



## Microbiology Division

### Mycology Reference Unit

#### BACKGROUND

The Mycology Reference Unit (MRU) aims to (1) contribute to the control of fungi of public health and clinical importance by undertaking relevant laboratory-based surveillance and research projects and (2) function as a reference laboratory in the field of clinical mycology

#### ACTIVITIES, HIGHLIGHTS AND ACHIEVEMENTS

In November 2007, the MRU surveillance laboratory was accredited by SANAS in accordance with ISO15189 standards.

#### THE GERMS-SA CRYPTOCOCCAL SURVEILLANCE PROJECT

The cryptococcal surveillance project, managed by the MRU, is nested within the national laboratory-based GERMS-SA surveillance programme. Since 1 January 2005, approximately 120 South African clinical microbiology laboratories have reported >15,000 laboratory-confirmed cases of cryptococcosis to the MRU. A surveillance audit performed for NHLS laboratories from eight provinces for the period 1 January to 31 December 2007 detected >1000 additional cases of cryptococcosis not previously reported. Since 2005, cryptococcal isolates obtained from cases where fungal culture was performed have been speciated and a subset of these isolates selected for further characterisation, e.g. antifungal susceptibility testing, PCR fingerprinting, mating type analysis and sequence analysis. Viable isolates have been stored at -70°C to facilitate retrospective isolate analysis. Clinical case data, obtained at 16 sentinel site hospitals, has allowed for more detailed epidemiologic analysis. In addition, surveillance data has been used to support development of clinical guidelines for the prevention, diagnosis and management of cryptococcal meningitis and disseminated cryptococcosis in HIV-infected patients. Surveillance data, presented to the National Department of Health Comprehensive HIV/AIDS Care, Management and Treatment (CCMT) Programme Team, were used to motivate for use of cryptococcosis as an indicator disease for monitoring the long-term effectiveness of the CCMT Programme.

#### Antifungal susceptibility testing

Global surveillance for resistance of *Cryptococcus* species to antifungal drugs has shown minimal development of resistance to commonly used drugs, e.g. fluconazole over time. Data from South Africa were lacking. Sixty-two isolate pairs from cases with recurrent cryptococcal episodes were tested concurrently for susceptibility to fluconazole using a reference standard method (CLSI M27-A2). Despite the lack of interpretive breakpoints, four cases had isolate pairs with a significant (fourfold) increase in minimum inhibitory concentration between incident and recurrent isolate, suggesting that "fluconazole resistance" may have developed. These findings require further investigation.

#### Guidelines for the prevention, diagnosis and management of cryptococcal meningitis and disseminated cryptococcosis in HIV-infected patients

In response to an identified need to improve overall management of AIDS-associated cryptococcosis in Southern Africa and hence entry of patients into the public-sector antiretroviral treatment programme, clinical practice guidelines were developed in August 2007. The guidelines have been published in the Spring 2007 edition of the Southern African Journal of HIV Medicine. A thirteen-person writing committee evaluated available data on management of cryptococcosis in HIV-infected patients and developed best practice guidelines tailored to a resource-limited setting. The guidelines are intended to provide practical advice to clinicians in Southern Africa, working without specialist support, who encounter patients with AIDS-associated cryptococcosis in their routine practice. The guidelines include seven recommendations covering all aspects of management of patients with AIDS-associated cryptococcosis: (1) Diagnosis of cryptococcosis, (2) Initial treatment of cryptococcosis, (3) The role of the laboratory, (4) Supplementary management, (5) The role of antiretroviral therapy, (6) Special populations and (7) Drug information, interactions and toxicities.

#### Paediatric cryptococcosis

Cryptococcosis is rarely described in children. Cases of cryptococcosis in children (<15 years), reported to MRU between 1 January 2005 and 31 December 2007, were compared to adult cases. A large series of paediatric cryptococcal cases was identified and described. From the analysis, it is evident that South African children with

cryptococcosis were more likely to be HIV-uninfected, on highly active antiretroviral therapy (HAART) at time of diagnosis, diagnosed on blood culture, and treated with amphotericin B induction therapy than adults.

### Molecular Analysis of Cryptococci

In 2007, sixty three clinical isolates of *Cryptococcus neoformans* were subjected to PCR fingerprinting (to establish molecular type) and mating type analysis. The majority of isolates belonged to the VNI type. A significant bias towards the MAT $\alpha$  mating type was established. In addition, 2 rare *Cryptococcus gattii* clinical isolates have been analysed in conjunction with similar global isolates, in collaboration with a worker at the University of Sydney (Sydney, Australia).

### THE CRYPTOCOCCAL ECOLOGY PROJECT

In collaboration with workers from Duke University (Durham, North Carolina, USA), the MRU embarked on a project to explore the ecology of cryptococcosis in 2007. More than 250 cryptococcal isolates were obtained from 24 examined environmental sites, e.g. soil, bird excreta, tree bark, etc. These isolates have been partially typed and will be compared with clinical isolates in the future.



Collecting environmental samples for the cryptococcal ecology project: Professor Thomas G Mitchell and Dr Anastacia Litvinseva, Department of Molecular Genetics and Microbiology, Duke University, North Carolina, USA



### MYCOLOGY EXTERNAL QUALITY ASSESSMENT (MEQA) PROGRAMME

In 2007, the MRU coordinated 3 MEQA surveys for 102 laboratories participating in the yeast sub-programme and 29 laboratories participating in the mould sub-programme. Results of laboratory performance are available in summary format in a separate report.

### COLLABORATIONS

Listing of collaborating institutions/individuals and the appropriate project/programmes:

#### ANTIFUNGAL SUSCEPTIBILITY TESTING

Beth Arthington-Skaggs, PhD, Head of Antifungal Drug Testing Unit, Mycotic Diseases Branch, Division of Bacterial and Mycotic Diseases, Centers for Disease Control, Atlanta, USA

Mary Brandt, PhD, Chief, Mycotic Diseases Branch, Division of Bacterial and Mycotic Diseases, Centers for Disease Control, Atlanta, USA

#### MOLECULAR ANALYSIS OF CRYPTOCOCCI

Wieland Meyer, PhD, Associate Professor, Head of Molecular Mycology Research Laboratory, Centre for Infectious Diseases and Microbiology, Western Clinical School, University of Sydney

#### THE CRYPTOCOCCAL ECOLOGY PROJECT

Thomas G. Mitchell, PhD, Associate Professor, Department of Molecular Genetics and Microbiology, Director, Molecular Mycology and Pathogenesis Training Program, Duke University, North Carolina, USA

Anastacia Litvinseva, PhD, Department of Molecular Genetics and Microbiology, Duke University, North Carolina, USA

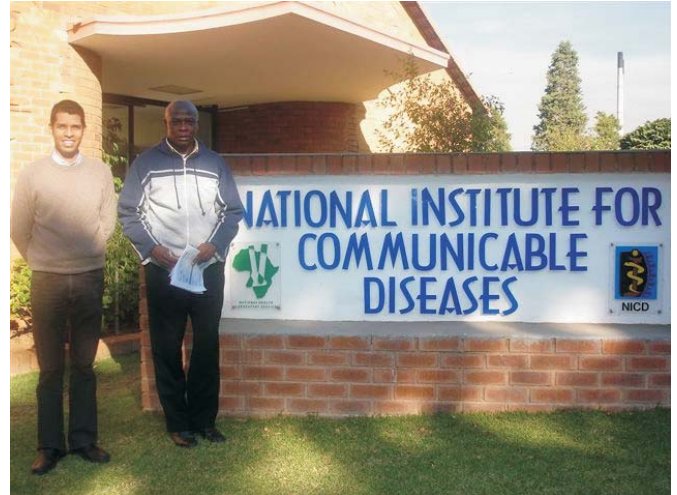
### CAPACITY BUILDING

#### PATHOLOGY REGISTRARS

The MRU participates in the NICD training programme for pathology registrars. In 2007, 6 clinical pathology and mono-speciality registrars were trained for a day (8 June) during the short NICD training programme and 2 mono-speciality registrars were trained for 1 week (13-17 August) during the long programme. Training included practical identification of clinically important yeasts and moulds, a review of antifungal susceptibility test methods, an introduction to molecular epidemiology and molecular diagnostic techniques as well as an overview of relevant clinical infectious disease issues.

**LABORATORY TRAINING**

Mr Frederick Kechia (Laboratory Scientist, Medical Mycology Laboratory, Faculty of Medicine and Biomedical Sciences, University of Yaoundé I Cameroon) participated in a two week laboratory training programme (14-25 May 2007) coordinated by the MRU. The training programme included observation and performance (under supervision) of diagnostic and antifungal susceptibility testing techniques on a clinical mycology bench (Johannesburg Hospital Clinical Microbiology Laboratory), attendance at a two day training course in diagnostic clinical mycology (coordinated by the NHLS Continuing Education Unit at Johannesburg Hospital Clinical Microbiology Laboratory), observation of laboratory techniques used for characterisation of *Cryptococcus* species (MRU), observation and overview of aspects of the national cryptococcal surveillance programme (Johannesburg Hospital and MRU) and a theoretical overview of antifungal susceptibility testing techniques (MRU).



**Dr Nelesh Govender (Head, MRU) and Mr Frederick Kechia (Laboratory Scientist, Medical Mycology Laboratory, Faculty of Medicine and Biomedical Sciences, University of Yaounde, Cameroon)**