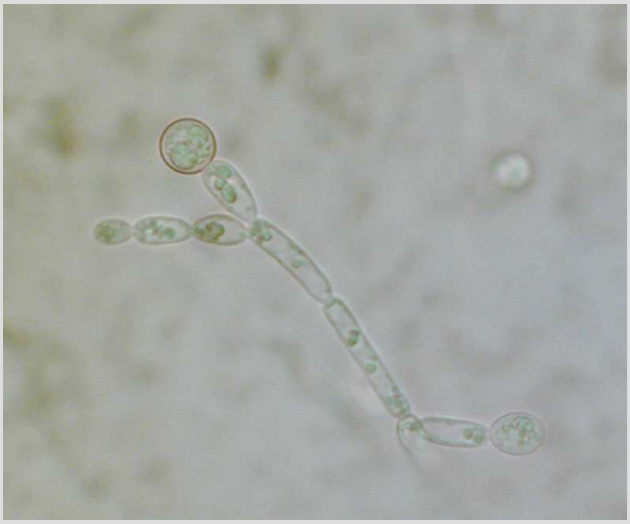
5th Nordic Society for Medical Mycology and 28th Swedish Society for Clinical Mycology Joint Scientific Meeting



Program and Abstracts

Impacts of Diagnostics on Antifungal Treatment.

Teaterskeppet, Stockholm, Sweden

May 22, 2008

Introduction

Dear Friends and Colleagues,

It is with great pleasure that we welcome you to the 5th Nordic Society for Medical Mycology and 28th Swedish Society for Clinical Mycology joint scientific meeting. The meeting this time takes place at Teaterskeppet, a beautiful ship taking us on a short cruise from the Stockholm harbour to the archipelago.

After setting the scene with lectures covering fungal infections in the different patient groups the scientific programme focus on the Impacts of Diagnostics on Antifungal Treatment and if New Developments have brought us any further. We are proud to welcome Theoklis Zaoutis from the Children's Hospital of Philadelphia in the US and Rosemary Barnes from the Cardiff University in the UK as key-note speakers together with a distinguished faculty of speakers from the Nordic countries who are ready to share their knowledge in their area of expertise.

At the end of the meeting we hope that every participant has learned something new, has been refreshed on something old and has had the opportunity to meet other Nordic colleagues within the field of medical mycology.

On behalf of the NSMM board

Lena Klingspor
Meetings Secretary NSMM & SSCM

Maiken Cavling Arendrup
President of NSMM

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This meeting would not have been possible but for the generous support of our sponsors. We gratefully acknowledge their contributions.

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Program

Impacts of Diagnostics on Antifungal Treatment.

Opening Ceremony And Fungal Infections In Selected Patient Populations

10:00 **Opening remarks and welcome**
Lena Klingspor, meetings secretary, *Maiken Cavling Arendrup*, president, NSMM.

10:15 **Fungal Infections in the ICU population.**
Tom Hartvig, Rigshospitalet, DK

10:40 **Fungal Infections in the Haematological population**
Per Ljungman, Karolinska University Hospital, SE

Keynote Lectures

11:05 **Fungal Infections in the Neonatal and Pediatric population**
Theoklis Zaoutis, Children's Hospital of Philadelphia, US

11:50 Coffee break

12:15 **Impacts of Diagnostics on Antifungal Treatment: Have New Developments brought us any further ?**
Rosemary Barnes, Cardiff University, UK

13.00 Lunch

Laboratory and imaging drivers for antifungal treatment decision

14:00 **Epidemiologic trends and Impact on Initial Antifungal Treatment.**
Peter Gaustad, Rigshospitalet, Oslo, NO

14:30 **Antifungal Drug Monitoring – Current status**
Filip Josephson, Karolinska University Hospital, SE

15:00 **The Impact of Histopathology on Antifungal Treatment**
Henrik Elvang Jensen, Dansk Veterinær Institut, Copenhagen

15:30 **Imaging options in invasive fungal infections**
Kirsi Volmonen, Helsinki University Hospital, FI

16:00 Coffee break

16:30 **Recent experiences with the Nordic EQA Programme**
Klaus Leth Mortensen, SSI, DK

Free Papers; Oral and Poster Presentations

16:45 **Oral *Candida* species in Pakistani adults in relation to type 2 diabetes and gender**
Fawad Javed, Lena Klingspor, Ulf Sundin, Mohammad Altamash, Björn Klinge, Per-Erik Engström

17:00 **Candida guilliermondii fungaemia and endophthalmitis in a patient with acute myeloid leukaemia**
Jon Peiter Saunte, M la Cour, Ditte Marie Saunte, Maiken Cavling Arendrup

Annual General Meeting For NSMM

17:15 General Assembly for members of the society

18:00 Farewell Dinner (25 €)

Abstracts

Fungal Infections In Selected Patient Populations

Fungal Infections in the ICU population

Tom Hartvig MD
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The importance of fungi as a pathogen in the ICU is a challenge for the intensivist.

Data from USA 1980-1990 show that's yeast accounts for 11% of all bloodstream infections in the ICU and that intensive care treatment is an independent risk factor of fungemia even when other risk factors such as central catherisationen is controlled. Other important known risk factors are patients with neutropenia, abdominal surgery, exposure to multiple or broad spectrum antibiotics, colonization with *Candida*, steroid use and immunosupression in transplanted patients.

In Europe no comprehensive study exists. The EPIC study a compromised single-day point prevalence study from 1400 ICUs Europe. Yeast was reported as the major organism in 9% of bloodstream infection. A shift in pattern of bloodstream infection with a decrease in *C. albicans* infection an increase of non albicans have been reported. The widespread use of azoles has been implicated in the trend, but other factors such as an increase in immunocompromised patients are probably involved. *Aspergillosis* in non-neutropenic patients is uncommon (0,6-0,8%). In neutropenic patients in the ICU the prognosis are poor if the neutrophil count does not recover. According to IDSA guidelines voriconazole should be the preferred drug, but liposomal amphotericin B as alternative primary therapy could be considered in some patients.

The diagnosis of invasive fungal infection in the ICU can be a cumbersome task. Early therapy is important and often the diagnosis is based on clinical suspicion in high risk patients. Surveillance cultures can help in early identification of the fungus. In general the blood cultures for yeast are positive in 3-4 days, but a significant proportion of patient with invasive fungaemia have negative blood cultures. DNA and RNA-based diagnostic methods hold promise for improved sensitivity and specificity, but these methods will require extensive validation in clinical studies.

Adjunctive methods are often used: CT scan/X-ray in diagnosing invasive fungal infection is unspecific (except pulmonary *Aspergillus* infection). The early identification of fungus species is important to predict susceptibility and resistance. Empiric treatment of invasive fungus infection with antifungal drugs should according to guidelines include broad spectrum antifungal drugs: Amphotericin B in lipid formulation or echinocandins given intravenously. Azoles in invasive fungal infections should only be used if the species is known, because non albicans species demonstrate resistance to azoles.

Data from ICU 4131 Righospitalet Denmark in the year 2006 and 2007 will be presented.

Fungal Infections in the Haematological population

Per Ljungman, Karolinska University Hospital, SE

No abstract submitted at time of printing the programme

Keynote Lecture 1

Fungal Infections in the Neonatal and Pediatric population

Theoklis Zaoutis, Theoklis Zaoutis, MD, MSCE
Assistant Professor of Pediatrics and Epidemiology
Director, Pediatric Infectious Diseases Fellowship
Associate Director, Center for Pediatric Clinical Effectiveness (CPCE)
The Children's Hospital of Philadelphia

There are increasing data to suggest that pediatric fungal infections differ from their adult counterparts in terms of epidemiology, risk factors and outcomes. Furthermore, even among pediatric patients with fungal infections there are differences. For example, fungal infections in neonates have a unique epidemiology. In addition, the pharmacokinetic and pharmacodynamic properties of antifungal agents are different in children. Despite the development of newer antifungal agents, there is a paucity of data on their efficacy and safety in pediatric patients. This session will provide an overview of the unique epidemiologic features of invasive fungal infections in children and provide an update on the treatment of these infections.

Keynote Lecture 2

Impacts of Diagnostics on Antifungal Treatment: Have New Developments brought us any further ?

Rosemary Barnes, Cardiff University, UK

Accurate diagnosis of invasive infection particularly aspergillosis remains problematic and mortality is high if diagnosis is delayed. This has resulted in the practice of empiric antifungal treatment in at-risk patients with refractory fever. Although still standard practice, there is little evidence to suggest that it confers a survival benefit and reduces invasive fungal infection in patients not on antifungal prophylaxis.

Development of molecular (antigen and PCR based) diagnostic tools has led to the view that antifungal management should be diagnosis-based. Antigen testing (but not PCR) is included within the European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) consensus definitions for diagnosing fungal infection although proposed modifications downgrade antigen positive patients without specific signs and symptoms into an unclassified category.

Semi-automated, standardised real-time PCR methods are being evaluated globally through the European Aspergillus PCR Initiative. The various PCR techniques perform consistently but variability in the efficiency of DNA extraction relate to volume used and methods of cell wall disruption.

Early studies suggest that use of biomarkers can lead to a reduction in empirical antifungal usage. Serial screening by antigen testing and HRCT has been evaluated as a guide for pre-emptive therapy. Maertens and colleagues[1] used daily EIA GM testing combined with early CT scanning in neutropenic febrile episodes. Antifungal treatment was given if two consecutive EIA GM results were positive and confirmed by bronchoalveolar lavage or CT findings. The approach suggested it was possible to reduce empiric amphotericin B without increased mortality or fungal-related death. Cordonnier and colleagues[2] randomised 293 patients with haematological malignancies to receive either empirical or pre-emptive therapy Patients were screened for GM and those on the empirical arm received antifungals if they had persistent fever. The pre-emptive patients were given antifungal agents only if they showed clinical signs or had a positive GM test. Survival was not significantly different between the two groups and although pre-emptive patients received significantly less antifungals than the empirical group, no significant cost savings were achieved.

Our experience has shown antigen testing and PCR to be clinically useful and cost-effective tools. The limitations, particularly the impact of prevalence of invasive fungal infection on the performance of these assays need to be understood[3]. The consistently high negative predictive value allows exclusion of invasive fungal disease and renders empiric antifungal drug treatment unnecessary. Other potential benefits include improved diagnosis and survival and decreased hospital stay.

References

1. Maertens J, Theunissen K, Verhoef G, *et al*. Galactomannan and computed tomography-based preemptive antifungal therapy in neutropenic patients at high risk for invasive fungal infection: a prospective feasibility study. Clin Infect Dis 2005; 41:1242-50.
2. Cordonnier C, Pautas C, Maury S, *et al*. Empirical versus pre-emptive antifungal therapy in high-risk febrile neutropenic patients: A prospective randomized study. Blood 2006;108: 572A.
3. Pfeiffer CD, Fine, JP, Safdar N. Diagnosis of invasive aspergillosis using a galactomannan assay: a meta-analysis. Clin Infect Dis 2006; 42; 1417-1427.

Laboratory and imaging drivers for antifungal treatment decision

Epidemiologic trends and Impact on Initial Antifungal Treatment.

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Invasive mycoses are a serious concern - the infections are difficult to diagnose, and, until recently, options for therapy have been limited. *Candida* species are the most common cause of fungal infections and have a high mortality rate. *C. albicans* is the most common species, but non-*albicans* strains are becoming more prevalent. The change in the epidemiology of invasive *Candida* infections to species less susceptible to fluconazole has impact on the empirical treatment.

Risk factors for systemic fungal infections, in particular *Candida* infections, include long-term central venous lines, neutropenia, abdominal surgery, exposure to multiple or broad-spectrum antibiotics, colonization by *Candida*, steroid use and immunosuppression.

The clinical symptoms and signs indicative of fungal infections are non-specific. Fever, in particular persistent, is an important sign, but not always present. Colonization by *Candida* in patients with unexplained fever, leukocytosis, and hypotension may be an important indication of invasive candidiasis

The diagnosis of invasive fungal infections poses significant clinical challenges. Early therapy is critical to improved outcomes and in most cases antifungal therapy is started on the basis of non-specific clinical symptoms and signs. Diagnosis of *Candida* infection relies on the fungal blood culture, but a significant proportion of patients with invasive candidiasis will have negative blood cultures. Blood culture methods are positive for yeasts by a mean time of 3 days. Recent efforts have focused on non-culture-based methods and adjunctive methods (x-ray) to improve diagnosis. Despite advances in non-culture-based methods for aspergillosis, only limited success has been seen for *Candida* and other opportunistic mycoses.

Species identification of fungi is an important tool to predict susceptibility or resistance. Amphotericin B is a broad spectrum antifungal and lipid formulations are less toxic and as microbiologically active as the parent compound. For the *Candida* species*, C albicans* is usually susceptible to most antifungals, whereas other species as *C. krusei, C. glabrata* and *C. norvegensis* are more likely to demonstrate resistance or reduced susceptibility to azoles. Most of the *Candida* species have a low MIC to the echinocandins. The MICs of echinocandins are highest against *C. parapsilosis* and *C. guillermondi*.

Breakthrough candidemia and recurrent candidemia is an increasing problem. To handle these infections the importance of fungistatic versus fungicide antifungals, pharmacokinetics and immunomodulatory therapy on the patient outcome have to be elucidated.

Antifungal drug monitoring – focus on voriconazole, itraconazole and posaconazole

Filip Josephson, Karolinska University Hospital, SE

Therapeutic Drug Monitoring (TDM) refers to the use of plasma drug concentration measurements for dosage adjustments, in order to achieve the drug exposure assumed to give the optimal efficacy in relation to toxicity. TDM may be utilised *electively* in situations of altered pharmacokinetics (e.g. in renal or hepatic failure, in the presence of significant metabolic drug-drug interactions, in pregnancy, in pediatric patients), in order to achieve the drug exposures generally seen with the recommended doses with documented efficacy. TDM-guided therapy may also be implemented *routinely* in order to achieve a defined target drug exposure assumed to be optimal in terms of efficacy and toxicity.

The definition of upper and lower plasma concentration targets for TDM is often rather problematic, and is dependent both on inferences from dose-ranging studies and on observational concentration-effect studies. MIC values (or other indices of *in vitro* susceptibility) for the infecting organism are sometimes used as putative target plasma concentrations in antimicrobial therapies. These, however, are generated under highly artificial conditions, not taking into account important pharmacological aspects such as variability of drug exposure during the dosing interval, plasma protein binding, tissue penetration and inoculum-size effects. Furthermore, the required plasma drug exposure for maximal effect in anti-infective therapy may vary considerably depending on host immunity and the anatomical locale of the infection. Therefore, direct use of the MIC or other parameters related to *in vitro* drug susceptibility as targets for optimal *in vivo* drug exposure, may not be appropriate.

Voriconazole has qualities that both illustrate the potential utility and the present limitations of TDM. Voriconazole exposure at a given dose varies considerably between patients, to a large extent due to different hepatic CYP450 enzyme activity, and to the presence or absence of putative interacting drugs. Also, the hepatic and CNS toxicities of voriconazole are to a certain extent exposure-dependent. Thus, TDM of voriconazole could be of substantial clinical value.

The interpretation of plasma drug concentration data for voriconazole, however, is not clear-cut. The clinical efficacy of lower voriconazole doses than those recommended by the manufacturer appear not to have been systematically evaluated. Also, the largest available pharmacokinetic/pharmacodynamic dataset for voriconazole (from the premarketing trials), failed to identify any lower target drug concentration with an adequate predictive value for treatment success or failure (FDA, Briefing Document for Voriconazole, 2001). Mainly, but not exclusively, based on MIC values for *Aspergillus* and *Candida spp*, sometimes adjusted for protein binding, lower target trough values from 0,25-2 mg/L have been suggested (Denning et al 2002, Trifilio et al 2005, Pasqual et al 2008, Smith et al 2006). Furthermore, animal models seem to suggest that voriconazole AUC rather than trough concentration may be the pharmacokinetic parameter that correlates most closely to effect (Andes et al 2003).

On the other end, the risks of hepatotoxicity and encephalopathy does seem to increase with increasing trough plasma concentrations. However, there are no clear target values highly predicitive of toxicity, though recently 5.5 mg/L was suggested as a possible threshold for the risk of encephalopathy (Pasqual et al 2008). Voriconazole shows non-linear pharmacokinetics and the intraindividual variability of drug exposure is great (Vfend Scientific Discussion, EMEA, 2005); therefore, one

expects difficulties in predicting the quantitative effect of a dose modification on drug exposure.

Itraconazole exposure at a given dose is also highly variable and dependent on gastric pH, food intake, immune status and CYP450 enzyme activity. Itraconazole has an active metabolite which is present in concentrations similar to those of itraconazole. Several studies have investigated the relation between itraconazole concentration and effect, many without being able to establish any such relation (reviewed by Buchowski et al 2005). On the basis of data mainly from studies of prophylactic treatment in immunocompromised patients, target trough levels of itraconazole >0.25-1 mg/L or a summary concentration of itraconazole and its active hydroxyl metabolite >1 mg/L have been suggested (e.g Goodwin and Drew 2008). The weakness of the documentation for any particular lower itraconazole target level must, however, be borne in mind when interpreting data. No upper cut-off level for itraconazole toxicity has been identified.

For posaconazole the premarketing trials have demonstrated relations between exposure and response both in prophylaxis and in treatment of invasive fungal infections; thus TDM might be helpful. In prophylaxis, data have suggested that an average posaconazol concentration exceeding 0.7 mg/L correlates with greater effect, and a target concentration of 0.350 mg/L 3-5 hours after dosing has been suggested (FDA-CDER Posaconazole, Clinical Pharmacology and Biopharmaceutics Review 2005). In the treatment of invasive aspargillosis, increasing global responses were reported by quartile of drug exposure, with mean average concentrations in the first and the fourth quartile of 0.134 and 1.25 mg/L respectively. Importantly, the bioavailability of posaconazole is strongly dependent on food requirement, and seems to be considerably lower the more ill the patient (Noxafil Scientific Discussion, EMEA 2005); thus confounding may affect these data. Given that dose increases above 800 mg/d have been shown not to give higher drug exposures, increasing the number of dosing occasions and optimising food intake may be a more effective intervention than increasing the total dose, in case of low posaconazole exposure. No upper cut-off level for posaconazole toxicity has been identified.

The Impact of Histopathology on Antifungal Treatment

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Invasive fungal infections have emerged as a major cause of morbidity and mortality in especially immunocompromised and debilitated patients over the past two decades. In susceptible patients, invasive fungal infections are often difficult to diagnose and treat. Especially three factors contribute to the high mortality of invasive fungal infections:

1) Lack of a reliable and timely diagnosis.
2) Clinical signs and symptoms are insignificant and often absent until the infection is at an advanced stage.
3) Antifungal therapies are currently suboptimal, and the response is depending on the correction of the underlying immune deficiency.

The availability of well-tolerable and highly efficacious systemic antifungals has improved the spectrum of therapeutic options and the success rates of antifungal treatment. However, with respect to high treatment costs associated with these new agents, it is mandatory to specify indications and limitations for the use of these substances that require accurate diagnoses, sometimes even to the species level.
In cases of candidosis, four overlapping forms of invasive behaviour are recognized: 1) catheter-related candidosis; 2) acute disseminated candidosis; 3) chronic disseminated candidosis including hepatosplenic candidosis; and 4) deep organ candidosis. A rational, early systemic antifungal treatment can be based on imagining diagnostic techniques as well as on conventional mycological and non-culturally based procedures. Radiology and different forms of scanning (e.g. CT) are highly efficient for the exact localization of nodules in e.g. hepatosplenic candidosis; however, the causes of "nodules" are numerous. Therefore, in order to obtain a diagnosis of e.g. hepatosplenic candidosis it is in most cases mandatory to take out tissue by trans-peritoneal needle aspiration biopsy, usually performed with CT guidance. In such cases, the diagnostic accuracy is very high, but of cause demands the presence of fungal elements within the actual specimen taken. Moreover, in each individual sample, cultivation may only be successful if viable elements are present, whereas histology and subsequent immunohistochemistry in most cases are independent of fungal viability. Similarly, "lesions" in other solid organs are in many cases not diagnosed as cases of mycoses until biopsy material has been examined histologically. Moreover, often the agent is not identified correctly until immunohistochemistry or other molecular-based techniques are applied.
In a recent retrospective study of invasive mycoses (n = 109), the histomorphological diagnoses were compared with diagnoses obtained immunohistochemically. In that study, a correct diagnosis of aspergillosis, candidosis, and zygomycosis was upheld in 69%, 88%, and 67%, respectively. Moreover, a correct diagnosis of dual fungal infections could only be confirmed in 17% of the histomorphologically based diagnoses. In the present series of cases, administration of different anti-fungal drugs had no significant influence on the misassignment of morphologically based diagnoses. With respect to identification of aspergillosis, candidosis, and zygomycosis, respectively, it appeared that the diagnostic specificity, when based solely on histomorphological criteria, was especially challenged in chronic lesions, lesions with massive necrosis, and lesions containing only few fungal elements.

Imaging options in invasive fungal infections

Kirsi Volmonen, Central University Hospital of Helsinki, FI

Imaging is a strong diagnostic tool when acute invasive fungal infections are suspected in an immunocompromised patient. Before laboratory tests even become positive, the only way to make an effort to diagnose an invasive fungal infection is often radiological imaging. However, it should be emphasized, that any imaging is unspecific and the only way to make a definitive diagnosis of invasive fungal infection is histological prove of fungus in tissue. The best imaging option depends on the area on infection.

In brain, the most useful option is MRI due to its superior soft tissue contrast compared to CT. CT may be used to follow any gross changes, and it can show fresh haemorrhage quite nicely, but MRI is strongly recommended in neurological problems of an immunocompromised patient. The infection spreads either haematogenously or from paranasal sinuses to the brain. The most common invasive fungal infections in the brain are Aspergillosis, Candidasis and Mucormycosis in Finland, only found in immunocompromised patients. Cryptococcosis is rare, is especially seen in AIDS, but can also be seen in immunocompetent hosts. Imaging findings are variable and unspecific mimicking other infections and even tumors. Necrosis, abscesses, hemorrhage are typical findings, contrast enhancement is variable. Cryptococcosis may cause meningitis. Liquor samples may be negative.

The lung is the most common site of invasive fungal infections. Whether conventional X-ray is positive or not, CT, preferably HRCT, should be performed. The most common invasive fungus is *A. fumigatus*, which may cause different kinds of infections, depending on the state of immunosuppression of the patient. The chronic semi-invasive form is found in mildly immunosuppressed patients, but the more acute invasive form is mainly found in severely immunocompromised patients. Usually it causes consolidations and nodules, often with a halo sign, but cannot be differentiated from other fungal infections and bacterial/mycobacterial infections/lobar pneumonias. Sometimes aspergillosis causes a brochopneumonia, which in radiological terms could be caused by any other infective agent, too, making differential dg impossible. BAL may be negative, but CT-guided thin needle biopsy may prove the diagnosis. *Pneumocystis jiroveci* (PCP) is a common fungal infection, too, though not truely invasive, causing ground glass infiltrations seen in HRCT. *Mucor* is very rare, radiology does not differ from *Aspergillus. Candida* is also rare, seen mainly in hematological malignancies and drug abusers. The radiological findings in candidasis of lung are highly variable. Endemic fungal infections like coccidioidosis, blastomycosis, histoplasmosis and paracoccidioidosis are mostly encountered in North- and South-America, but a travelling patient may get the disease and in immunocompromised patients may cause atypical, especially miliary/disseminated forms of these fungal infections.

Truly invasive fungal infections in the sinus area are rare, mainly caused by *Aspergillus* and *Mucor*. First imaging of choice is conventional x-ray followed by CT and/or MRI. CT, in contrast to MRI, is capable of showing calcifications, (air bubbles) and bone destruction often encountered in fungal infections but on the other hand MRI is better in showing the soft tissue involvement of the infection.

The fungal infections of the liver, spleen and kidney all share the same attributes. Ultrasound is the first imaging of choice due to its availability and low cost, but is less sensitive and less specific than CT and MRI. However, US guided biopsy and drainage are possible. If iodine cannot be given to the patient, MRI is especially recommended in suspicion of fungal infection of the mentioned organs. *Aspergillus, Candida* and *Mucor* are encountered in immunocompromised patients.

Recent experiences with the Nordic EQA Programme

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Background: A Nordic External Quality Assessment program in medical mycology was established in 2005. In order to monitor the level of routine diagnostics, not "best practice", the specimens were designed to resemble clinical samples and the laboratories were asked to handle the samples like routine samples.

Materials and Methods: Five simulated clinical samples were distributed at no cost to 63 Nordic laboratories of clinical microbiology of whom 54 submitted results. The specimens contained the following microorganisms: 1) *Fusarium solani* (corneal scraping), 2) *Candida (C.) albicans* and *C. krusei* in a ratio of 10:1 (blood culture), 3) *C. glabrata* and *Stenotrophomonas maltophilia* (tracheal aspirate), 4) *Aspergillus (A.) fumigatus* and *C. dubliniensis* (BAL), and 5) *C. inconspicua* (vaginal secretion). A brief clinical information was given for each specimen.

Results: Specimen 1: 48% (26/54) of the laboratories detected the mould, of whom 6 correctly identified it to the species level (23%). Specimen 2: 83% (45/54) reported the presence of the *C. krusei* isolate, but only 57% (31/54) also reported the *C. albicans* isolate even though the ratio was in favour of *C. albicans*. Specimen 3: 94% (51/54) detected the yeast, of whom 86% (44/51) correctly identified it as *C. glabrata*. Specimen 4: The presence of *A. fumigatus* was correctly reported by 59% (32/54) of the laboratories while 6% (three laboratories) did not report growth of a mould at all. Specimen 5: 17% (9/54) correctly reported the isolate as *C. inconspicua*, and 35% of the laboratories reported either the closely related *C. norvegensis* or non-albicans yeast. Thirty-four laboratories reported 528 susceptibility results. 22% (5/22) incorrectly reported the *C. glabrata*-isolate as fluconazole-susceptible. One laboratory incorrectly reported the *C. albicans*-isolate in specimen 2 as fluconazole-resistant.

Discussion: The results of this third distribution of simulated clinical samples emphasise that mycological diagnosis is difficult especially in the routine and polymicrobial setting and that there is a need for the continuous training of laboratory technicians and clinical microbiologists. However, a higher proportion of laboratories succeeded this year detecting the polymicrobial nature of the blood culture and the Aspergillus-isolate in the presence of yeast. This suggests that quality assessment programmes including simulated clinical samples are valuable for the improvement of mycological skills in clinical laboratories.

This work was also presented in part as poster (P1349) at the 18th European Congress of Clinical Microbiology and Infectious Diseases, 19-22 April 2008, Barcelona.

Oral *Candida* species in Pakistani adults in relation to type 2 diabetes and gender

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Background: In Pakistan, the prevalence of type 2 diabetes mellitus (T2D) is ~ 10%. Although oral candidal colonization is associated with T2D, the association of glycemic levels and gender with oral yeast carriage are scarcely addressed. The aim was to investigate the oral yeast carriage among Pakistani adults in relation to T2D and gender.

Materials and Methods: Oral yeast samples were collected from 68 type 2 diabetic and 78 non-diabetic (control) individuals by tongue surface scrapping. Identification was determined by a yeast identification system (API 32-C System bioMériux, Lyon, France) or with molecular recognition (18S rRNA gene). Random blood glucose levels (RBGL) were measured with a glucometer. Diabetic individuals with RBGL > and < 11.1 mmol/L were categorized as "hyperglycemic" and "non-hyperglycemic" respectively. Numbers of teeth (excluding third molars) were recorded. Smokers and individuals using antibiotics and steroids were excluded.

Results: Among hyperglycemic subjects, *C. albicans* colonization was higher in females compared to males ($P< 0.01$). These females had more teeth compared to hyperglycemic males with T2D ($P< 0.01$).

Discussion: In the present study, oral *C. albicans* colonization was increased in hyperglycemic females compared to males in the same group. The hyperglycemic females had approximately twice as many teeth as hyperglycemic males. The presence of more teeth may be associated with the increased *C. albicans* colonization in hyperglycemic females compared to males with hyperglycemia. Dental plaque, a composite biofilm which accumulates on the hard tissues in the oral cavity, also contains a variety of micro-organisms including *C. albicans* (Rosan B and Lamont RJ, 2002; Lofman H et al; 2003; Sissons CH, 2007). However, the association between *C. albicans* colonization and menopause can not be over-looked. The mean age of Pakistani females at menopause is approximately 47.1 years (Baig LA and Karim SA, 2006) compared to Sweden where menopause starts at the age of 50 years (Nedstrand E, 1995). In the current study, hyperglycemic females were in the post-menopausal phase.

Case Report: Candida guilliermondii fungaemia and endophthalmitis in a patient with acute myeloid leukaemia

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2 Unit of Mycology, Statens Serum Institut, Copenhagen.

A 51 year-old man with acute myeloid leukaemia in relapse presents with pneumonia and blurred vision.

He was treated in the ICU with 3 different antibiotics iv, and in two separate blood cultures obtained 3 days apart, Candida guilliermondii was found. Susceptibility pattern was determined by EUCAST susceptibility testing with the exeption of amphotericin B which was done using Etest The results is shown below.

	Fluconazole	itraconazole	voriconazole	Caspofungin	Amphotericin
MIC	>16	0.125	0.25	1	0.048
SIR	R	S	I	S	S
BP	2/4	0.125/0.5	0.125/NE	2/NE	1/NE

* Breakpoints are indicated as S≤X / R>Y. NE indicates Not Established.

All tests for viral, parasite and bacterial infection were negative. On funduscopy he presented with bilateral haemorrhagic and white multifocal lobular retinal infiltrates.

Antifungal treatment was initiated: Fluconazole + amphotericin B + 5-flucytosine combination therapy for four weeks iv followed by Voriconazole 800 mg orally for 4 days and then reduced to 400 mg for the following 4 weeks.

On follow up examination the white retinal infiltrates grew in both eyes despite ongoing voriconazole treatment. The serum concentration of voriconazole was 0.92 µg/ml (therapeutic level: 1-6 µg/ml).

Vitrectomy of both eyes was performed with intravitreal injection of amphotericin B, and visual acuity in the right eye increased from 0.9 to 1.0; in the left eye the visual acuity decreased from 0.1 to 0.02 upon treatment. The white retinal infiltrates diminished upon treatment with postoperative amphotericin B and 5-flucytosine iv. Microscopy and culture of speciemens obtained during vitrectomi were negative.

Conclusion

Fungaemia due to C. guilliermondii is rare. In Denmark 13/1562 blood isolates collected in the 4 year period 2004-7 were C. guilliermondii (0.8%) and only in two cases the fluconazole MIC was >16 µg/ml and in 3 the voriconazole MIC was >0.125 µg/ml (including the present isolate). The penetration of voriconazole over the blood Brain/eye barrier is app 46% (Lutsar CID 2003) and thus the combination of the serum level below the recommended range and the present isolates increased voriconazole MIC may have contributed to the progression during voriconazole treatment. Earlier studies have shown that vitrectomy is useful when Candida endophthalmitis is found. This together with species identification, susceptibility testing and drug level monitoring when voriconazole is used are recommended.

Posters

The comparison of Ketoconazole %2 solution effect with Ketoconazole %2 shampoo in treatment of seborrhoeic dermatitis

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Background: Seborrhoeic dermatitis (SD) is a chronic, inflammatory skin disorder, affecting areas of the head and body where sebaceous glands are most prominent and active.

The aetiology of SD is unknown, although hormones and the Malassezia spp. formerly known as Pityrosporum, are thought to be involved in the development of the condition

Previous researchs show that Ketoconazole (KCZ) is effective in topical applications for treating seborrheic dermatitis and dandruff. 2% Ketoconazole gel and shampoo has been developed for seborrheic dermatitis.

Because ketoconazole solution can be used in all surface body with minimum side effect, comparison of 2% ketoconazole solution and 2% ketoconazole shampoo on seborrheic dermatitis patients was studied.

Materials and Methods: 100 patients with seborrheic dermatitis (male:40, female:60 between 12-65 years) were enrolled to determine the comparison of 2% ketoconazole solution and 2% ketoconazole shampoo.The patients were evaluated according to itching, burning, erythema, scaling and seborrhoea as Severity index that was divided to three scales:1-mild(0-4),2-moderate(5-8).3-severe(9-12) in 0,14 and 28 days. Solution and shampoo was given twice a day for 4 weeks for each patient.the clinical response was graded as markedly mild, moderate and severe as scoring index according to itching, burning, erythema, scaling and seborrhoea. For isolation and identification of malassezia species, used direct examination with methylen blue staining, LNM(leeming&notman media), tweens assimilation and catalase test.

Results: Clinical improvement was evaluated (as markedly scoring index) in two group at 0,14 and 28 days. Scoring index varied from 3-11 in T0 and 1-8 in T14 and 1-8 in T28. There were statistically significant results between T0 and T14 and T28 in two groups(P-values < 0.0001).the frequency of isolation of Malassezia globosa was 43%, M. sympodialis 1% and M. furfur 25%. M. restricta 7%.

Discussion: The comparison of the 2% ketoconazole solution and 2% ketoconazole shampoo revealed that the efficacy of 2% ketoconazole solution was greater than 2% ketoconazole shampoo for 4 weeks after the end of treatment. These data approve other research and suggest twice-daily ketoconazole solution is an effective treatment for seborrheic dermatitis and a viable alternative to the 2% ketoconazole shampoo.