost of 2.6 billion US\$/year, growing 2-3% annually, with late diagnosis contributing substantially to these costs. Treatment costs in a single Australian Haematology centre alone are ~1.1 million AU\$/year.

Since current antifungal agents have limited efficacy and increasing drug resistance is encountered, correct and targeted antifungal therapy is of paramount importance. A key independent predictor of mortality and adverse disease outcome is the delayed initiation of effective antifungal therapy. Thus early accurate identification of the agents of mycoses is essential to optimise the chance of cure. As a consequence, there is a growing need for the rapid and

accurate identification of mycoses agents to enable early diagnosis and targeted antifungal therapy.

Conventional methods of fungal identification rely on subjective micro-, and macroscopic morphological and biochemical characteristics of fungi^{5,6}, which are notoriously time consuming (1-4 weeks), often challenging because of the complex and overlapping traits for identification and inaccurate. Serological methods are overall of little value⁷. Main limitations of these approaches are: **(a)** slow fungal growth rates and delayed production or lack of characteristic fruiting bodies/spores, necessary for their identification; **(b)** lack of clear-cut discriminatory tests since many macro-/micro-morphological characteristics overlap at the genus or species level⁶; this has become increasingly problematic with advances in molecular biology and the constant refinement of fungal taxonomy, resulting in the description of many new, clinically relevant taxa; **(c)** fungal elements in smears or tissue sections may be non-viable and not produce identifiable morphological characters; **(d)** most significantly, morphological-based diagnostic skills in the work force have diminished with subsequent lack of experience to provide correct identification.

Consequently, the mycology diagnostic laboratory is currently in transition between the use of conventional methods and emerging molecular identification methods. Molecular methods based on an accurate phylogenetic reference system offer big advantages over phenotypic methods since they rely on stable genotypic characteristics and do not require prior culture, isolation or specialised operator interpretation. They are rapid and can be directly applied to clinical specimens. Many molecular techniques have been utilised for reliable identification of pathogenic fungi⁸: PCR-RFLP analysis^{9,10}, RAPD¹¹, species-specific PCR¹², PCR-fingerprinting^{10,13} karyotyping¹⁴, hybridisation with genus/species-specific DNA or RNA probes¹⁵, real time PCR¹⁶, reverse line blots¹⁷, rolling circle amplification¹⁸, *in situ* hybridization¹⁹ and increasingly, DNA sequencing²⁰⁻²². New non-DNA based technologies are also entering the diagnostic laboratory, most notably Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry (MALDI-TOF MS)²³⁻²⁵. While accurate for common yeast species, its application for filamentous fungal identification is still problematic. Another drawback of this technology is that it can only be applied to pure cultures and identification is dependent on the availability of a reference spectral library of well-validated strains. Notably, the application of many of these techniques for the identification of fungi is still in its infancy – many remain in-house assays, which either have not been validated or standardised for general use, or are too expensive. Consistently reliable and universally applicable standardised molecular methods for the identification of medically important fungi are yet to be established.

Facing the challenge of growing numbers of fungal infections – sequence based fungal identification using the ISHAM-ITS reference database *(continued from page 9)*

Molecular approaches are increasingly used, with DNA sequencing of short DNA sequences - DNA barcodes - becoming the gold standard, offering a practical and simplified approach for species identification, which is less demanding in terms of taxonomical expertise. DNA barcoding consists of using short sequences (500-800 bp) for the identification of organisms at species level via comparison to a reference sequence collection of well-identified species. The concept of barcoding is that species identification must be accurate, fast, cost-effective, culture independent, universally accessible and feasible for non-experts. This approach employs sequence diversity in short standardised gene regions for species identification of all living organisms. Initially, the cytochrome *c* oxidase I (*COXI*) gene formed the primary barcode for members of the animal kingdom²⁶. Barcode sequences have since been used to 'sort' cryptic organisms to inform taxonomic revisions²⁷, and to identify plant insect pests for quarantine purposes²⁸. However, *COXI* proved ineffective for most fungi²⁹.

The current official fungal DNA barcode, the nuclear ribosomal Internal Transcribed Spacer region (ITS1/2), was selected, due to its widespread use and ease of amplification, as universal fungal DNA barcode in 2012, using state of the art Sanger sequencing³⁰, over other loci that showed better phylogenetic/taxonomic performance in a genome wide survey³¹. It is routinely used by the medical community for fungal identification at the species level on the basis of matching sequences in publicly accessible databases, such as GenBank. However, its wide-spread applicability is still limited by the absence of quality-controlled reference sequence databases. According to a recent study, 10% of the publicly available fungal ITS sequences were identified incorrectly at species level. Many of the ITS sequences deposited in public databases are incomplete or wrongly annotated prompting an increasing demand for a quality-controlled database.

An international consortium of medical mycology laboratories was formed in order to establish such a quality controlled ITS database under the umbrella of the ISHAM (International Society for Human and Animal Mycology) working group on "DNA barcoding of human and animal pathogenic fungi". The new database provides the medical community with a freely accessible tool via <http://www.isham.org/> or directly at <http://its.mycologylab.org/> to rapidly and reliably identify most mycoses agents (Figure 1). The new database currently contains 3200 complete ITS sequences representing 524 fungal species. It contains 226 species represented by one strain, 116 species by two strains, and 182 species by a minimum of three to a maximum of 115 sequences. The lengths of complete ITS sequences in the ISHAM-ITS reference database vary between 285 and 791 bp with an average of 500 bp. The generated sequences were used

to evaluate the variation and overall utility of the ITS region for the identification of pathogenic fungi at intra-and interspecies level. The average intraspecies variation of the ITS sequences currently included in the database ranges from 0 to 2.25%, with 170 species having a variation of less than 1.5% (Figure 2) making the ITS sequencing a useful genetic marker enabling accurate identification of ~75% of all fungal pathogens to the species level. At interspecies level, 13 taxa presented a clear barcoding gap (K2P distance). However, in four taxa, it was not possible to define a clear barcoding gap making their accurate identification based on ITS region unreliable (Figure 3). Critically, the remaining 25% include fungi responsible for serious fungal infections, such as emerging yeast species (e.g. *Clavispora*, *Kodamaea*) and filamentous fungi including the dermatophytes (e.g. *Aspergillus*, *Fusarium*, *Rhizopus*, *Mucor*) for which additional molecular methods are required for their reliable identification.

To read more about the ISHAM-ITS reference database see Irinyi *et al.* Medical Mycology 2015 (doi:10.1093/mmy/myv008).

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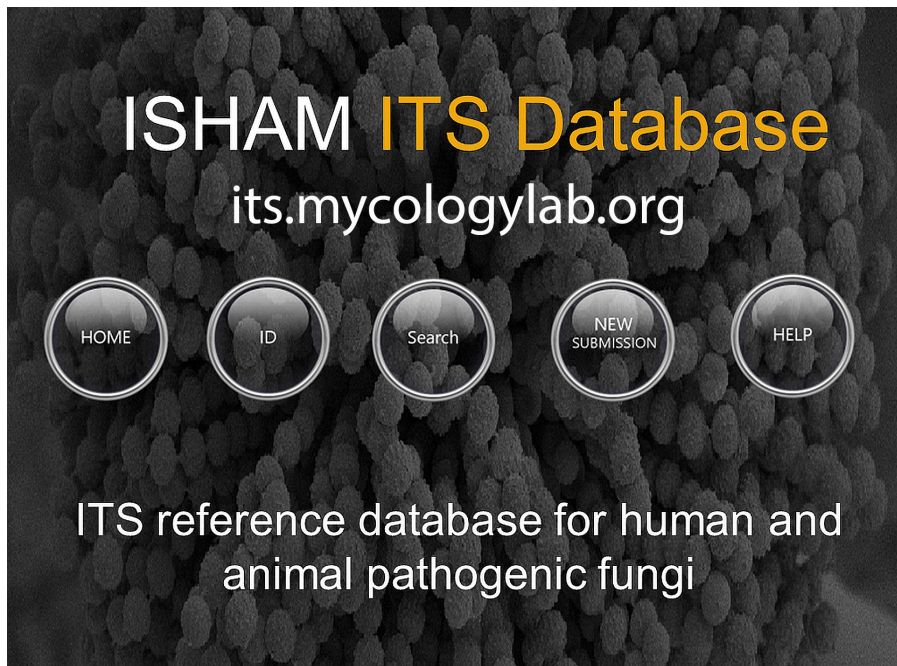


Figure 1. The ISHAM-ITS reference database for human and animal pathogenic fungi.



Figure 2. Average nucleotide diversity per species expressed as a percentage based on the value of π of the 176 clinically important fungal species. The error bars indicate the standard deviation of nucleotide differences.

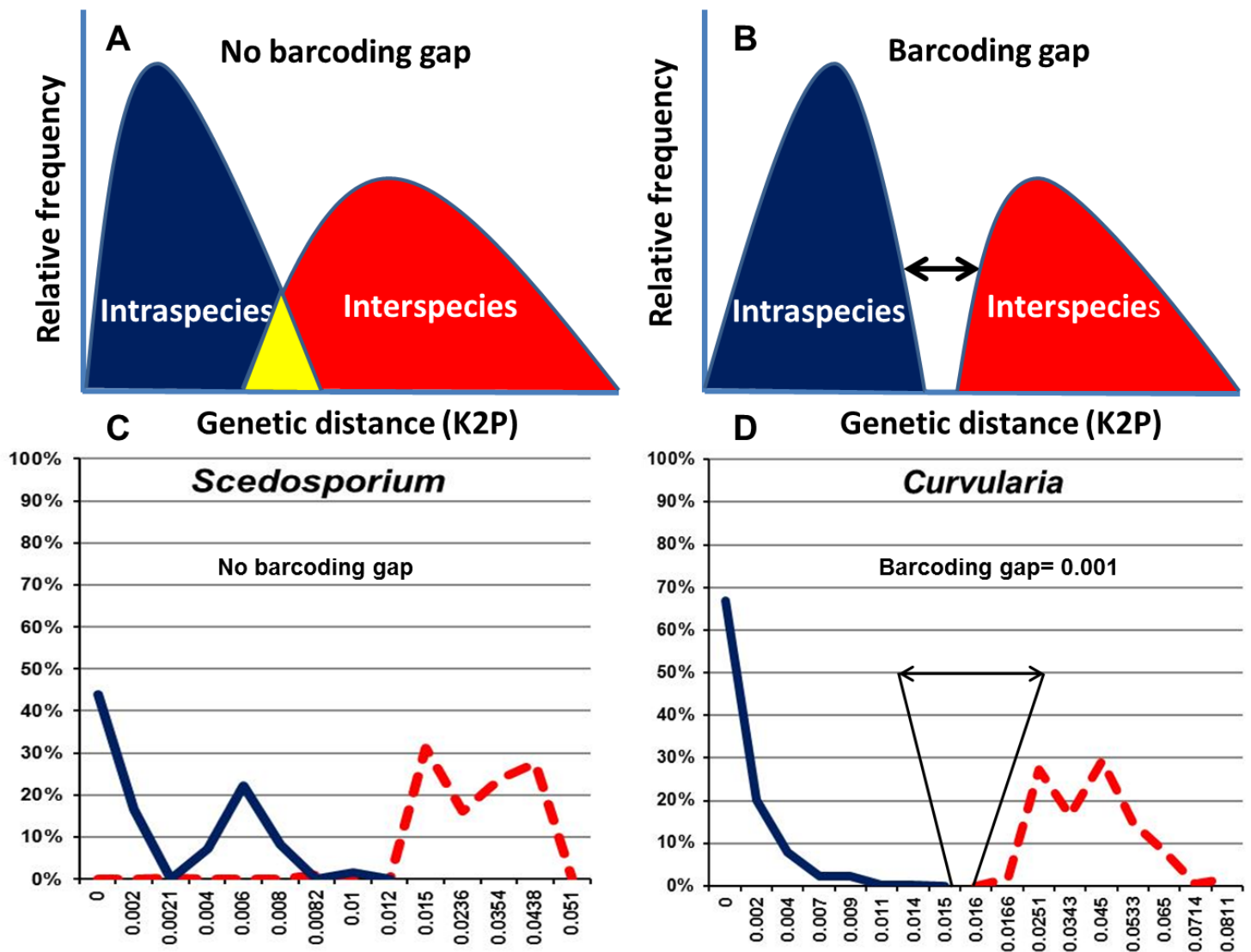


Figure 3. **A)** The distribution of intraspecific Kimura 2-parameter genetic distances shown in blue and interspecific in red overlaps (yellow). **B)** The distribution of intraspecific Kimura 2-parameter genetic distances in blue and interspecific in red creates a barcoding gap. **C)** Distribution of interspecies (red broken line) and intraspecies (blue solid line) pairwise Kimura 2-parameter genetic distances in *Scedosporium* including *S. angustum*; *S. apiospermum*; *S. aurantiacum*; *S. boydii*; *S. dehoogii*; *S. ellipsoideum*; *S. minutisporum*. **D)** Distribution of interspecies (broken line) and intraspecies (solid line) pairwise Kimura 2-parameter genetic distances in *Curvularia* including *C. aerea*; *C. borrieriae*; *C. inaequalis*; *C. geniculata*; *C. hawaiiensis* *C. inaequalis*; *C. lunata*; *C. protuberata*; *C. spicifera*; *C. sorghina*, *C. verruculosa*.

Facing the challenge of growing numbers of fungal infections – sequence based fungal identification using the ISHAM-ITS reference database *(continued from page 10)*

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Clinical and Translational Mycology on the southern shores – perspective and an unrecognised need?

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The hosting of the 19th International Society of Human and Animal Mycology (ISHAM) Congress, the premier international forum for medical, veterinary and basic science mycology, in Melbourne, Australia in 2015, has prompted the opportunity to journey through the beginnings and rationale of coordinated and systematic study clinical and applied mycology in Australia. The Australia and New Zealand Mycoses Interest Group (ANZMIG) is a special interest group of the Australasian Society for Infectious Diseases (ASID). This year, it has had the honour of the scientific and logistical organisation, of the ISHAM Congress, which includes symposia sessions co-badged with the Mycoses Study Group (MSG), USA and the International Immunocompromised Host Society. Australian speakers make a strong contribution in four key areas within the Clinical, Translational, Basic Science and One Health streams of the program.

BEGINNINGS OF CLINICAL MYCOLOGY

If one took the perspective that the position of virological research was within the book of *Revelations* in the common Bible, bacteriological research within book of *Acts*, then it would be reasonable to position mycology still attempting to “*Exodus*” across the Red Sea (authors: pers. comm.). Even though fungal infections contribute substantial burden to communities and indeed overall, cause similar number of deaths as tuberculosis and malaria, they often do not get the attention or support required to address their impact on human and animal health.

To address the gap in clinical mycology at least, the first iteration of ANZMIG (the Mycoses Interest Group) meeting was held in 1997, inspired by the energy of Emeritus Professor David Ellis, then, Head of Mycology at the Women’s and Children’s Hospital Adelaide and Ms. Jennifer Antonino, Area Manager (Australia and New Zealand) of Nexstar Pharmaceuticals who provided logistical and financial support. Starting with 8 members, interest in MIG steadily increased and its name changed to ANZMIG to reflect the contributions of New Zealand colleagues.

Today, ANZMIG scientific and business meetings are held every quarter and membership has expanded to represent hospital and research scientists and veterinarians. The focus is largely on clinical, epidemiological and translational aspects of mycology, with more recent aspects on antifungal stewardship including “how to report susceptibilities to antifungal agents” and guidelines for the use of antifungal drugs. Meetings are supported in part by each of Gilead Sciences, Pfizer and MSD Australia, with Astellas

also becoming a sponsor in 2015. Registrars in training are encouraged to attend the scientific sessions, and three are selected and supported to present cases or discuss their work. As a bonus, their presentation/work may be accredited by the Infectious Diseases Specialist Advisory Committee, Royal Australasian College of Physicians (RACP) as a project. ANZMIG activities are headed by a chair, immediate past chair, scientific chair and secretary.

OBJECTIVES

ANZMIG has two main objectives. It is a professional body to facilitate conduct of national mycology surveillance and clinical trials, including antifungal drug trials and antifungal guideline development. Its other primary goal is to promote and improve education in all aspects of mycology in Australia, the Asia Pacific region and elsewhere. Laboratory capacity building and training of scientists is likewise actively promoted.

EDUCATION

ANZMIG has conducted regular education programs, firstly as wet laboratory workshops at the Women’s and Children’s Hospital, Adelaide, Westmead Hospital and Concord Hospital, in Sydney.

The biennial Mycology Masterclasses, which began in 2007, are a centre piece for mycology teaching in Australia. A mix of basic, translational and clinical mycology takes place in a convivial atmosphere conducive to debate and discussion. These classes appeal to Infectious Diseases and Microbiology trainees, laboratory scientists from all over Australia and Asia and include participation from haematology, intensive care and organ transplantation specialists, which allow for cross-fertilization of ideas and the potential for collaborative projects. They are coordinated by the current Secretary of ANZMIG, Dr. Sarah Kidd, Head, National Mycology Reference Centre, SA Pathology. Mycology Masterclass members are also faculty for the Asia Pacific Mycology Masterclasses (supported by Merck) since 2012, and its members also act as faculty for the International Gilead CARE program on improving education and management of fungal infections

ANZMIG has organised a popular session at the annual scientific meeting of ASID for 15 years and awards an annual prize for the best mycology abstract at that meeting. Its members actively contribute to Australian Society for Microbiology and Australian Society for Antimicrobials.

Clinical and Translational Mycology on the southern shores – perspective and an unrecognised need? (continued from page 14)

MYCOLOGY SURVEILLANCE

A major legacy of ANZMIG has been the network established for national studies which have defined the epidemiology of serious fungal infections in Australia. This began with The Australian Candidemia Study in 2000 to which almost all microbiology laboratories in Australia contributed, and which resulted in publications describing the general epidemiology¹, as well as that in special groups such as paediatrics², cancer³, solid organ transplantation⁴, intensive care^{5,6} and uncommon species⁷; essential data for developing antifungal guidelines for invasive candidiasis. A rich repository of well-characterised bloodstream isolates was established, curated by Westmead Hospital and SA Pathology and available for future research. Recently the new CLSI breakpoints have been examined against this data set⁸. A follow-up to this study is underway now (see **Changing epidemiology of candidaemia in Australia: the Australian Candidaemia Study 10 years on**, in this issue) and we will compare epidemiology, susceptibility and outcomes to the earlier data.

An important local fungus *Scedosporium* spp., was chosen for the second epidemiological study and application of molecular testing to isolates allowed identification and clinical characterisation of *Scedosporium aurantiacum*, a relatively new species for which there was little clinical information including that of treatment⁹⁻¹¹.

Moulds other than *Aspergillus* were the focus of a more recent study (ongoing), showing that mucormycetes were the most common, closely followed by *Scedosporium* species and identifying the need for more rapid diagnostic tests and definitions targeted to patients without classical immunocompromised¹² and allowing a large body of data regarding susceptibility of these agents to antifungal drugs.

Cryptococcus gattii epidemiology, another fungus dear to Australia was also described¹³. Important prognostic factors were identified for the first time and treatment responses characterised¹⁴.

Although not ANZMIG studies, two successful NHMRC grants entailing several million dollars evolved from the ANZMIG collaborations: one a randomised trial of *Aspergillus* PCR and galactomannan as early diagnosis for aspergillosis in high risk haematology patients compared to standard diagnostic methods¹⁵. This study showed the safety of this approach (CIA Monica Slavin). A second grant related to risk prediction for candidemia in the ICU is ongoing (CIA Tania Sorrell). Notwithstanding these grants, clinical and applied mycology suffers the same underfunding and low profile described internationally¹⁶ and ANZMIG is working to improve NHMRC grant outcomes.

ANTIFUNGAL GUIDELINE DEVELOPMENT

Useful though the US and European antifungal guidelines are to assist with use of antifungal drugs, Australian and New Zealand Consensus guidelines for antifungal use in the haematology/oncology setting are at least equally highly regarded. These guidelines were first published in 2004 as a stand-alone paper¹⁷ and updated in 2008 as a supplement consisting of six separate articles¹⁸⁻²³. When recently compared to other international antifungal treatment guidelines, the 2008 guidelines ranked the highest overall when the Appraisal of Guidelines Research and Evaluation (AGREE) criteria for assessing the quality and methodological rigour of guidelines was applied²⁴. A recent survey of antifungal drug prescribers also highlighted the clinical relevance and applicability of the previous guidelines²⁵. These guidelines were updated most recently in 2014 and linked to Therapeutic Guidelines Australia, the standard national hospital-wide antimicrobial prescribing guide. The 2014 guidelines are comprehensive, incorporating 9 sections and including recommendations for paediatrics, *Pneumocystis jirovecii*, *Cryptococcus gattii* and a survey of diagnostic and prophylaxis practices²⁵⁻³³. Australian mycologists also contribute to other guidelines of the International Society for Host and Lung transplantation.

CONCLUSION

After a slow start, ANZMIG has expanded and become a collaborative group promoting education very successfully, and taken small steps in research in Mycology within Australia and our region. Attendance at the scientific sessions is open to all and is encouraged. Over the years, ANZMIG has had the honour of visiting clinicians, hospital scientists and academics attending, always imparting valuable advice to both the scientific content and business end of the special interest group. Yet the gaps in research vis-à-vis that in virology, bacteriology and parasitology are obvious with ever continuing playing of catch up in an increasingly harsh environment. A continued goal is to increase the profile of mycology and improve grant funding success in this relatively neglected area.

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Laboratory Profile: The Clinical Mycology Reference Laboratory

The Clinical Mycology Reference Laboratory is part of the Centre for Infectious Diseases and Microbiology Laboratory Services and Pathology West – ICPMR, based at Westmead Hospital.

The Clinical Mycology Reference Laboratory provides fungal pathogen detection and identification services across the Pathology West network of hospitals. The Laboratory specialises in both culture-based and culture-independent methods, working directly with clinical specimens. The Laboratory also operates as a reference laboratory, identifying referred isolates using conventional and sequence-based methods. Each year, the Laboratory undertakes > 3,000 conventional fungal isolations and ~ 900 conventional identifications. In addition, the Laboratory performs ~ 1,500 *Aspergillus* PCRs, > 500 panfungal PCRs and ~500 antigen tests each year. The Laboratory also specialises in antifungal susceptibility testing, performing around 150 tests annually. As required, the Clinical Mycology Reference Laboratory works closely with the Molecular Mycology Research Laboratory of Professor Wieland Myer, which is co-located within the Westmead precinct, and provides complementary capability in molecular typing of strains. An example of the collaboration between the two laboratories is the investigation of a nosocomial *Pneumocystis jirovecii* pneumonia (PJP) outbreak among kidney transplant patients in 2011.

In addition to providing diagnostic laboratory services, the Clinical Mycology Reference Laboratory is an important participant in a number of research and development efforts involving medically important fungi; present examples are the Second Australian Candidaemia study, and a prospective trial of the *Aspergillus* lateral flow assay. In these, clinical need is the key driver, and Laboratory staff work closely with medical staff specialists.

The Clinical Mycology Reference Laboratory is headed by Dr Catriona Halliday (see **Staff Profile**, page 18) and staffed by hospital scientists Vishal Ahuja, Joanne Nitschke, and Angie Winata.

Staff Profile

Name: Sharon Chen

Position: Director, Centre for Infectious Diseases and Microbiology Laboratory Services, Pathology West – ICPMR, Westmead Hospital

Email: sharon.chen@health.nsw.gov.au

Associate Professor Sharon Chen is a Medical Microbiologist and Infectious Diseases Physician at the Centre for Infectious Diseases and Microbiology (CIDM), Westmead Hospital, with research interests in medical mycology, new diagnostic tests in microbiology and laboratory automation. She is currently the Director of CIDM-Laboratory Services at the ICPMR, Pathology West, within which the **Clinical Mycology Reference Laboratory** operates.

Sharon is past chair and scientific chair of the Australia and New Zealand Mycoses Interest Group (ANZMIG) of the Australasian Society for Infectious Diseases (ASID), and is the principal site investigator at Westmead Hospital on numerous international antifungal trials. Sharon actively contributes to the development of Australian guidelines for the use of antifungal agents.





Staff Profiles

Name: Catriona Halliday

Position: Senior Hospital Scientist, Clinical Mycology Reference Laboratory, Centre for Infectious Diseases and Microbiology Laboratory Services, Pathology West – ICPMR, Westmead Hospital

Email: catriona.halliday@health.nsw.gov.au

Dr Catriona Halliday is the senior scientist in charge of the **Clinical Mycology Reference Laboratory** at CIDMLS. Catriona was awarded her PhD in the area of molecular mycology from The University of Sydney in 2001 and began working at CIDMLS that same year. At the time that Catriona joined CIDMLS, the Laboratory was a single person affair. Over the past fourteen years, and under the mentorship of Professor Tania Sorrell and Associate Professor Sharon Chen, Catriona has made significant contributions to the development of the Clinical Mycology Reference Laboratory as a unique facility.

Among Catriona's key achievements, over the past twelve years she has developed and implemented into routine use numerous culture independent methods to aid in the rapid diagnosis of invasive fungal disease (IFD). At the time Catriona joined the Laboratory, no culture independent tests were in use. Today, more than half of all identifications undertaken by the Laboratory are performed using culture-independent methods. These include PCR assays for *Aspergillus* spp and *Pneumocystis jirovecii*, as well as a panfungal assay. In addition, Catriona has led the evaluation and implementation of the *Aspergillus* galactomannan ELISA and Cryptococcal antigen lateral flow assay. The implementation of these methods into routine use has dramatically improved speed of diagnosis and targeting of anti-fungal therapy, resulting in better patient outcomes and reduced treatment costs.

Catriona has presented numerous posters, proffered and invited papers at both national and international conferences and workshops detailing her development of culture-independent methods. On a practical basis, Catriona is also involved in training medical registrars, undergraduate and post graduate students in practical and theoretical medical mycology. In the future Catriona anticipates expanding the culture independent tests offered by the laboratory to include the detection of resistance genes in *Candida* and *Aspergillus* species and is hopeful that with standardisation molecular assays will be incorporated into the diagnostic criteria for IFD.



Name: Wieland Meyer

Position: Chief Scientist, Molecular Mycology Research Laboratory, Centre for Infectious Diseases and Microbiology, Westmead Hospital

Email: wieland.meyer@sydney.edu.au

Prof. Dr. rer. nat. Wieland Meyer is the Head of the Molecular Mycology Research Laboratory at CIDM, Professor for Molecular Medical Mycology at Sydney Medical School – Westmead Hospital, The University of Sydney, Westmead Millennium Institute, Sydney, Australia and Guest Professor at Fundação Oswaldo Cruz (FIOCRUZ) in Rio de Janeiro, Brazil, and curator of the Westmead Hospital Medical Mycology Culture Collection housing more than 10,000 fungal strains.

His research focuses on the evolution, phylogeny, speciation, population genetics and molecular epidemiology of human and animal pathogenic fungi (especially of the genera *Candida*, *Cryptococcus*, *Scedosporium* and *Pneumocystis*), the development of fast, simple and reliable molecular identification techniques for human/animal fungal pathogens, and the understanding of fungal pathogenesis on a molecular level. He is investigating the global epidemiology of the *Cryptococcus neoformans* /*C. gattii* species complex, several *Scedosporium* species associated with Cystic Fibrosis and *Pneumocystis jirovecii* in renal transplant patients, establishing Multilocus Sequencing Typing (MLST) schemes, accessible at mlst.mycologylab.org.

He is also undertaking global whole genome based population analyses of agents of cryptococcosis in collaboration with TGen, Phoenix, US, and leading an international consortium of mycology reference laboratories, which established the first quality controlled ITS database (its.mycologylab.org) and identified new alternative genetic loci for fungal DNA barcoding.

He is the Vice-President and future General Secretary of International Society of Human and Animal Mycology (ISHAM) and a member of the executive committee of International Mycological Association (IMA).

Events....

CIDM-PH Seminar Series

Date: Wednesday, 3rd June 2015

Time: 3.00pm - 4.00pm

Location: Westmead Millenium Institute, Hawkesbury Road, Westmead, Sydney

Speaker: Dr John Besser, Deputy Chief of the Enteric Diseases Laboratory Branch (EDLB), CDC

Topic: Foodborne and Zoonotic Disease Surveillance: Genomics, Metagenomics, and the Road Ahead

Enquiries: WSLHD-CIDM-PH@health.nsw.gov.au

Medical Entomology Symposium

Date: Friday, 11th September 2015

Time: 8.30am - 5.00pm

Location: Lecture Theatre 3, Westmead Education & Conference Centre, Westmead Hospital, Sydney

Program: Coming soon

MBI Colloquium

Date: Friday, 6th November 2015

Time: 8.30am - 4.30pm

Location: New Law Lecture Theatre 101 - Main Campus, University of Sydney

Program: Coming soon

In the media.....

The Conversation: Dr Cameron Webb "Is climate change to blame for outbreaks of mosquito-borne disease?" explains the role of climate change (as well as other factors) in the recent emergence of mosquito-borne disease risk in Australia, particularly our current outbreak of Ross River virus disease in QLD and NSW that looks likely to be our worst outbreak over 20 years.

The link to the article is: <https://theconversation.com/is-climate-change-to-blame-for-outbreaks-of-mosquito-borne-disease-39176>



19th ISHAM Congress 4-8 May 2015 Melbourne Convention Centre

Do not miss the unique opportunity to learn the latest progress in medical mycology, mycoses and antifungal treatment at the 19th International Congress of the International Society of Human and Animal Mycology (ISHAM) from 3 – 9th May 2015 in Melbourne. The Australian and New Zealand Mycoses Interest Group (ANZMIG) of the Australasian Society for Infectious Diseases (ASID), under the chair of Prof. Wieland Meyer, Dr. Sarah Kidd, A/Prof. Sharon Chen and Prof. Monica Slavin, welcomes the experts of human and animal pathogenic fungi and infectious disease physicians interested in mycoses from a around the globe to an exiting four days scientific/clinical program, which will be preceded by two days of workshops, including: histopathology, MALDI-TOF, antifungal susceptibility, mycoses in the immunocompromised host, and therapeutic drug monitoring and a one day Young ISHAM meeting (3 May 2015), followed by a BioloMICS workshop (9 May 2015). The Keynote speaker will be Prof. Sarah Gurr (UK) "The threats and tribulations of fungi to humankind".

Plenary speakers include: Prof. Tania Sorrell (Australia) "New Insights in Candidaemia", Prof. Marillene Henning Vainstein (Brazil) "Insights in fungal pathogenicity drawn from proteomics studies", Prof. William Hope (UK) "Modeling of Human Antifungal Dosages", Prof. Isabella Dib Gremiao (Brazil) "Animal to human transmission new threat of Sporotichosis", Prof. Richard Cannon (New Zealand) "Antifungal resistance – threats, trends and targets", Prof. John Perfect (USA) "How can Animal Models Inform Clinical Practice?", Prof. Olivier Lortholary (France) "Advances in Mucormycosis" and Prof. Joe Heitman (USA) "Virulence mechanisms shared by fungi that infect humans, animals and plants".

For more information and registration see www.isham2015.com.au. The workshops can also be attended independently of the conference.

CONTACT US

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

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