

# **Medical Mycology: The African Perspective**



**25 January, 2005  
Hartenbosch, South Africa**

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## **Medical Mycology: The African Perspective**

Over the last few years the significance of medical mycology in Africa has grown enormously. The dramatic increase of Cryptococcosis in the Southern part of the continent is an obvious example. Mycetoma, Histoplasmosis and dermatophyte infections are other major disease categories. The newly founded Africa Fund for Fungal Biodiversity and Mycotic Infections has taken the initiative to organize a symposium on mycological problems which are endemic to the African continent, including themes where African scientists have achieved major breakthroughs.

Major aim of the symposium is to establish long-term relations between scientists from Africa and abroad. Invited speakers will be from the African continent presenting their latest research data, and speakers from outside Africa working on topics concerning African fungi.

The organizers,

Sybren de Hoog (Utrecht), Abdallah Ahmed (Khartoum), Jacques Meis (Nijmegen), Hester Vismer (Cape Town)

This event is supported by the International Society for Human and Animal Mycology (ISHAM), the European Confederation of Medical Mycology (ECMM) and the Centraalbureau voor Schimmelcultures (CBS). It is organised in cooperation with the 43rd Congress of the Southern African Society for Plant Pathology (SASPP)

## Programme

Monday 24 January

**17:00-18:00**

Registration at the "Walvis" Hall

Putting up of posters at the "Dolfyn" Hall

Loading and pre-viewing of presentations at the "Indaba" Hall

Tuesday 25 January

**07:00-08:00** Registration at the "Walvis" Hall

Complete loading and pre-viewing presentations at the "Indaba" Hall

**08.00** Opening & Welcome

*Session 1. Chairs: Hester Vismar and David Soll.*

**08.00 - 08.30** \_\_\_\_\_ **p. 6.**

Prof. El Sheikh Mahgoub (*Mycetoma Research Centre, University of Khartoum, Khartoum, Sudan*): Mycoses in Africa

**08.30 - 08.50** \_\_\_\_\_ **p. 7.**

Prof. E. Blignaut (*Department of Stomatological Studies, Faculty of Dentistry, Medical University of Southern Africa, Medunsa, South Africa*): *Candida albicans* clades among clinical isolates in Southern Africa

**08.50 - 09.10** \_\_\_\_\_ **p. 8.**

Prof. Dr. A. Moharram<sup>1</sup>, Dr. R.C. Summerbell<sup>2</sup> and Prof. Dr. G.S. de Hoog<sup>2</sup> (<sup>1</sup>*Faculty of Science, Assiut University, Egypt*; <sup>2</sup>*Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands*): Diagnostic molecular studies of pathogenic and non-pathogenic isolates of *Sporothrix*.

**09.10 - 09.30** \_\_\_\_\_ **p. 9.**

Dr. R.C. Summerbell (*Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands*): *Fusarium* and *Acremonium* infections in Africa: what do we know and what should we expect?

**09.30 - 09.50** \_\_\_\_\_ **p. 10.**

Prof. Dr. A. Al-Zahraa Karam El-Din (*Microbiology Department, Faculty of Science, Ain-Shams University, Abassia, Cairo, Egypt*): Cryptococcosis in Egypt.

**09.50 - 10.10** \_\_\_\_\_ **p. 11.**

Dr. A.O.A. Ahmed<sup>1</sup>, Prof. Dr. G.S. de Hoog<sup>2</sup>, Prof. Dr. A.H. Fahal<sup>1</sup> and Prof. Dr. A. van Belkum<sup>3</sup> (<sup>1</sup>*Mycetoma Research Centre, University of Khartoum, Khartoum, Sudan*; <sup>2</sup>*Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands*; <sup>3</sup>*Erasmus Medical Centre, Rotterdam, The Netherlands*): *Eumycetoma* due to *Madurella mycetomatis*: current experience.

**10.10 - 10.30** \_\_\_\_\_ **p. 12.**

H.M. Al-Abdely, MD (*King Faisal Specialist Hospital and Research Center Riyadh, Saudi Arabia*): Therapeutics for eumycetoma.

**10.30 - 11.00** Tea/coffee break

*Session 2. Chairs: Ifomea Enweani and Ahmed Fahal.*

**11.00 - 11.20** \_\_\_\_\_ **p. 13.**

Prof. Dr. G.S. de Hoog<sup>1</sup>, Dr. S.-m. Choi<sup>2</sup> and Dr. R. Horré<sup>3</sup> (<sup>1</sup>*Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands*; <sup>2</sup>*Department of Dermatology and* <sup>3</sup>*Institute for Medical Microbiology and Immunology, University of Bonn, Germany*): Severe infections by black yeast-like fungi illustrated by a case from Libiya.

**11.20 - 11.40** \_\_\_\_\_ **p. 14.**

Dr. H. Vismer (*Medical Research Council, Cape Town, South Africa*): *Sporothrix schenckii* infections in South Africa - A clinical, epidemiological, ecological and molecular taxonomic overview.

**11.40 - 12.00** \_\_\_\_\_ **p. 16.**

Dr. K. McCarthy (*National Institute for Communicable Diseases, Johannesburg, South Africa*): Population-based, active surveillance for cryptococcosis in Gauteng, a province of South Africa, from 2002-2004.

**12.00 - 12.20**

Dr. H. Crewe-Brown (*Chris Hani Baragwanath Hospital, Witwatersrand, South Africa*): A follow-up study on cryptococcosis in Gauteng province South Africa to assess long term outcome and access to routine care.

**12.20 - 12.40** \_\_\_\_\_ **p. 17.**

Prof. Dr. A.H. Fahal (*Mycetoma Research Centre, Khartoum, Sudan*): Mycetoma in the Sudan.

**12.40 - 13.00** \_\_\_\_\_ **p. 18.**

Prof. I. Enweani<sup>1</sup>, Dr. Y. Gräser<sup>2</sup> and Prof. D. Agbonlahor<sup>1</sup> (<sup>1</sup>*Department of Microbiology, Ambrose Alli University, Ekpoma, Nigeria*; <sup>2</sup>*Institut für Mikrobiologie und Hygiene, Humboldt Universität, Berlin, Germany*): ABO blood groups and dermatophytosis.

**13.00 - 14.00** Lunch.

*Session 3. Chairs: Kerrigan McCarthy and Hail Al-Abdely.*

**14.00 - 14.20** \_\_\_\_\_ **p. 19.**

Dr. A. Botha<sup>1</sup>, A. Botes<sup>1</sup> and Dr. T. Boekhout<sup>2</sup> (<sup>1</sup>*University of Stellenbosch, South Africa*; <sup>2</sup>*Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands*): Natural habitats of *Cryptococcus*.

**14.20 - 14.40** \_\_\_\_\_ **p. 20.**

Prof. Dr. D.R. Soll (*The University of Iowa, Iowa City, U.S.A.*): The *Candida albicans* clade South Africa (SA).

**14.40 - 15.00** \_\_\_\_\_ **p. 21.**

Prof. Dr. H.C. Gugnani (*Dr. B.R. Ambedkar Centre for Biomedical Research, University of Delhi, Delhi, India*): Ecology of three varieties of *Histoplasma capsulatum* and human and animal infections caused by them in Africa.

**15.00 - 15.30** Tea/coffee break

*Session 4. Chairs: Alf Botha and Ahmad Moharram.*

**15.30 - 15.50** \_\_\_\_\_ **p. 22.**

J. Nzamba, S. Nzenze Afene, E. Ibinga and Prof. P.M. Kombila (*Département de Parasitologie-Mycologie et Médecine Tropicale, Faculté de Médecine, Université des Sciences de la Santé, Libreville, Gabon*): Evaluation of the fungal flora isolated from nails in the Medicine School of Libreville (Gabon).

**15.50 - 16.10** \_\_\_\_\_ **p. 23.**

Dr. M. Richardson (*Department of Bacteriology & Immunology, University of Helsinki, Finland*): Increasing awareness of fungal infections: role of education and the internet.

**16.10 - 16.30** \_\_\_\_\_ **p. 24.**

Ph.A. van Damme (*University Hospital Nijmegen and Canisius Wilhelmina Hospital Nijmegen, The Netherlands*): Noma.

**16.30 - 17.30**

Poster viewing with wine and cheese at the "Dolfyn Hall".

**17.30 - 18.30**

Inaugural meeting Pan-African Society for Medical Mycology (PAMM) at the "Indaba Hall".

**19.00:**

Congress dinner (Dress: Smart Casual - no jackets required) at the "Skoffelsaal".

## ORAL PRESENTATION

### **Mycology in Africa**

**Elsheikh Mahgoub\***

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As much as in several geographical locations, studies and reporting of mycoses and individual fungi in Africa depends on the interest of scientists in the locality. Although few clinicians, or pathologists or mycologists were available in Africa during the 20<sup>th</sup> Century, yet important studies, that have contributed to the body of knowledge in mycology, came from Africa.

With the advent of the HIV / AIDS epidemic, increased numbers of compromised individuals from disease and chemotherapy, better methods of diagnosis and management of fungal infections, mycoses have increased in number. Similarly awareness about mycoses and interest have increased among physicians. It will be difficult to tell the story of all mycoses reported from Africa, and we will therefore focus on mycoses that have a relatively high and serious morbidity namely: sporotrichosis, mycetoma, cryptococcosis, histoplasmosis due to *Histoplasma duboisii* and paranasal *Aspergillus* granuloma. Epidemiology, diagnosis and management of these mycoses will be discussed in the paper.

## South African oral *Candida* isolates

### E. Blignaut\*

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An estimated 7.6 million (16%) South Africans are infected with the human immunodeficiency virus (HIV). Oral candidiasis is the most frequently encountered oral complication and *Candida albicans* the most commonly isolated species from HIV/AIDS patients. Among 554 isolates of *Candida*, *C. albicans* constituted 91.5% and 73.2% in adult HIV/AIDS patients and healthy individuals respectively; *C. krusei* 5.1% and 9.3%; *C. tropicalis* 1.2% and 2.2%; *C. parapsilosis* 0.9% and 2.6%; *C. glabrata* 0.6% and 3.1% and other *Candida* spp. 0.6% and 9.3%. The prevalence of the various species isolated from institutionalised paediatric HIV/AIDS patients differed significantly from those found in adults. *C. dubliniensis* was isolated more frequently from paediatric patients (26.6%) than from adult, black HIV/AIDS patients (1.5%). This species was more prevalent in healthy, white adults (16.4%) and white, adult HIV/AIDS patients (9.1%) but was not isolated from healthy, black individuals. Using the broth microdilution method (NCCLS), no resistance to fluconazole (MIC>64 µg) was found among *C. albicans* isolates obtained from South African adults. However, 8.4% of *C. albicans* isolates displayed a natural resistance to amphotericin B (MIC>1µg) *in vitro*. DNA fingerprinting of oral *C. albicans* isolates, with the moderately repetitive Ca3 probe, revealed five different clades, or genetic subtypes, with a unique South African clade being significantly more prevalent among black HIV/AIDS patients and black healthy individuals. Future research is directed at establishing phenotypic characteristics among the different clades and determining any possible clinical significance.

## Studies on pathogenic and non-pathogenic species of *Sporothrix*

A.M. Moharram<sup>1\*</sup>, R.C. Summerbell<sup>2</sup> and G.S. de Hoog<sup>2</sup>

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Morphological and molecular studies were made to revise 72 fungal strains belonging to *Sporothrix* and *Ophiostoma*. Cultures were obtained from the CBS culture collection. The data base showed that only 13 strains are clinical isolates from humans and the remainder was recovered from animals, insects, plants, other fungi, sewage sludge or non-specified sources. All cultures were allowed to grow at temperatures ranging from 18 to 45°C. Extraction of fungal DNA was made either by FastPrep kit using the FastPrep instrument or by grinding with micropestles and utilization of CTAB buffer. The second method was found to be better for obtaining good quality of DNA. Universal primers such as V9G, LR5 and LS266 were used for PCR amplification of the internal transcribed spacer (ITS) region covering the ITS1, ITS2 and 5.8S of the ribosomal DNA. Addition of dimethylsulfoxide (DMSO) proved to be the optimal means to achieve good PCR product. Other primers such as ITS1 and ITS4 were used for sequencing.

Results showed that the majority of isolates exhibited their best growth at 24 or 28°C. Isolates described as *Sporothrix inflata*, *S. curviconia* and *Ophiostoma stenoceras* were not able to grow at 36°C. Most isolates described as *S. schenckii* showed limited growth at 37°C with partial or complete conversion to yeast-like phase. Only one strain described as *S. catenata* showed growth at 40 and 45°C. Sequence based identification was done in comparison to sequences of type strains studied in this work or accessioned in GenBank. There are some strains appear to be unrelated to *Sporothrix* but their identity could not be established.

## ***Fusarium* and other hypocrealean human disease in Africa**

**R.C. Summerbell\***

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*Fusarium* has long been known as one of the most common and dangerous human mycotic pathogens throughout Africa, being considered the second most common cause of microbially-induced corneal damage and blindness after onchocerciasis. Principally members of the *Fusarium solani* complex have been implicated, with other species such as *F. dimerum* and *F. oxysporum* being uncommonly reported. Traumatic accident involving the cornea precipitates infection, as elsewhere in the world. Because HIV-positive patients are especially susceptible to *F. solani* keratitis, according to J. Mselle, this problem is likely to be increasing in importance. Treatment of *F. solani* ocular mycoses is problematical because responses to therapy are non-uniform even under ideal conditions. Now thanks to molecular study, we know that this is probably connected to the fact that this "species" is an amalgam of 50 or more separate phylogenetic species. It is not yet known which of these entities are responsible for human disease in Africa; in general, they are just beginning to be delineated worldwide. *F. dimerum* is also a species complex. *Fusarium verticillioides* and *F. nygamai*, which both cause disease in the neutropenic host, are very common in Africa mainly on corn and sorghum, respectively, but the medical system in Africa has not yet generated enough severely immunocompromised patients to make these fungi a common problem. Persons with HIV are not known to be particularly susceptible to these fungi. *Acremonium kiliense*, an agent of mycetoma mainly in the Sahel area, is a unitary species, but other species are often misidentified under this name, a situation now readily clarified with molecular techniques. A unique intertriginous dermatomycosis mainly affecting toe webs of persons from West Africa is caused by *Fusarium solani*, *F. lichenicola*, and *F. oxysporum*. It is not clear why all cases seem to be in otherwise healthy Africans, including persons who have moved to Europe.

## **Cryptococcosis in Egypt**

**A. Al-Zahraa Karam El-Din\***

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Cryptococcosis is recognized as one of the most common life-threatening opportunistic fungal infections in immunocompromised patients. Few reports on cryptococcosis in Egypt although the region possesses many factors that would predict the country to be an area where the fungus would be common. This review attempts to present an overview of the status of cryptococcosis in Egypt from its first description [environmental isolates by Refai et al., 1983] to the most recently times [clinical isolates by Elias et al. (in press)]. The epidemiology of cryptococcosis in Egypt is still far from understood. Awareness of the disease and an integrated approach to patient management with active interaction between the clinicians and the laboratory personnel would be highly beneficial to highlight the spectrum of cryptococcosis in Egypt.

## **Recent developments in the clinical, epidemiological, and diagnostic management of *Madurella mycetomatis* induced mycetoma**

**A.O.A. Ahmed**<sup>1,2\*</sup>, W. van Leeuwen<sup>3</sup>, A. Fahal<sup>1</sup>, W. van de Sande<sup>3</sup>, H. Verbrugh<sup>3</sup>, G.S. de Hoog<sup>4</sup> and A. van Belkum<sup>3</sup>

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Eumycetoma in arid climates is mostly caused by the fungal species *Madurella mycetomatis*. The disease is characterized by extensive subcutaneous masses, often in combination with sinuses which drain pus, blood and fungal grains. The disease affects individuals of all ages, although the burden is most severe among working class adults, since it is extensively invalidating. Amidst major plagues including tuberculosis, malaria and the HIV pandemic, *M. mycetomatis* can be considered an underestimated, but still socio-economically important disease. The international literature is crowded with case reports on mycetoma manifestation, but fundamental research was lacking until recently. We here present a review on the developments in the clinical, epidemiological, and diagnostic management of *M. mycetomatis* induced mycetoma. This involves the recent description of molecular diagnostics and genetic typing procedures, methods which were not only used for patient management but also for environmental research. In addition, fungal susceptibility tests have been presented and a mouse model of infection was developed. All these features will significantly promote our understanding of the molecular basis of eumycetoma.

## Therapeutics for Eumycetoma

**H.M. Al-Abdely\***

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Fungal mycetoma (Eumycetoma) is a chronic subcutaneous infection, mostly of the lower extremities that is prevalent in tropical and subtropical regions. Accurate estimates of incidence or prevalence are lacking. The disease is caused by a wide range of fungal species including hyaline and dematiaceous fungi. *Madurella mycetomatis* remains the most common cause of eumycetoma in Africa. Clinical pharmacological research on therapeutics for eumycetoma is generally limited. This is mainly due to limited market for antifungals in typically lower-income societies. Therefore all the drugs that have been used for the treatment of fungal mycetoma were developed and approved for other fungal infections. Data on efficacy of the antifungals against eumycetoma have come mainly from anecdotes and case series. Comparative studies are lacking. The data are scares on the relatively newly marketed antifungal agents. The clue towards possible efficacy of such agents can only be suggested from a few in vitro studies or other clinical forms of disease caused by the fungi that also cause eumycetoma. The typical example is activity of voriconazole against *Fusarium species* and *Scedosporium apiospermum*.

Success in therapy is dependent on several factors: these include the extent of local destruction, especially bone involvement, the causative organism and host immune status. Surgery remains a mainstay treatment of patient with eumycetoma with adjuvant antifungal therapy. Identification of the causative organism is critical in selecting the best antifungal agent. Broad-spectrum triazoles such as voriconazole and posaconazole have shown activity against several fungi that can cause eumycetoma in vitro and in few patients. However, strong data in not available on such agents, and still most of the experience has been obtained with the older imidazoles and triazoles like ketoconazole and itraconazole. Terbinafine has been shown to have synergy with azoles and may have a future a role in treatment of eumycetoma. Parenteral agents like amphotericin B or echinocandins are inconvenient for therapy because of the need for prolonged therapy. Amphotericin B is less active and more toxic than azoles against many fungi that cause mycetoma. Duration of therapy is arbitrary and depends on the extent of disease, patient's response and drug toxicity. Most cases require several months or longer courses of antifungal therapy. Amputation would be the last option after failure of debulking surgery and appropriate antifungal therapy.

## **Severe infections by black yeast-like fungi illustrated by a case from Libiya**

**G.S. de Hoog**<sup>1\*</sup>, S.-M. Choi<sup>2</sup> and R. Horré<sup>3</sup>

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We report on a severe, verrucous facial mycosis and sinusitis in a 12 year-old Libyan girl. Her disease started with verrucous, hyperkeratotic plaques and subcutaneous violet nodules of unknown origin on her face and upper extremities. Despite topical antimycotic therapy she needed in-hospital treatment because of severely progressive tumorous cutaneous and nasal lesions. Microscopic examination of scale samples taken from the upper extremities and the face revealed brown, thick-walled fungal elements. Under the assumption of a chromoblastomycosis, an oral treatment with itraconazole and fluorocytosin was initiated, with significant improvement of the lesions. The etiologic agent was identified as *Phialophora verrucosa*. The etiology of further black yeast-like species will be elucidated.

## ***Sporothrix schenckii* infections in South Africa – a clinical, epidemiological, ecological and molecular taxonomic overview**

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Sporotrichosis is a subcutaneous fungal infection caused by the dimorphic, pathogenic fungus *Sporothrix schenckii*. It constitutes the most common subcutaneous fungal infection in the general population in South Africa (SA). Sporotrichosis in SA dates back to 1914 when the disease was first diagnosed in the Guateng gold mines. Thus, two epidemiologic forms of human sporotrichosis exists in SA, i.e. infections acquired from a source in nature, and infections acquired in the gold mines from timber underground. The epidemic proportions that sporotrichosis took in the mines have contributed significantly to our present day knowledge of the clinical features of the disease, the causative organism and its mode of spread. Similarly, circumstances leading to the infection in the general population are reasonably well understood. Sporotrichosis is reported throughout SA with a higher incidence in the Northern Province, Gauteng, Mpumalanga and Kwazulu Natal with only small numbers of cases recorded annually in the rest of the country. The disease occurs at all ages, ranging from less than one year to 90 years old, with males predominating. Exposure to possible sources of the fungus, either from recreational or occupational activities in males, is the main determining factor in acquiring the disease. Females seem to be at lesser risk, mainly becoming infected through gardening injuries, insect bites or other minor injuries due to outdoor activities. The lymphocutaneous and localized forms of the disease are most often recorded, while extracutaneous and systemic disease are rarely encountered. Although no pronounced seasonal variation is noted in occurrences, the onset of the disease seems to be mainly in the cooler and dryer months of the year. Morphological differences have been recorded in strains of *S. schenckii* isolated from underground timbers, compared to strains isolated from the general population, but the latter could be converted on moist wood to produce triangular and pigmented conidia, similar to those seen in the gold mine isolates. Pigmented conidia proved to be more viable than unpigmented ones, and pigment production is potentially present in all conidium types. Larger conidium volumes were unrelated to genetic material but constituted and increased cytoplasmic content and cell wall thickening in the older conidia, as confirmed by fluorescent nuclear DNA staining and electron microscopy. Research therefore suggests an intrinsic polymorphism in *S. schenckii*, as the two types of pathogenic strains could be interconverted by altering the nutritional conditions alone. Molecular research indicate that (i) Mitochondrial DNA (mtDNA) restriction fragment length polymorphism (RFLP) in clinical strains of *S. schenckii* classified the species into

23 mtDNA types, and clustered them into two major groups by phylogeny, i.e. Groups A and B. Group A isolates are predominant in SA. Environmental isolates, morphologically identified as *S. schenckii*, are rarely identified as such by RFLP, while (ii) DNA sequence comparisons between human pathogenic and environmental strains, with similar morphology to *S. schenckii*, represent different species. (iii) Furthermore, 18s rDNA sequences reveal significant differences between *S. schenckii* and *Ophiostoma stenoceras*. The latter species was previously considered as the possible teleomorph of *S. schenckii*.

## ORAL PRESENTATION

### **Cryptococcosis in Gauteng Province (South Africa): Results of population-based active surveillance 2002-4**

**K. M. McCarthy**<sup>1\*</sup>, R Hajjeh <sup>2</sup>, HH Crewe-Brown<sup>1</sup>, M Brandt<sup>2</sup>

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Population-based active surveillance of cryptococcosis was conducted for two years from 1 March 2002 until 29th February 2004 to establish the incidence of cryptococcosis, an AIDS-defining illness, and to describe its demographic and clinical characteristics in Gauteng Province (population 8.8 million), South Africa, an area with a high HIV seroprevalence. A case of cryptococcosis was defined by the isolation of *Cryptococcus neoformans* from any specimen, a positive India ink or cryptococcal latex agglutination test on CSF. Cases were identified prospectively by regularly visiting all laboratories in Gauteng. Patient demographics, clinical characteristics, treatment, diagnostic tests and outcome at the end of hospitalization were collected. Census data and the ASSA model for HIV/AIDS seroprevalence in South Africa were used to provide denominators for incidence calculations. After exclusion of non-Gauteng residents, 2756 incident cases were identified over 2 years. Females accounted for 1372 cases (50%), and 2702 cases (98%) were black. The mean age was 35 years (range 0-74 years, median 34). The incidence of cryptococcosis was 15.6/100,000 population. In the population of 31-35 year-olds the incidence was 38/100,000. Amongst people living with HIV and AIDS, the incidence was 95/100,000. Amongst AIDS patients only, the incidence was 14/1000. Cryptococcal meningitis accounted for 2638 (96%) of cases. Cryptococcaemia occurred in 344 cases (12%). Altered mental status/confusion was present in 840 cases (30%). Of the 642 cases who had an HIV test during the incident admission, 606 (94%) were HIV seropositive. (In 22 cases (4%) the results were unknown.) CD4 test results were available in 555 cases (21%). The mean CD4 count was 62 (range 0-955). Twenty four cases (<1%) were receiving antiretroviral therapy. Mortality at the end of hospitalization was 752 (27%). The high incidence of cryptococcosis reflects the high seroprevalence of HIV in the Gauteng population. These data highlight the significant morbidity and mortality associated with cryptococcosis in South Africa.

## **Mycetoma in the Sudan**

**A.H. Fahal\***

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Mycetoma is a chronic, granulomatous, subcutaneous, inflammatory disease caused by true fungi (eumycetoma) or filamentous bacteria (actinomycetoma). Tropical eumycetoma is frequently caused by the fungus *Madurella mycetomatis*. The disease occurs in the mycetoma belt stretching between the latitudes of 15 degrees South and 30 degrees North and is endemic in relatively arid areas. The organisms are present in the soil and may enter the subcutaneous tissue by traumatic inoculation. The true incidence of the disease is under-estimated and generally it is a disease which is badly neglected by medical community. It has many socio-economic impacts both on the community and patients. Mycetoma commonly affects adults aged 20 to 40 years, predominantly males. The clinical triad of subcutaneous nodule, sinuses and discharge usually leads to diagnosis; the disease is commonly painless. The foot is most commonly affected. Both forms of mycetoma present as a progressive, subcutaneous swelling, although actinomycetoma has a more rapid course. Multiple nodules develop which may suppurate and drain through sinuses, discharging grains during the active phase of the disease. Diagnosis may involve radiology, ultrasonic imaging, cytology, culture, histology or immunodiagnosis.

Actinomycetoma is amenable to treatment by antibiotics, preferably by combined drug therapy for long periods. Eumycetoma is usually treated by aggressive surgical excision combined with medical treatment. Medical treatment may be used on its own or as an adjunct to surgery; those affected by eumycetoma have a poorer prognosis and may require many years of drug therapy. Recurrence is common, rates ranging from 20 to 90 per cent.

Many scientific case reports on mycetoma exist, but fundamental research was lacking until recently. We present a review on developments in the clinical, epidemiological, and diagnostic management of *M mycetomatis* eumycetoma. We describe newly developed molecular diagnostic and gene typing procedures, and their application for management of patients and environmental research. Fungal susceptibility tests have been developed as well as a mouse model of infection. These advances should greatly further our understanding of the molecular basis of eumycetoma.

## Association of ABO blood group and dermatophytosis in Nigeria

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ABO blood group system has been reported to have some association with some disease conditions. In relation to dermatophytosis, some authors reported that there is a relationship with Blood group A while others said that there is no relationship. Dermatophytosis is an endemic disease in Nigeria with different strains prevalent in specific parts of the country. *Microsporum audouinii* is most prevalent in Eastern and Western Nigeria, *T. schoenleinii* in Northern Nigeria, while the “soudanense”-like variety *T. violaceum* occurs in the middle belt. Tinea capitis is an infection of the scalp and is very common in prepubertal children. Other forms of tinea are also frequent. Incidence of dermatophytes range between 10-55%. Isolates of scrapings from lesions were subjected to KOH mounts, culture was done on SDA impregnated with chloramphenicol and cycloheximide. Molecular methods included PCR with a primer pair amplifying a microsatellite region (GT-repeat) that is specific for the *T. rubrum*/*T. violaceum* species complex; ITS-PCR in conjunction with RFLP analysis and sequencing was performed to identify the remaining dermatophyte species. The dermatophyte isolates from tinea capitis on school children from Edo State, Nigeria which were subjected to molecular typing using the microsatellite marker revealed that the genotype A was most prevalent. In combination with a large set of biochemical tests (e.g., CEA, BCP) we have been trying to subdifferentiate the isolates assigning them to the following morphotypes: “*gourvillii*-like” (34%), “*soudanense*-like” (26%), “*raubitschekii*-like” (4.7%), “*megrinii*-like” (2.7%). The genotype B was much less frequent and included 11.3% of *T. rubrum* isolates that did not produce the red pigment (yellow variant) and would have been misidentified without the molecular tests. The causative agents other forms of Tinea included *M. audouinii* (9.3%), *M. canis* (4.0%), *T. verrucosum* and *E. floccosum* (2.0%) each, *T. schoenleinii* and *T. mentagrophytes* (1.3%) each and *M. gypseum* (0.7%). A higher percentage (69%) of blood group O children suffered from dermatophytes in comparison with group A (25.2%), B (1.9%) and AB (0.9%). A specific pattern of infection according to sex was not observed. Children below the age of 12 years are most prone. Control of dermatophytosis in Nigeria needs an urgent attention. Spectrum has changed from *M. audouinii* to the species of the *T. rubrum*/*T. violaceum* complex in Edo State. Surveillance and proper treatment are advocated.

## Natural habitats of *Cryptococcus neoformans*

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Despite the fact that *Cryptococcus neoformans* has been isolated from many clinical samples, as well as from a wide diversity of plant and soil samples originating from all over the globe, relatively little is known about the natural habitat of this yeast, especially in Africa. Literature on in vitro studies regarding cardinal growth temperatures, nutrient assimilation and antibiotic resistance, as well as other phenotypic characteristics, provides us with a glimpse of the intrinsic abilities and fundamental niche of this opportunistic human pathogen. To elucidate the ecological niche of this yeast in Africa, we initiated a preliminary study in which soil and plant samples are screened for the presence of *C. neoformans*. By using conventional and molecular techniques, characteristically pigmented yeasts isolated on birdseed agar are being identified and characterized. Analysis of the amplified D1/D2 regions revealed that, thus far, the majority of isolates are representatives of *C. neoformans* var. *neoformans*, while the remainder was identified as *C. neoformans* var. *grubii*. These findings corroborate other studies conducted globally. Since we were so far unable to isolate *C. neoformans* from *Eucalyptus*, the results suggest that the ecological association known to occur in Australia between *Eucalyptus* and this yeast species may not exist in South Africa. However, the different variants of the yeast species may have evolved to occupy other, yet undiscovered ecological niches that may serve as natural reservoirs of this pathogen. To fully understand the impact of this pathogen on human society, future studies may have to focus on elucidating the natural ecological interactions displayed by the individual variants.

## **The *Candida albicans* clade South Africa (SA)**

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*Candida albicans* remains the most pervasive fungal pathogen in man. It colonizes over 70% of healthy individuals as a benign commensal, and in response to a change in the host that usually affects defense systems, opportunistically multiplies and invades tissue, causing superficial to life-threatening infections. Unfortunately, we have tended to consider all *C. albicans* strains as similar, and have treated the organism as of one phenotype in laboratory experiments. However, DNA fingerprinting methods have revealed five general clades of *C. albicans* (I, II, III, SA, E). These clades exhibit geographical specificity to some extent. Clade E is concentrated in Europe and clade SA is concentrated in South Africa. These clades are deep-rooted and exhibit phenotypic differences including resistance to specific drugs. Population structure studies have revealed that there is little recombination between clades. I am interested in recruiting medical mycologists throughout Africa to collect *C. albicans* samples so that we can genetically map the distribution of *C. albicans* clades throughout the African continent as is being done in the United States. Knowing the clades that inhabit a locale, their drug resistance and other phenotypic characteristics, provide a definite advantage in treatment.

## **Ecology of three varieties of *Histoplasma capsulatum* and human and animal infections caused by them in Africa**

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The three varieties of *Histoplasma capsulatum*, viz. var. *capsulatum*, var. *duboisii* and var. *farciminosum* are endemic in several parts of the world including Africa, and show different geographic distribution. The *capsulatum* variety is known to occur naturally in bat-infested caves in some parts of South Africa. A natural reservoir of *H. capsulatum* var. *duboisii* in soil admixed with bat guano has been found in a bat cave in Nigeria (West Africa). Natural microfoci of the fungus possibly exist in some other parts of Africa. Infection caused by var. *capsulatum* (classical histoplasmosis) occurs predominantly in South Africa and sporadically in several other African countries. The lung is the primary site of infection, and the symptoms vary greatly. Outbreaks of histoplasmosis have been reported in cave explorers. Most of the infections are asymptomatic; however disseminated disease occurs in otherwise healthy people, and especially in the immunocompromised hosts. Surveys of histoplasmin skin sensitivity carried out in different parts of Africa have shown the rate of positive reactors to be 0.0% to 28%. Infection due to var. *duboisii* (African histoplasmosis) is endemic in Western and Central Africa and the island of Madagascar. The sites of infection are mainly the skin, subcutaneous tissue and the bones, and rarely the internal organs. In the AIDS patients, the infection manifests as disseminated disease. *H. capsulatum* var. *farciminosum* causes ulcerated lesions of the skin and subcutaneous tissue in horses and mules (epizootic lymphangitis); infection is widespread in North Africa and has also been reported from some other African countries. The fungus has been recovered from soil in some parts of Africa. Molecular genomic studies of geographically diverse isolates of *H. capsulatum* var. *capsulatum* have identified five clades in this species, while only one clade has been identified in the isolates of *H. capsulatum* var. *duboisii*. There have been no such studies on the isolates of *H. capsulatum*.

## Evaluation de la flore fongique isolée des ongles à Libreville (Gabon)

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Les onychomycoses représentent les étiologies les plus fréquentes des onychopathies au Gabon. Chez les malades concernés, la mycose unguéale est une réelle préoccupation en raison de son caractère, au mieux inesthétique, au pire récidivant, parfois douloureux et donc mal supporté. La prise en charge de ces maladies impose un examen mycologique rigoureux (prélèvement de qualité, examen direct et cultures). Plusieurs champignons sont responsables de ces pathologies.

Notre évaluation de la flore fongique responsable de ces pathologies au Gabon, repose sur une étude rétrospective de 1979 à 2002 (23 ans). Celle-ci a pris en compte les prélèvements d'ongles ayant une culture positive avec une ou plusieurs espèces fongiques que l'examen direct soit positif ou négatif. Sur 1552 malades recensés âgés de 0 à 82 ans, 780 (50%) ont eu un prélèvement d'ongles des doigts, 646 (42%) ont eu un prélèvement d'ongles des orteils et 126 (8%) ont eu les deux à la fois. Au total 1678 prélèvements ont été réalisés, soit 906 (54%) prélèvements d'ongles des doigts et 772 (46%) prélèvements d'ongles des orteils. Les onyxis causés par les levures prédominent et représentent 71,8% de tous les prélèvements. Ils touchent essentiellement les ongles des doigts. Les espèces candidosiques isolées sont principalement représentées par *Candida albicans* (41,4%) suivi par *C. parapsilosis* (20,7%), *C. guilliermondii* (6,1%) et *C. tropicalis* (4,2%). Le genre *Trichosporon* (11,6%) infecte plus les ongles des orteils.

Les onyxis dermatophytiques retrouvés dans 29,7% des cas et ceux dus aux moisissures rencontrés dans 19,5% des cas touchent essentiellement les ongles des orteils. *Trichophyton rubrum* (43,5%) et *T. interdigitale* (24,8%) infectent plus les ongles des orteils. *T. soudanense* (20,8%), *T. gourvilii* (4,21%) et *Microsporium langeronii* (3%) causent essentiellement des onyxis des ongles des doigts. Les moisissures recensées que sont *Scytalidium dimidiatum* (67,3%) et *S. hyalinum* (6,1%), *Fusarium solani* (16,5%) et *Cylindrocarpon tonkinense* (3,7%) sont plus prévalents au niveau des ongles des orteils.

## **Increasing awareness of fungal infections: role of education and the internet**

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Teachers in the area of medical mycology are clearly keen to share the delights of their subject with a wider community. As a result the internet is well populated with educational materials relating to this area of human and veterinary medicine. Furthermore, we are now beginning to see the benefits of distance learning as a way of creating interest and educating many people from a variety of backgrounds. Internet education has many positive attributes to offer to medical mycology.

Many educational internet web sites contain subject matter from basic morphology and biology, through pathology and treatment, to details of sequencing and genetic databases. The study of medical mycology is very much image based. Many sites have large collections of pictures. Video banks are now being set up. Other web sites present information on specific organisms and the diseases they cause. Arguably, the best example of a specialized resource is the elegant "Aspergillus web site". An extensive collection of articles, image banks, guidelines and a very impressive publication data base is presented with automatic email alerts when new material has been posted. The internet also allows the possibility of discussion forums. Lectures can be downloaded, reviewed and discussed with the presenter. Individual medical mycology societies also have their own web sites which host a wealth of educational material. The International Society for Human and Animal Mycology (ISHAM) web site provides a portal to many mycology internet sites and educational material.

The potential role of the internet in increasing the awareness of medical mycology globally is exemplified by distance learning courses and diplomas. One of the first initiatives in this area is the Diploma Course in Medical Mycology that is now provided by University College London and the British Society for Medical Mycology.

Due to the enthusiasm of many individuals, as much as centres of excellence, medical mycology teaching is well served on the internet. There is now an impressive array of teaching materials, research data, and healthcare and practical guidance resource.

## Noma

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Noma is a devastating facial “infectious” disease of yet unknown etiology of the poorest among the poor in Sahel Africa, which might be generally prevented by Third-World-Aid programmes aimed at more and better water, food, (health) education, hygiene, vaccination, medicines, and which should attract more attention from the general public and doctors in the African continent and the western world.

In the early stage, the clinical features are a striking fetid odour, a progressive necrotising stomatitis and the typical cone shaped necrotic zone - *cône gangréneux* - in the faces of anxious, drooling children. In later stages of the disease, striking defects of the lips, cheeks, mucous membranes, gingiva, jaws and nose occur with scar tissue formation and contractures, and consequently trismus and fibrous and bony ankylosis. Quite often the diagnosis of noma is erroneously ascribed to cleft lip, alveolus and palate disorder, Burkitt’s lymphoma, and other benign (e.g. pleomorphic adenoma of the nose and palate) and malignant neoplasms. The differential diagnostic options also include orofacial conidiobolomycosis caused by *Conidiobolus coronatus* or *C. incongruus* infection. From a diagnostic point of view, apart from histo-pathological analysis, specimens of the microbiological flora of the intra- and extra-oral lesions should be sampled and cultured. A mixed predominantly anaerobic flora is to be anticipated.

Ideally, specialised laboratories and application of molecular genetic tools with microarray and sequencing techniques might answer at least the question of which microorganisms are causatively involved. Clinical and surgical aspects will be discussed.

## **Problems of mycoses in a war torn country**

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Somalia is one of the countries in the horn of Africa where has no been no functional government over the past 14 years. Hence the infra-structure has collapsed in all aspects. Health institutions were destroyed irreparably or were looted, while the health professionals either died, fled to other parts of the world, or retired. Mycoses are one of the important areas in the field of medicine in this country, but unfortunately it received insufficient attention from the world community. In the African continent is no better. In my home, land due to reasons mentioned above, mycoses have yet to be recognized, although all factors favouring severe mycotic infections are available. A preliminary survey focused on the level of dermatomycotic infections has been carried out. The sites investigated were barber shops and one small private clinic that deals with dermatomycoses. The result was amazing and prompted me to expand the study by focusing other areas such as TB patients and others with underlying illnesses. The current event, establishing a society of medical mycoses for Africa, will certainly be a golden opportunity for mycologists like me who have an isolated laboratory in this area.

## Original aspects of dermatophyte infections in Senegal

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Use of depigmenting agents for cosmetic purposes is a common practice in West African women. In a representative sample of senegales women, 52.7% were using bleaching products. In 425 women using these products dermatophyte infection was observed in 128 (30%). Steroid use was significantly associated with the presence of dermatophytosis. During a twelve months period we observed 20 adults with tinea faciei at the dermatologic clinic of the university hospital of Dakar. All patients were female, 16/20 cases were a complication of cosmetic use of bleaching agents which were topical corticosteroids. *Trichophyton soudanense* was the most frequently isolated agent. In Europe or in USA tinea capitis is very unusual in adults. Most of cases are detected in post-menopausal women with often an underlying immunosuppressive disease. There is a lack of data concerning adult tinea capitis in Africa. During a five years period we observed 158 adults (140 women and 18 men) with tinea capitis in Dakar, Senegal; the mean age was 33.1. *T soudanense*, *Microsporum langeronii*, *T rubrum*, *T violaceum*, *T schoenleinii* were also isolated. Tinea capitis in female adults is not exceptional in subsaharian Africa, this data was also recently found in Gabon. As gabonese authors we noted the atypical or mild clinical manifestation of tinea capitis in adults.

## **Ethnomycological knowledge in Burkina Faso**

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Ethnomycological information was gathered in Burkina Faso, West Africa, among three ethnic groups. Gathering of the wild edible mushrooms is a traditional activity, but today this tradition seems to be dying out. Mainly elder people know about and appreciate mushrooms. Women are more knowledgeable than men. There are significant differences between ethnic groups and villages as well; people in more remote areas possess more knowledge on mushrooms than in villages near urban areas. The system of naming and classifying mushrooms of the three ethnic groups is based on morphological, colour and habitat considerations and correspond well with scientific definitions. The edible mushrooms belong to the following genera: *Agaricus*, *Termitomyces*, *Phlebopus*, *Russula*, *Amanita*, *Lactarius*, *Psathyrella*, *Macrocybe* and *Leucocoprinus*. There are only few indications on the use of mushrooms in medical practices.

## **Fungal dermatological infections of inmates at a prison facility in Awka Anambra State, Nigeria**

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Skin infection is considered a major public health problem in Nigeria and is even more so in the prison system with the overcrowding, humidity and minimal medical attention. A survey of dermatological fungal infections of male prisoners was carried out over a 12-month period. Samples were collected from 17 incarcerated males aged between 17-40 years. The respondents were randomly chosen from a prison population that consulted with the medical personnel on biweekly basis. Questionnaires and skin scrapings were the experimental tools used. Scrapings were collected from the respondents at the beginning of the study and again at the end of the study. Samples were inoculated onto Sabouraud dextrose agar alone or supplemented with chloramphenicol (0.05 mg/mL) and or cycloheximide (0.5 mg/mL). Yeasts constituted 25.8% of the isolates, other isolates included *Aspergillus*, *Cladosporium*, *Fusarium* and *Trichosporon*. A number of bacterial isolates were also identified. Antifungal susceptibility tests showed that polyene antifungals were the most effective at MIC of 3.125 mg/mL. The study indicates that opportunistic infections may be quite prevalent among prison inmates and that these infections can be effectively treated using easily available antimycotics.

## **Bovine ringworm in the Shahrekord area, Iran**

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Dermatophytosis (Ringworm) is caused by the invasion of dermatophytes on keratinized epithelial cells and hair fibers. The induction factor is fungi, which grow on the hair or skin or both. This survey was conducted to determine and isolate the different *Trichophyton* species which cause dermatophytosis in cattle of Shahrekord areas. The study was carried out during Autumn and Winter (2002) and in this research hair and skin scrapings have been taken from 50 suspected cows. Laboratory experiments carried out by examination of skin scrapings and hairs, through direct microscopic means and by culture.

The highest percentage of infection was seen in 2-5 months old calves (34%). Infection rates in females were 45 cases (90%) and in males, 5 cases (10%). In most cases damages were seen in head and neck (64%). The numbers of damages were variable between 1 and 7 in one animal and the sizes of the damages were 2-5 centimeters in average. These fungi were isolated and determined in direct skin scrapings, culture of 47 samples out of total 50. *Trichophyton verrucosum* was isolated from 36 samples, and *Trichophyton mentagrophytes* was isolated from 11 samples.

## Favourable medium for sporulation of keratinophilic fungi

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**Introduction and objectives:** Dermatophytes are keratinophilic fungi that are able to attach to and decompose keratin. We describe a simple and inexpensive method for preparation of a low cost medium which permits abundant sporulation of keratinophilic fungi.

**Materials and Methods:** Materials were: *Ipomoea batatas* L. (sweet potato) that was bought from the Melen food-market, and hoof was collected from the Yaoundé city abattoir. Medium was prepared in similar technique used to prepare Potato Dextrose Agar (PDA) in the laboratory. Formulation per litre of medium consisted of extracts from sweet potato (300 mL) prepared from 200 g of peeled and diced potatoes, and hoof (200 mL) obtained from 15 g of ground hoof. Agar (15 g) and glucose (20 g) were added. Sabourauds dextrose agar (Oxoid Ltd., England) was used as positive control. Inoculation of medium was done with single spore obtained by standard dilution plate method. Tubes were inoculated in 5 replicates and incubated in a randomised block design on laboratory bench at room temperatures ( $25 \pm 2^\circ\text{C}$ ). Descriptions and spore count were based on 15-day and 7-day old cultures respectively for dermatophytic strains and *Fusarium* spp. isolates. Spore suspension was prepared and spore count done using the Hawksley BS 748 haemocytometer.

**Results and Discussion:** The pH of the medium was between 6-7. The medium after setting was consistent and transparent. There was no visible difference from medium appearance and the Sabouraud\_s medium control. A total of 30 strains that is, 25 dermatophytes (17 *Trichophyton rubrum* plus 8 *Microsporum canis*), and 5 isolates of *Fusarium* species all obtained from clinical specimens were studied. Mycelia were abundant on control medium and very scanty on experimental formulation. Pigmentation when present was also more conspicuous in the control than in experimental medium formulation. On the contrary spore count was significantly higher in experimental medium than in the control. From the above it is evident that these fungi can utilise keratin from hoof source which contains 12-14% nitrogen and sweet potato for growth and sporulation.

**Conclusion:** Having established the keratin requirement we preliminarily conclude that this medium can be used to obtain abundant spores of these species. Meanwhile studies continue to determine the optimal conditions.

## Imported *Histoplasma capsulatum* histoplasmosis in two French university hospitals

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Histoplasmosis is the commonest systemic mycosis imported in France. Between 1970 and 1994, 94 cases of *Histoplasma capsulatum* infection and 23 cases of *Histoplasma duboisii* infection were seen in France. With the emergence of HIV infection a large number of *H. capsulatum* histoplasmosis were observed in AIDS patients: 51 over 94 (54.2%), 26 over 51 were reported in 1993 and 1994. During a four years period, from 2001 to 2004, 8 *H. capsulatum* histoplasmosis were diagnosed in Saint-Antoine and Tenon hospitals, Paris. All were disseminated histoplasmosis: 7 were observed in HIV-infected patients with CD4 <100, the last one had a liver transplantation. The most frequent presentation of our patients was febrile pancytopenia. The disease was acquired in Africa (4), South-America (2), Carribean (1). The case following a liver transplantation concerned a frenchman who never left France. Initial diagnosis of histoplasmosis was made by positive findings on direct examination of May-Grünwald-Giemsa smears in 6 cases. In two patients diagnosis was established only by cultures of blood or bronchoalveolar lavage. Four patients received initial treatment with amphotericin B and four with oral itraconazole. A patient died after four months of antifungal treatment, two were lost of view. Surviving patients were followed for an average range of 16-44 months.

## **Dermatophyte pseudomycetoma in Senegal (West Africa)**

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Dermatophyte pseudomycetoma are very uncommon. Most of the described cases were observed in black African patients. Dermatophyte pseudomycetoma is a chronic lesion of the scalp or the nape suggestive of lipoma or epidermal cyst. In the majority of cases, diagnosis was done on histopathological findings. Granules are numerous, white, with hyphae, frequent vesicules and cement. They are surrounded by foreign body giant cells. In two of our five cases cultures of granules were done. *Microsporum langeronii* was identified in one case and *Trichophyton soudanense* in the other. In these two adult female patients tinea capitis was also present, the same agents were isolated from infected hairs. Treatment of dermatophyte pseudomycetoma is surgical excision as antifungal therapy alone is often insufficient to eradicate it

## **Black fungi in our drinking water; clinical potential of the main agents**

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Municipal drinking water in NW Europe contains a large diversity of fungal species. We isolated the fungal flora with a selective protocol. Many of them proved to be melanised. The preponderant black species was previously identified on morphological grounds as *Phialophora* (order *Chaetothyriales*), a fungal genus containing human pathogenic species. Molecular identification using ribosomal DNA ITS and SSU sequences showed that the fungus should be classified in *Cadophora* (order *Leotiales*). One of the striking properties of this fungus is its psychrophily and its wide distribution in the temperate climate and sub-arctic zones on the northern and southern hemispheres. In search of the natural niche of this fungus contaminating drinking water, we included closely and more distantly related species in our studies. The fungus seems to be a potential plant pathogen. Its frequent occurrence in drinking water indicates its main mechanism of dispersal. The wide distribution of the fungus will enable population genetic studies in the near future.

## **Recent update in the etiology of dermatophytoses amongst children in Southern Nigeria**

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A recent survey of dermatophytoses was carried out amongst children in two Nigeria states of Anambra and Delta in the south east and south south geographical flanks respectively during the period January 2003 to December 2003. The study involved 1263 (Delta) and 1624 (Anambra) children aged 4-16 years. Incidence was significantly higher ( $p < 0.05$ ) in young children aged 7-11 years and 4-6 years than in older children aged 12-16 years among samples proven to be mycologically positive by microscopy, culture or both in both states. There were significant differences in the incidence of dermatophytoses amongst children in urban and rural areas in and between the two states investigated ( $p < 0.05$ ). *Tinea capitis* was the predominant clinical type in both cases. While *Trichophyton tonsurans* was the most prevalent etiological agent in Anambra state, *T. soudanense* prevailed in Delta state. Comparative analysis with my recent study in the northern geographical zone established that incidence of dermatophytoses is higher in northern Nigeria. These data suggest a changing pattern in the etiology of dermatophytoses in southern Nigeria.

## **Distribution of dermatomycosis in Cross-River upstream bank of Eastern Nigeria**

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A total of 2522 pupils were clinically examined for presence of skin lesions suspected to be dermatophytic infections. Fourteen primary schools were visited and one hundred and seven samples were collected. Out of the samples collected 51 were *Tinea capitis*, 34 were *Tinea corporis* and 22 were *Tinea versicolor* (Pityriasis versicolor). The disease occurred more in males (73%) than in females (27%). Infection were found more in pupils of age limit 6-14 years except for Pityriasis versicolor that occurred between the ages of 12-15 years. Based on mycological studies of the collected samples, seven different species of dermatophytes were isolated namely: *Trichophyton tonsurans*, *Trichophyton mentogrophytes*, *Trichophyton violaceum* and *Microsporum audouinii* in *Tinea capitis* lesions. *Trichophyton schoenleinii* and *Trichophyton rubrum* were isolated from samples from *Tinea corporis* lesions. Pityriasis versicolor lesions gave only one biotype from the samples: *Malassezia furfur*. Cultural and religious practices of this region seem to enhance the spread of this disease.

## Searching the natural life cycle of human pathogenic black yeasts in Thailand

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The black yeast *Exophiala dermatitidis* commonly occur in in public steam baths and is occasionally seen as agents of systemic, potentially fatal human disease. We revealed the natural ecological niche of the fungus in the tropical rain forest. It occurs in local foci on the surface of wild fruits and berries, subsequently passes through the stomach and intestinal tract of fruit-eating, warm-blooded animals such as birds and bats, and is dispersed on a small scale via the faeces. This ecology was established by selective isolation involving an acidic step in Raulin's solution and a high temperature step at 40°C on ECA medium. Incubation was for several weeks. This procedure excludes the ubiquitous fruit-inhabiting contaminants, which cannot survive at temperatures above 35°C. Samples taken in the Khao Khaew Open Zoo, Bala Natural Park, Narathiwat Province in Thailand proved to be positive. Also saunas in Bangkok were positive, similar to steam baths in Europe. Most strains belong to ITS rDNA genotype B, whereas nearly all invasive, neurotropic strains are of the pathogenic A genotype. The bathing facilities harboured the genotypes A and B in more or less equal frequency.

**Chemical composition of *Ganoderma lucidum* (Leys.:Fr.) Karst. and *Phellinus pachyphloeus* (Pat.)Pat., macrofungi used in traditional medicine in Burkina Faso (West Africa)**

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An enquiry was conducted in local markets of Ouagadougou (Burkina Faso) in 2003. A total of 30 herbalists were interviewed. The aim was to gather information on medical macrofungi. In order to know composition of useful species *Ganoderma lucidum* and *Phellinus pachyphloeus* were chosen for biochemical analyses. There are few records of macrofungi in traditional medicine. Most reported medicinal uses concern Polypore species. The biochemical composition *Ganoderma lucidum* and *Phellinus pachyphloeus* are almost similar. Anthracenosides, Polyphenols, sterols and triterpens, emodols, essential oil, saponosides and carotenoids were isolated.

## Comparison of the microscopic MEC, macroscopic MEC and spectrophotometrically MIC of caspofungin for dermatophytes

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Background: Caspofungin (CAS), a member of the echinocandins, is an antifungal agents which blocks the synthesis of 1,3- $\beta$ -glucan of the fungal cell wall. Previous studies of the minimum effective concentration (MEC) of CAS against *Aspergillus* spp. was assessed by macroscopic and microscopic observations. However it is easier to determine the MEC macroscopically, and it would be even better if it can be measured with a spectrophotometer. Therefore the aim of this study was to see if there is a concordance between the results of the microscopic MEC, macroscopic MEC and spectrophotometrically read MIC of CAS for dermatophytes.

Methods: The in vitro susceptibility of the species *Microsporum canis* (4), *Microsporum gypseum* (2), *Trichophyton mentagrophytes* (5), and *Trichophyton rubrum* (6) for CAS was assessed with a broth microdilution (BMD) method (Fernández-Torres et al. J Clin Microbiol 2002,40:3999) in three different ways, macroscopic and microscopic (Espinel-Ingroff J Clin Microbiol 2003,41:401) and spectrophotometrically at 405 nm. Results: The agreements of the comparisons were (see table):

Species (number of isolates tested)	Agreement (%)	
	micro/macro	micro/spectro
<i>M. gypseum</i> (2)	50	33
<i>M. canis</i> (4)	75	50
<i>T. mentagrophytes</i> (5)	80	13
<i>T. rubrum</i> (6)	100	6

Conclusions: Spectrophotometrically reading is not an option to determine the MEC. The MEC for *Trichophyton* ssp. can be determined macroscopically. The MEC for *Microsporum* ssp. has to be assessed microscopically.

## Comparison of the E-test with broth microdilution for antifungal susceptibility testing of amphotericin B for dermatophytes

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Background: The E-test is an easy method to test the in vitro susceptibility of moulds. In a previous study (M-1217, 2003, 43rd ICAAC) the susceptibility for amphotericin B (AMB) of dermatophytes using the E-test on RPMI 1640 agar was compared with a published broth microdilution (BMD) method. A low agreement (0-50%) was found. Aim of this study was to compare the results of the E-test performed on antibiotic medium 3 (AM3) for AMB with the BMD method for dermatophytes. Methods: Antifungal activity of AMB was tested against clinical isolates of the species *Microsporum canis* (4), *Microsporum gypseum* (2), *Trichophyton mentagrophytes* (5), and *Trichophyton rubrum* (6). For the E-test we used an inoculum concentration of 65-70% T at 530 nm, an incubation temperature of 30°C, and an incubation time of 7 days. The MIC was read at 100% growth inhibition. Results: The agreements of the E-test compared to the BMD method were (table):

Species (number of isolates tested)	Agreement (%)
<i>M. gypseum</i> (2)	100
<i>M. canis</i> (4)	100
<i>T. mentagrophytes</i> (5)	73
<i>T. rubrum</i> (6)	100

Conclusions: A previous study has shown that the agreement between the results of the E-test on RPMI 1640 agar and the BMD method for AMB were low. This study the agreement between the E-test on AM3 agar and the BMD method for AMB was good for 3 out of 4 species (73-100%). If susceptibility testing for AMB of dermatophytes is warranted the E-test on AM3 agar has the preference.

## Yeast and dimorphic fungi associated with human infections in South Eastern Nigeria

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A total of 1,924 specimens from 9 different types of samples were examined by direct microscopy and culture to recover yeast and dimorphic fungi associated with human infection. Identification of yeasts was based on their carbon assimilation patterns, using API 20C AUX and ID 32C (bioMérieux, France) commercial kits. A total of 179 specimens (9.3%) were positive for yeasts. Most of the yeast isolates were recovered from urine samples and genital swabs. Prevalence was significantly higher in females (14.7%) than in males (1.5%)( $p < 0.05$ ). The age 21-30 years group recorded the highest prevalence of yeast infection (16.2%) followed by age group 11-20 years (11.9%) and >40yrs (5.6%). Difference in percentage prevalence of yeasts among the age groups was statistically not significant ( $p > 0.05$ ). However, when genital samples were considered, prevalence was significantly higher in the age group 21-30 years than in older ones ( $p < 0.05$ ). Isolates recovered included seven species of *Candida* and *Trichosporon inkin*. *C. albicans* accounted for the highest number of isolates (128) followed by *C. tropicalis* (23) and *C. parapsilosis* (9). Two isolates each of *C. famata* and *C. norvengensis* were recorded and reported for the first time in South Eastern Nigeria. The two isolates of *T. inkin* were recorded from perianal lesions and are also reported for the first time in this area. *C. albicans*, *C. glabrata*, *C. parapsilosis* and *C. krusei* were found to be the most common yeast species that act as agents of human disease in South Eastern Nigeria. The only dimorphic fungus met in the study, *Histoplasma capsulatum* var *duboisii* was recovered from a case of African histoplasmosis with subcutaneous lesions.

## **Study of *Malassezia pachydermatis* contamination of domestic dogs referred to a pet clinic in Sharekord**

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*Malassezia pachydermatis* is a lipophilic yeast. It is commonly found on normal and abnormal skin and within the ear canal, chin, lips, anal sacs, vagina and rectum of the dog. Factors that may contribute to *Malassezia* proliferation include: excessive sebum production, allergy, bacterial skin disease, endocrinopathy, alternation of T cell response, disruption of the normal skin flora, disruption of the epidermal barrier, accumulation of moisture. Otitis externa and *Malassezia* dermatitis result from *Malassezia pachydermatis*. Since dogs are one of the most popular pets among people we decided to evaluate these animals for infection of *M. pachydermatis*. We examined 200 dogs which are referred to a pet clinic in Sharekord, randomly, and we took samples from their bodies and ear canals by scrape and swab and tooth brush methods. Samples were collected and put in sterile pockets and sent to the mycology lab of the sanitary college of Sharekord Azad University for differential diagnosis. After the evaluation of 200 samples which were collected from 200 indoor dogs in Sharekord city during January 2002 to August 2003, some saprophytes, 15 cases of *M. pachydermatis*, two cases of *Candida albicans* and six cases of other yeasts were found.

## **Reduction of fumonisin B1 and zearalenone by lactic acid bacteria in fermented maize meal**

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*Fusarium* species of fungi infecting maize worldwide elaborate mycotoxins, which have been associated with cancer incidence worldwide. This study was carried out to investigate the potential of lactic acid bacteria (LAB) fermentation in reducing mycotoxin concentration and toxicity in maize meal products. Maize meal was spiked separately with fumonisin B1 and zearalenone, and fermented for four days. The potential cytotoxicity of the mycotoxin-spiked fermented extracts was also investigated using an SNO cell line. A significant decrease ( $p < 0.001$ ) in the concentration of the two mycotoxins was observed, with a 56-67% and 68-75% reduction in the third and fourth days respectively. In addition, the two mycotoxins were not detectable in commercially fermented maize meal (amahewu) samples. After fermentation, mycotoxin-spiked maize meals containing LAB culture were comparatively less toxic to SNO cells than those without LAB. However, this observation was not statistically significant ( $p = 0.295 - 0.681$ ). These results indicate that lactic acid bacteria fermentation can significantly reduce the concentration of mycotoxins in maize to trace levels. However, such a reduction may not significantly alter the possible chronic toxic effects of such toxins.

## Histoplasmosis in Southern Africa

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Histoplasmosis, a fungal disease caused by *Histoplasma capsulatum*, is well documented in southern Africa. It presents in five different forms:

Acute benign pulmonary – not uncommon

Acute disseminated – rare

Chronic pulmonary – rare

Chronic localised – rare but increasing

Chronic disseminated – rare but increasing

This poster documents the distribution of acute benign pulmonary histoplasmosis in southern Africa, viz. Zambia, Zimbabwe, the former Transvaal, and the southern Cape. It describes the clinical features of, and recommends symptomatic treatment for, immune competent patients. Immune compromised patients, and the other forms of histoplasmosis, require specific anti-fungal therapy. Despite 25 years experience of this uncommon disease in Cape Town, there are several unanswered questions viz.:

What are the optimal conditions for the survival of *H. capsulatum* spores in bat guano?

Why do the antibody studies not correlate with the clinical and radiological features?

Why should exposed children not get the disease while simultaneously exposed adults become infected?

Why are we now seeing histoplasmosis in immune compromised patients who give no history of exposure to bat guano?

## **Susceptibility of clinical isolates of *Cryptococcus neoformans* to commonly used antifungal agents at Dr. George Mukhari Hospital, Pretoria**

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Cryptococcal meningitis is treated with amphotericin-B and fluconazole during the induction phase and fluconazole is used thereafter for lifelong prophylaxis. With the continued use of any antimicrobial, there is concern for the development of resistance. In the case of fluconazole, reports are emerging of resistance developing after long term use. The aim of this study was to assess the susceptibility profile of *Cryptococcus neoformans* to fluconazole (Diflucan Patnership Programme), amphotericin-B, and voriconazole after the introduction of free fluconazole for cryptococcal meningitis and to evaluate the use of the disc diffusion method for susceptibility testing. Fifty consecutive clinical isolates obtained from patients with cryptococcal meningitis at the Dr. George Mukhari Hospital, Pretoria from January 2001 to June 2002 were analyzed. The micro broth dilution (BMD) test, the Etest, and the disc diffusion susceptibility test were performed on the isolates. All isolates were susceptible to amphotericin-B and voriconazole with MIC90 of 0.25 mg/L and 0.125mg/ respectively. MIC90 for fluconazole was 8 µg/mL. Two isolates were in the 16-32 µg/mL and 2 isolates had MIC of 64 µg/mL and 128 µg/mL for fluconazole. There was a 98% agreement between the disc diffusion and the Etest for fluconazole. Only one isolate that was susceptible dose dependent (SDD) by the Etest was shown to be resistant by disc diffusion testing. The majority of the isolates are still susceptible to fluconazole and there is no adverse impact as yet on the susceptibility profile for prophylaxis. The disc diffusion method is shown to be useful for rapid testing for clinical management of cryptococcal meningitis. In view of this, ongoing surveillance is essential to monitor trends in the susceptibility profile.

## **Clade related Amphotericin B resistance among South African *Candida albicans* isolates**

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Oral candidiasis is the most commonly encountered oral manifestation in HIV/AIDS patients and *Candida albicans* the most frequently associated species. Recently DNA fingerprinting established 5 sub-types, or clades, with regional specificity, among clinical isolates of *C. albicans*. The SA clade predominated among isolates obtained from black, healthy individuals as well as HIV/AIDS patients in South Africa. Antifungal resistance testing of South African oral yeast isolates revealed that 8.2% of isolates were resistant to amphotericin B.

**OBJECTIVE:** The purpose of this study was to determine whether the observed natural resistance to amphotericin B among oral isolates of *C. albicans* was related to a particular clade, and whether these isolates demonstrated phenotypic switching.

**METHODS:** Thirty eight *C. albicans* isolates which were previously found to exhibit a natural resistance to amphotericin B (MIC >1 µg/mL) were DNA fingerprinted using the moderately repetitive Ca3 probe. The isolates were also grown on Lee's medium to determine phenotypic switching through the identification of different colony morphologies.

**RESULTS:** Of the 38 resistant *C. albicans* isolates, group SA constituted 29 (76%), while groups I, II and NG (isolates not belonging to any of the groups), constituted 3 (7.9%) each

Chi-square 11.93;  $p < 0.02$ ). None of the resistant isolates belonged to group III. All isolates revealed phenotypic switching as was evident from a variety of different colony morphologies.

**CONCLUSION:** A statistically significant number of amphotericin B resistant isolates belonged to the SA clade and this resistance is probably related to phenotypic switching.

## **Species of *Phaeoacremonium* associated with human infections and environmental reservoirs in infected woody plants**

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Three species of *Phaeoacremonium* have been associated with phaeohyphomycosis, namely *P. parasiticum* (formerly *Phialophora parasitica*), *P. inflatipes* and *P. rubrigenum*. Numerous unknown isolates resembling *Phaeoacremonium* spp. have in recent years been isolated from human patients, as well as from woody plants that appear to be the main environmental source of these fungi. Nine new *Phaeoacremonium* species, of which six were obtained as etiologic agents of human opportunistic infection, are reported. Mostly post-traumatic subcutaneous invasion and occasional deep infection have been found associated with isolates of *Phaeoacremonium* isolated from humans. The new species from human sources show a similar pattern where the majority of the isolates were from subcutaneous sites, but some from joints and one from a blood culture. The species of *Phaeoacremonium* can be identified based on their cultural and morphological characters, and the identifications are strongly supported in phylogenetic analyses of partial sequences of the actin and beta-tubulin genes. A multiple-entry electronic key based on morphological, cultural and beta-tubulin sequence data was developed to facilitate routine species identification. Re-examination of all isolates of *P. inflatipes* associated with human disease showed them to be misidentified and to belong to new, previously undescribed taxa.

## **Pediatric cryptococcosis in Gauteng Province, South Africa: results of population-based active surveillance**

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**Objectives:** To describe the clinical and demographic characteristics of children with cryptococcosis in Gauteng Province. Prior to the HIV epidemic, cryptococcosis was rarely if ever seen in children.

**Methods:** Population-based surveillance for cryptococcosis is currently conducted in Gauteng (population 9 million), South Africa starting 1 March 2002. Incident cases were defined by the isolation of *Cryptococcus neoformans* from any specimen, India Ink or latex agglutination, in a resident of Gauteng. Cases were identified prospectively by regularly visiting laboratories throughout the province. Information collected included demographic and clinical characteristics, treatment, diagnostic tests and outcome at end of hospitalization.

**Results:** During March 1 to November 30, 2002, 933 cases were identified, of whom 9 (1%) cases occurred in children less than 15 years old. The estimated overall annual incidence rate was 0.6/100,000 children <15 years old, compared to 11/100,000 in the overall population. The median age of these children was 8 (range 0-14 years), and 2 were neonates. Six (66%) were male. Cryptococcosis was diagnosed by the isolation of *C. neoformans* from cerebrospinal fluid in all children. The most common presentation was cough (7/9), followed by fever, nausea and vomiting (5/9), neck stiffness and altered mental status (4/9). Among children older than 1 year, headache was the most common presentation (6/9). Seven children were known to be HIV-infected prior to diagnosis, and 3 had other AIDS defining illnesses. CD4 lymphocyte counts were known in only 2 patients (6 and 103/956;L). Treatment included amphotericin B (6 patients), and fluconazole only (3 children). Four (44%) children died during hospitalization.

**Conclusions:** Although rates of disease in children are lower than adults in South Africa, cryptococcosis occurs among children, in particular those infected with HIV. Physicians should be aware of this, and consider cryptococcosis in the differential diagnosis of HIV-infected children presenting with meningitis.

## POSTER PRESENTATION

### **Use of larger Fungi in traditional medicine by Nagot people in Benin (West Africa)**

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Larger fungi in Benin are diverse and commonly used by local inhabitants as food, in trades at local market and for traditional medicine. Local people hold traditional ethnomycological knowledge relatively and those from the Nagot people, located in central part of Benin, is deep, amazing and full of tuition, including some elements of pharmacology. The use of larger fungi by local people is widespread and include many species that are used either alone or in mixture but always by adding some others ingredients, leading to the so called receipt that fail to cure the disease when even one of the ingredients lacks. At local level, many *Polyporaceae* species are used for curing various diseases such as Oedemas and Scabies. Some *Gasteromycataceae* such as *Phallus* spp are ground and the outcome powder is applied during burn from fire. Such species could be found in trade at local market as medicinal items.

## **Assessment of the diversity of *Tomentella* species with great emphasis on their ectomycorrhizae with *Azelia africana***

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*Tomentella* spp. are telephoroid fungi whose ecological importance have been worldwide ignored until recently, as many researches highlighted the dominance of this group in ectomycorrhizal communities. Very few have been reported about the diversity and systematic of *Tomentella* in Africa. This group seems to present however great diversity and constitute undoubtedly putative partners of forest trees species of *Ceasalpiniaceae* (according to our recent collection trips). The studies of the diversity within this group and their ectomycorrhizae with *Azelia africana*, a multipurpose but endangered and threatened forest tree specie distributed in the whole west Africa, is a joint PhD project including the Laboratory of Applied Ecology of the University of Abomley-Calavi in Benin and the Laboratory of Systematic Mycology of the University of Munich in Germany. This study is aiming at the identification of the *Tomentella* fungi partners of *A. africana*, the anatomo-morphological characterization of their ectomycorrhizae according to both the age of the tree and the soil types. During three collection trips, *Tomentella* sporophores, ectomycorrhizae and soil samples will be collected under different aged individuals of *A. africana*. The sporophores will be dried as herbarium whereas the ectomycorrhizae will be kept in Formol-atmosphere, FAA and CTAB-buffer for further laboratory works. The identification of the sporophores will be realised through micro- and macroscopical features. The identity and the affiliation of the ectomycorrhizae to *Tomentella* species will be processed through molecular techniques notably the ITS-RFLP procedure and through the comparative ribosomal-DNA sequencing of both the sporophores and ectomycorrhizae. The ribosomal-DNA sequencing will be processed using BioEdit, a special sequencing software. Multivariate ordination techniques of the software CANOCO and/or PC-ORD will be applied to test the relationship between the ectomycorrhizae, the age of *A. africana* tree and the type of soil.

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