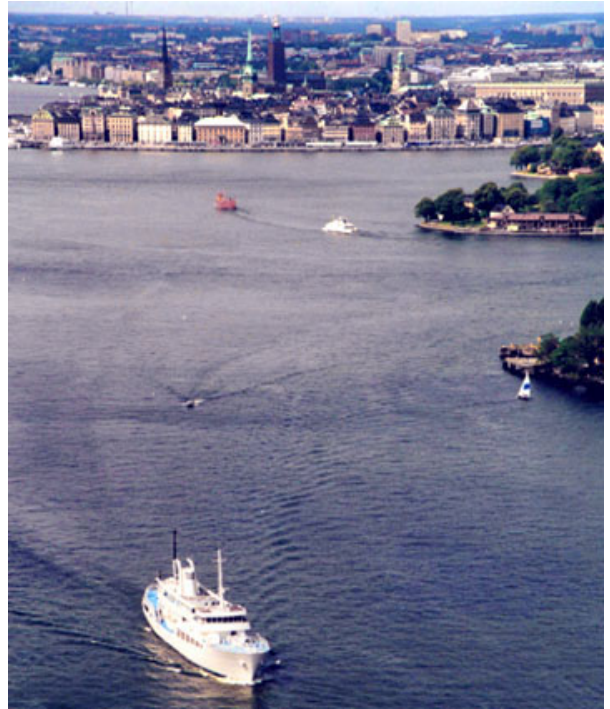


**1st Scientific Meeting of the
Nordic Society for Medical
Mycology
NSMM
& 24th Annual Meeting of the
Swedish Society for Clinical
Mycology
SSKM
A joint meeting**



Trends in Nordic Medical Mycology.

Program and abstracts

Teaterskeppet, Stockholm, March 12, 2004

Introduction

Dear Friends and Colleagues,

It is with great pleasure that we welcome you to the first joint meeting between the Nordic Society for Medical Mycology and the Swedish Society for Clinical Mycology in Stockholm, Teaterskeppet, Gamla Stan. <http://www.teaterskeppet.se/>.

The scientific programme focus on invasive candidiasis, and it has been our goal to cover as many aspects as possible of this important infection, i.e. clinical features, diagnostic markers and the different clinical presentations depending on different clinical settings. We are proud to welcome Frank Odds as key-note speaker 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 organizing committee

Maiken Cavling Arendrup
President of NSMM

Lena Klingspor
Meetings Secretary NSMM and SSKM

Sponsors

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

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Program

Trends in Nordic Medical Mycology.

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 intensive care setting**
Lars Heslet, Rigshospitalet, Copenhagen
- 10:40 **Fungal infections in the patient with hematological malignancy**
Niels Anker Peterslund, Århus Amtssygehus, Århus
- 11:05 **Fungal infections in the solid organ transplant patient**
Jan Tollemar, Karolinska Institutet, Stockholm
- 11:30 Coffee break

Keynote Lecture

- 12:00 **Candidiasis today.**
Frank Odds, University of Aberdeen, Aberdeen:
- 12.45 Lunch (free for members of the society)

Clinical Visual Markers And Diagnostic Approaches

- 14:00 **Skin manifestations in invasive fungal infections**
Jan Faergemann, Sahlgrenska University Hospital, Göteborg
- 14:30 **Oral manifestations in invasive fungal infections**
Olav Bergmann, Amtssygehuset i Herlev, Herlev
- 15:00 **In situ identification as a diagnostic tool in fungal infections**
Henrik Elvang Jensen, Royal Veterinary University, Copenhagen
- 15:30 **Culture and non-culture based diagnostics of fungal infections**
Lena Klingspor, Huddinge University Hospital, Stockholm
- 16:00 Coffee break

Free Papers; Oral And Poster Presentations

- 16:30 To be announced

Annual General Meeting For NSMM

- 17:30 General Assembly for members of the society
- 18:00 Dinner (free for members of the societies)

Abstracts

Oral Presentations

Fungal infections in the intensive care setting

Lars Heslet

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Invasive candidiasis is an important infection with a mortality similar to that of septic shock (40–60%). There is therefore a therapeutic imperative for treating severe mycotic infections. Mycosis is, however, difficult to recognise clinically leading to treatment omission or delay. This stresses the importance of the quality of microbiological recognition of *Candida* spp. in time.

The incidence of *Candida* infections has increased the last decade and *Candidaemia* is the fourth most frequent agent. In spite of an increasing body of evidence based on controlled trials the gap between the present state of the art of treating and evidence based prevention and treatment of invasive mycosis in critically ill patients is still too wide.

Use of antimycotic therapy is increasing and many new antimycotic agents have been marketed. The challenge for the ICU clinician, however, is to set up criteria for initiating and selecting the right antimycotic therapy for the right patient at the right time.

For patients with a lower initial risk, pre-emptive therapy should be based on a management strategy that takes into account the presence of definite risk factors and the quantitative evaluation of *Candida* colonisation exposure inasmuch as mycotic infection most often is preceded by colonisation. Azole prophylaxis is effective but may be associated with the emergence of resistance. Consequently such strategies must be restricted to highly selected groups of patients exposed to a high risk only.

Risk factors such as *Candida* colonisation, exposure to antibiotics, intravascular catheters etc., are significantly associated with the development of *candidaemia*. A predefined microbiological surveillance policy must include *Candida species diagnosis* and *susceptibility testing* in all surveillance cultures in order to ensure a timely and correct choice of antimycotic therapy in a sufficient dose.

Each ICU department should develop a protocollized antimycotic strategy for the clinical diagnosis and therapy in respect to 1) prophylaxis, 2) preemptive therapy and 3) goal directed antimycotic therapy based on a predefined mycological surveillance policy and focus on specific predefined patient categories at risk and on common risk factors for the prevention and treatment of severe *Candida* colonization and infection.

References

Eggimann P, Garbino J, Pittet D. Management of *Candida* species infections in critically ill patients.

Lancet Infect Dis. 2003 Dec;3(12):772-85.

Fungal infections in the patient with hematological malignancy

Niels Anker Peterslund

Århus Amtssygehus, Århus, Denmark

Abstract not available at time of printing

Fungal infection in the solid organ transplant patient

Jan Tollemar

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Invasive fungal infections remain a substantial cause of morbidity and mortality in solid organ transplantation. The fungal pathogens causing infections in transplant recipients are due to *Candida* or *Aspergillus* species. Fungal infections depend on which organ is transplanted, but is also affected by clinical progress. This is obvious in kidney recipients, with incidences of 45% reported during the 1960s, and in recent years, well below 10%. In liver recipients, the incidence ranges between 4% and 42% and for pancreas recipients, between 6% and 38%. Intestinal transplants recipients still have a high incidence of 53%.

The posttransplant course can be divided into three periods in terms of risk for infection: the first month, the 1–6-months, and more than 6 months after transplantation. During the first month, *Candida* infections mainly wound infections and, in case of contamination during surgery, invasive infections may develop. In the period 1–6 months, patients are susceptible to aspergillosis, *Candida* infections are uncommon. More than 6 months following transplantation, patients with poor graft function are at risk.

The choice of antifungal treatment for patients with organ transplants must be guided by the toxicity and interactions with the immunosuppressives used. We are currently witnessing an intense development of new antifungal drugs, but most as of yet not evaluated in transplant recipients.

To improve outcome of fungal infections in transplant recipients a high degree of awareness, aggressive use of diagnostics, and early institution of antifungal therapy must be utilized.

***Candida* today: state of the art**

Frank C. Odds

Department of Molecular and Cell Biology, University of Aberdeen, Scotland, UK

Although oral and genital *Candida* infections are still numerically the most common clinical problems caused by *Candida* species, the main focus on *Candida* for the last 10 years has been on disseminated forms of the disease in immunocompromised hosts, where attributable mortality rates remain high at above 30%. Considerable efforts to improve diagnostic methodology have still not yet resulted in any non-culture laboratory test that can be relied on to confirm accurately that a neutropenic patient with fever unresponsive to antibiotics has a *Candida* infection. Meanwhile, the introduction of novel antifungal agents in very recent years offers unparalleled new opportunities for management of *Candida* infections.

Molecular technologies have led to fascinating advances in the study of the epidemiology of *Candida* infections. DNA fingerprinting and multi-locus sequence typing have given us new insights into geographical distribution of clades of *Candida* species and their relation to phenomena such as antifungal resistance. On the basic science front there has never been a more exciting and productive time for the study of the pathogenesis of diseases caused by *Candida* and the interaction of the fungi with mammalian immune systems. Many possible molecular virulence factors have been identified, and the complexity of adaptive immune responses to *Candida* is becoming increasingly apparent. We can force strains of *Candida albicans* to mate in the laboratory (but not yet to produce stable progeny that complete a meiotic cycle).

Candida species, in particular *C. albicans*, have been a fascinating topic for study in both science and medicine for many years. That fascination looks set to continue for many years more.

Skin manifestations in invasive fungal infections

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Sweden

Invasive fungal infections are often a complication in the immunocompromised host. The reason for immune defects may be due to various immunosuppressive treatments as in organ transplant recipients, malignancy or auto-immune disorders. They may also be seen in patients with various malignancies, HIV-infection, chronic granulomatous disease, other immune defects and in breakdown of anatomical barriers such as the skin. *Aspergillus* and *Candida* accounts for more than 80 % of fungal infections. However, in bone marrow transplant patients *Fusarium* is often present. Cutaneous manifestations of invasive fungal infections are rather uncommon. In one study with *Aspergillus* infections in a large material with patients with acute leukaemia cutaneous signs of infections were found in 4 %. In secondary cutaneous aspergillosis *A. fumigatus* is the most common species but in primary cutaneous aspergillosis *A. flavus* is the most common species. Skin lesions of invasive fungal infections may be typical, but are very often non-specific or ambiguous. However, fungal skin lesions may be the first marker of a disseminated, potentially lethal fungal illness, so great attention should be given to their early recognition. Treatment is not different in patients with or without skin manifestations of invasive fungal infections. However, skin involvement together with systemic involvement is indicating a poor prognosis.

Oral manifestations in invasive fungal infections.

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Oral candidiasis is by far the most common type of fungal infection in the oral cavity. The incidence of oral candidiasis has increased during the last decades due to increased use of cancer chemotherapy, immunosuppressive agents and antibiotics, and to the emergence of HIV disease. The development of oral candidiasis can be explained by both systemic risk factors, and by a number of local risk factors such as increased colonisation density with *Candida* spp., decreased salivary flow rate and presence of dentures. A number of clinical subtypes exists, and several classification systems have been proposed. Oral candidiasis can be treated effectively by antifungal agents, and prophylaxis is effective in both granulocytopenic haemato-logical patients and in HIV patients. Other oral fungal infections such as oral zygomycosis and oral aspergillosis may be found in immunocompromised patients.

In Situ Identification as a Diagnostic Tool in Fungal Infections

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As antimycotic drugs are becoming more selective with respect to the panel of susceptible fungi, there is an increasing need for specific and reliable diagnosis of systemic mycoses. Accurate diagnosis may not only be for the benefit of optimal therapy, but is also essential for the study of pathological and epidemiological aspects of mycoses. Ideally, diagnosis of a fungal pathogen is made by observation of typical clinical symptoms, by demonstrating the presence of a fungus within lesions accompanied by a host reaction, and by subsequent isolation in culture of the infectious agent. Unfortunately, this has been achieved comparatively seldom in deep-seated mycoses. As a consequence of the difficulties of even suspecting the presence of deep-seated mycoses clinically, many cases are not diagnosed until tissue specimens (biopsies or autopsy material) are examined. Isolation of fungi from tissues as a means of diagnosing is especially problematic because isolation attempts are often negative, and when dealing with saprophytic fungi, contamination is a problem. In such cases it may be difficult to judge whether an isolate is of significance as a pathogen. Histologically, distinctive morphological details may provide a tentative diagnosis, but the appearance of fungi in sections is affected by steric orientation, age of the fungus, type of infected tissue, and host reaction. Moreover, the hyphal form of some of the most emerging saprophytic fungal pathogens, i.e. species of *Aspergillus*, *Fusarium*, and *Scedosporium* cannot be differentiated in tissue due to morphological similarities. Moreover, also the presence of sparse and/or atypical fungal elements may hamper a clear-cut diagnosis and may result in confusion of e.g. aspergilliosis, fusariosis, and scedosporiosis with zygomycosis and candidosis, respectively.

Several highly sensitive and specific indirect immunohistochemical techniques, and to a limited level in situ hybridisation techniques, have been developed for the identification of the most prominent causes of mycoses. As a range of different forms of fungal elements are frequently observed within lesions, especially when more organs are studied, dual immunostaining techniques are useful tools for obtaining a reliable diagnosis.

An important limitation to the widespread application of immunohistochemical techniques and their use in the routine diagnosis of mycoses lies in the fact that sensitive and specific reagents are usually derived from multiple heterologously absorbed polyclonal antibodies, which are not commercially available. However, in recent years more specific monoclonal antibodies have been developed, some of which can be obtained through companies offering diagnostic reagents.

Culture and non-culture based diagnostics of fungal infections

Lena Klingspor

Mycology Unit, Division of Clinical Bacteriology, Karolinska Institutet, Karolinska University Hospital, Huddinge, Stockholm, Sweden.

Invasive fungal infections (IFI), such as aspergillosis and candidosis, are important causes of morbidity and mortality in transplant recipients and other immuno-compromised patients. Invasive candidosis (IC) is also an increasing problem in the intensive care units. Infections with *Candida* spp. are the fourth leading cause of nosocomial blood stream infections in the United States. *Candida albicans* accounts for the majority of all such infections, but there has been a shift toward more non-albicans spp. in BSI. In a recent report from Sweden the crude mortality rate of candidaemia was 31%. The highest mortality rate was observed in patients with haematological malignancies (41.2%), age >70 years (41%), surgery (38.5%) and infections with > 1 *Candida* species (40%) or *C. glabrata* (38%). Autopsy studies have shown that the incidence of IFI is 15 to 25% among patients with leukaemia or those undergoing bone marrow transplant and 10% among those with lymphoma.

Because of lack of reliable diagnostic method, it has been common to treat high-risk patients with empiric anti-fungal treatment whenever there is clinical suspicion of invasive aspergillosis (IA) or IC. However, this is associated with significant overtreatment at high medical costs.

Although conventional diagnostic tests, such as microscopy and culture, remain the cornerstone of mycology diagnosis, the sensitivity in immuno-compromised patients is low. (Blood cultures for *Candida* spp. are positive in < 50%) . IA is common in allogeneic SCT recipients, with an incidence of 4-10%. The majority of these infections are diagnosed several months after SCT and they are frequently associated with GVHD. The diagnosis is difficult and established IA is difficult to treat with a death rate of 80-90%. There has been some progress in the early diagnosis of IA in recent years, mainly due to use of high-resolution CT-scanning and other imaging procedures.

New rapid methods that can detect IFI early in the course of disease, with high sensitivity and specificity, are needed. Non-culture based techniques that has been used in the past, such as the detection of antigens and anti-*Candida* antibodies, has lacked sensitivity and specificity in immuno-compromised patients.

Among the most promising of the new tests are the detection of fungal antigens such as Platelia *Aspergillus* and *Candida* (antigen and antibody) tests, (BioRad, France) and the detection of fungal DNA using methods as PCR. Molecular diagnosis using universal fungal PCR primers and species-specific probes have been developed and evaluated for the detection of fungal DNA in clinical specimens.

Recent advances including real-PCR, which allows for the online quantification of the DNA-load, as well as the DNA-chip technology and different sequencing methods will help to establish nucleic acid-based detection methods in the routine mycology laboratory.

Prospective studies evaluating the potential benefits of early therapy based on real-time PCR in patients at high risk for IC and IA are ongoing at the Karolinska University Hospital, Huddinge.

Free Oral Presentations

The ultimate opportunist: *Acanthamoeba castellani* Encephalitis following pediatric Hematopoietic Stem Cell Transplantation (HSCT) for Dyskeratosis Congenita

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We present the first Nordic case of fatal parasitic meningoencephalitis caused by *Acanthamoeba castellani* in a 13 yrs-old boy (1st of 2 sibs) with Dyskeratosis Congenita (X-linked syndrome of late onset bone marrow failure) following HSCT.

Two years after onset of marrow failure with transfusion dependency the boy underwent HSCT following reduced intensity preparative regimen with partially T-cell depleted PBSC- graft from an HLA- and CMV-matched unrelated donor. Prior to grafting, an intense anti-CMV treatment was necessitated for pneumonitis & retinitis lasting for 3 months. While on CsA immuno-suppression and four weeks commencing the graft, bilateral endoscopic maxillar antrostomy was performed in order to drain the obstructed sinuses. Graft rejection occurred 8 weeks later. While waiting for re-grafting, purulent meningitis developed 2 weeks onward. *Actinomyces odontolyticus* was isolated and ascribed being the causative agent identified both from the sinuses and cerebrospinal fluid. Meningitis was thought to be endogenous infections arising from the mucous membranes primarily involving the cervico-facial regions, probably due to hematogenous spread facilitated by surgery. *Actinomyces* spp. extremely rarely invade the CNS and did occur probably due to an appropriate, but far too short anti-microbial coverage. While on i.v. Penicillin treatment for the commencing 4 weeks, the pleocytosis and CSF protein contain raised. An alternative pathogen identified now was *Cladosporium spp.* and parenteral combination treatment with liposomal amphotericin B and voriconazole was instituted. The signs and symptoms of meningitis first improved but gradually got worse, accompanied by hypernatremia >160 mMol/L. The boy got gradually confused. Six weeks after onset of meningitis he got visual hallucinations and became consciousness with fits. Multi-organ failure developed and he unfortunately died 2 days after re-grafting. The clinical course, laboratory, imaging- and specific fungal and amoeba PCR studies as well findings at sterile autopsy performed will be presented.

Immunological failure due to primarily depressed cell-mediated immunity seems to play a major role in the etiology of this infection. While purulent meningitis was diagnosed and *Actinomyces ssp* was identified in the CSF followed by *Cladosporium ssp*, the boy was doing fine. It seems thus conceivable that endosymbiosis/cellular in vivo interaction occurs between amoeba and bacterial-, fungal pathogens respectively, which could precede manifest clinical signs and symptoms of encephalitis. Still the significance of this striking idea is highly speculative. The proper diagnosis will be overlooked unless pathological specimens are routinely examined for amoeba.

Primary granulomatous amoeba meningo-encephalitis is described in less than 100 patients and seems to be an emerging problem in HSCT patients. Further studies would be appropriate to evaluate its incidence as well as possible mechanisms of invasive infection.

Diversity of *Pneumocystis jiroveci* genotypes and prevalence of DHPS polymorphisms associated with sulfa resistance in Stockholm and Johannesburg

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Objectives: *Pneumocystis jiroveci*, formerly named *P. carinii* f. sp. *hominis*, remains an important cause of pneumonia in immunosuppressed hosts, including patients with AIDS, transplant recipients, patients receiving cytotoxic drugs, and malnourished children. Co-trimoxazole, a drug combination of trimethoprim and sulfamethoxazole, is the drug of choice for prophylaxis and treatment of *Pneumocystis* pneumonia (PCP). Dapsone, another sulfa derivative, is a second-line drug. In addition to sporadic reports of prophylaxis breakthrough and decreased efficiency of PCP treatment, recent studies show a significant association between exposure to sulfa drugs and the occurrence of non-synonymous nucleotide polymorphisms in the dihydropteroate synthase (DHPS) gene of *P. jiroveci*. The aims of the present study were (1) to assess the frequency of polymorphisms at codons 55 and 57 of the *P. jiroveci* DHPS gene in respiratory specimens from immunosuppressed patients in Stockholm and Johannesburg, and (2) to characterize the *P. jiroveci* strains/genotypes prevalent in these two populations.

Methods: Bronchoalveolar lavage (BAL) and sputum diagnostic samples from PCP cases confirmed by IFL and microscopy entered the study. DNA was extracted and the DHPS genotype analyzed by PCR amplification of the entire gene, followed by nested PCR of a fragment comprising codons 55/57 and sequencing of nested product using pyrosequencing technology. Allele proportions in specimens with mixture of genotypes were estimated using quantitative SNP analysis. Genotyping at the internal transcribed spacer (ITS) locus was performed by amplification using oligonucleotides priming at flanking regions of rRNA genes 18S and 26S.

Results: One hundred and ninety BAL and sputa PCP⁺ specimens (103 HIV⁻ and 41 HIV⁺ from Stockholm, 46 HIV⁺ from Johannesburg) were evaluated. In this sample, the prevalence of DHPS mutations linked to sulfa resistance was 0% in Stockholm and 48% in Johannesburg where high fungal load and high frequency of co-infections (86%) was observed. Infections with two or more genotypes were confirmed by analysis of ITS types. Genotyping at the ITS locus showed that despite a few "common" genotypes are predominantly recovered in the respiratory specimens, a large number of *P. jiroveci* strains seems to circulate in the population.

New lipid-based Amphotericin B formulations for oral use – efficacy in a mouse model of invasive Candidiasis

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Amphotericin B (AmB) is a broad-spectrum antifungal compound, which clinical use is limited by its poor oral bioavailability. We have developed new lipid-based formulations intended for oral use. Unlike other AmB formulations, our formulations truly dissolve AmB, which hypothetically would improve bioavailability and efficacy of orally dosed AmB.

Mice (n=15 per group) were challenged with *Candida albicans* (5.5×10^5 CFU i.v.). Conventional deoxycholate-formulated AmB (Fungizone[®]) and two of Camurus' AmB formulations (CamAmB-G and CamAmB-H, respectively) were dosed (1 mg/kg) by gavage once daily for three consecutive days starting one day after infection. The control group received glucose (5%). Four days after infection, the kidneys were collected and the number of colony forming units (CFU) was assessed in kidney homogenates by spot technique on Sabouraud plates.

The geometric mean of CFU/g kidney tissue in control mice was 3.3×10^4 (95% confidence limits $L_1 = 7.7 \times 10^3$, $L_2 = 1.4 \times 10^5$). CamAmB-G (CFU/g = 3.7×10^3 , $L_1 = 1.2 \times 10^3$, $L_2 = 1.1 \times 10^4$) and CamAmB-H (CFU/g = 7.3×10^2 , $L_1 = 3.1 \times 10^2$, $L_2 = 1.7 \times 10^3$) significantly decreased CFU count compared to control ($p < 0.05$; ANOVA on log-normalised data with Student-Newman-Keuls test *post hoc*). Furthermore, the H-formulation significantly decreased CFU count when compared to oral Fungizone[®] (CFU/g = 1.1×10^4 , $L_1 = 2.8 \times 10^3$, $L_2 = 4.2 \times 10^4$).

In conclusion, this study suggests that AmB can successfully be administered via the oral route for treatment of systemic fungal infections, when formulated in the new lipid-based carrier system. Assuming an acceptable safety profile, Camurus' oral AmB formulations may for example be used for prophylactic therapy.

Invasive fungal infections at The Norwegian Radium Hospital 1998 – 2003.

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The Norwegian Radium Hospital is a 350-bed tertiary care cancer center. We have searched the microbiological and pathology laboratory reports and identified 19 cases of invasive fungal infections (IFI) in the period 1998 – 2003. 17 IFI were due to candida species, of which 14 were diagnosed on the basis of positive blood cultures and three were diagnosed post mortem. The majority (12 cases) of the candidemia cases were caused by *Candida albicans* and two were *Candida tropicalis*.

- Ten IFI occurred in patients with febrile neutropenia. Nine had candidiasis and one had pulmonary aspergillosis. Eight of the ten patients had non-hodgkin lymphoma. Surprisingly, in three of the ten patients yeasts had not been detected in any surveillance culture previous to the candidemia episode. Amphotericin B deoxycholate was as effective initial therapy as liposomal amphotericin B in the yeast infections. Two patients died from the IFI within the first few days. A total of five patients developed chronic candidiasis and all had their infections well controlled, mainly with fluconazol. Five of the ten patients died from recurrence or progression of their cancer and one has recurrence of the lymphoma, but is alive 15 months later. Two patients were long time survivors.
- Five patients with IFI had had complicated surgery. Four had a gynecological cancer and one had a urinary bladder cancer. These patients were 59-85 years old. None died from their IFI, but all were dead within one to fourteen months from their underlying diseases. Their IFI, all *Candida albicans* candidemias were controlled with fluconazol for 10 to 20 days following removal of central venous catheter.

The last four patients were one patient with Hodgkins lymphoma who had a cryptococcal meningitis and three terminal cancers (Hodgkin, sarcoma and prostate) patients who surprisingly had invasive candida infection which, in few days, contributed to their fatal outcome.

Poster Presentations

The Fungi Screen Test: Rapid detection and identification of *Candida spp*, *Cryptococcus neoformans* and *Aspergillus fumigatus* by real-time PCR and melting point analysis

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Aim of the study

The main objective of the Fungi Screen Test is to detect and identify a number of different fungi in a single PCR run.

Materials and Methods

Fungal isolates are pretreated with lyticase enzyme, DNA is extracted in the BioRobot M48 (Qiagen) and real-time PCR is performed in the Rotorgene 3000 (Corbett Research).

Two pairs of fungal universal primers are used to amplify two highly variable sections of the rDNA gene; the ITS 1 region and the ITS 2 region. Each amplicon has a species-specific melting temperature. Plotting the melting temperatures of both amplicons in a two-dimensional diagram generates a unique signature for each pathogen.

Results

Strains of the following fungi have been tested, detected and identified by the Fungi Screen Test: *C. albicans*, *C. tropicalis*, *C. glabrata*, *C. parapsilosis*, *C. guilliermondii*, *C. intermedia*, *C. norvegensis*, *C. krusei*, *C. kefyr*, *A. fumigatus*, *A. niger*, *C. neoformans*. In cases of a positive result in the screen test, the identity may be confirmed by another more specific test. Work on such PCR-based confirmation tests have been initiated by designing primers and probes targeting the Top II gene.

Conclusion

The amplification of two different regions of the rDNA gene by real-time PCR combined with the benefits of melting point analysis gives the capability of fungal species identification. The fungi screen test is a rapid, low-cost and broad-range fungal detection method. Further studies of the screen test are needed to improve the sensitivity of the method for different clinical samples.

The fungi reducing effect of BenRad water purifier model M 300 and M600, on *Candida albicans* and *Aspergillus fumigatus* in water

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Introduction

Fungi are common contaminants in water pipe system. In severely immunocompromised patients these fungi can cause serious infections.

Aim

To investigate the fungi reducing effect of BenRad water purifier model M 300 and M 600 for *Candida albicans* and *Aspergillus fumigatus* in water.

Materials and methods

Solutions of approximately 10 000 CFU/ml *C. albicans* was prepared in a vessel and was passed through the M 300, at a flow of approximately 8 l/ minute.

Different solutions of approximately 3 000 CFU/ml to 100 000 CFU/ml *A. fumigatus* were passed through the M600, at a flow of approximately 8 l/ minute.

Cultures from the samples were taken before and after M 300 and M 600.

Results *Candida Albicans*

M 300

Three passages through the M 300 with a water flow of 8 l/minute reduced the number of *Candida albicans* cells from approximately 10 000 CFU/ml to 300-12 CFU /ml. After six passages to 0 CFU/ml except in one experiment, there the reduction was to 10 CFU/ml.

M 600

After three passages through the M 600 with a water flow of 8 l/minute the reduction of *C. albicans* cells was from 10 000 CFU/ml to 0 CFU/ml, except in one experiment, there the reduction was to 10 CFU/ml

Results *Aspergillus fumigatus*

M 600

Three passages through the M 600 with a water flow of 8 l/minute reduced the number of fungi spores from 27 000 CFU/ml to 3 CFU/ml. In one experiment the reduction was from 100 000 CFU/ml to 50 CFU/ml after four passages and after seven passages to 5 CFU/ml.

Conclusion

Especially the BenRad water purifier M 600 has a very good and rapid fungi reducing effect on both *Candida albicans* and *Aspergillus fumigatus* in water.

Comparison of NCCLS broth microdilution method with EUCAST broth microdilution procedure

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Objective: To compare two methods for the susceptibility testing of yeast isolates: the M27-A2 procedure of the National Committee for Clinical Laboratory Standards (NCCLS) and the procedure of the European Committee for Antimicrobial Susceptibility testing (EUCAST).

Methods: In-vitro susceptibility testing was performed using the NCCLS M27-A2 method and the procedure of EUCAST. Nine antimycotics (amphotericin, caspofungin, itraconazole, fluconazole, miconazole, econazole, ketoconazole, clotrimazole and voriconazole) were included. The methods were compared with 5 *Candida* species (ten isolates of each *C. albicans*, *C. glabrata*, *C. tropicalis*, *C. parapsilosis*, *C. krusei*) and 1 species of *Saccharomyces* (ten isolates of *Saccharomyces cerevisiae*). The *C. parapsilosis* (ATCC 22019) was used as quality control strain.

Results: There were agreements between MICs within +/-3 dilutions obtained by the NCCLS method and the EUCAST standard. The MICs were lower for the fungistatic antimycotics, when using the EUCAST procedure. Of the total MICs obtained, 28 % deviated within +/-1 dilution, 10 % deviated +/- 2 dilutions and 1 % deviated +/- 3 dilutions. Trailing were seen only, when the NCCLS method was used.

Conclusion: In conclusion, this study has demonstrated that antifungal susceptibility results obtained by using the EUCAST procedure are in close agreement with those achieved by the NCCLS M27-A2 procedure. In addition, the proposed EUCAST standard has the advantage of reducing the incubation time needed to determine the MIC.

Two cases of *Candida albicans* isolates developing pink colonies on CHROMagar plates

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Candida albicans, the most commonly isolated yeast species, is presumptively identified by its green colony-colour on CHROMagar plates. We here report two cases of *C. albicans* infections, in which the initial identifications were non-*albicans* isolates due to a pink colour of the colonies on CHROMagar plates.

Case 1: A 17-year-old woman suffering from vulvovaginitis.

Case 2: A 70-year-old woman, who underwent abdominal surgery because of bile-pancreatitis.

Methods and Results: In both cases the isolates produced chlamydoconidia, had positive germ tube tests and were identified as *C. albicans* by the API identification program (99.9%). Surprisingly the isolates developed pink colonies on the CHROMagar plate, when incubated at 35-37°C for 48h.

The internal transcribed spacer regions (ITS1 and ITS2) were sequenced in Case 1 and the 18S rRNA gene was sequenced in Case 2 from the pink isolates. Comparison with sequences in the Gene Bank database confirmed that the isolates were *C. albicans* isolates.

Minimal inhibition concentration's were in agreement with the typical susceptibility pattern for *C. albicans* using the NCCLS*.M27-A2 microdilution method in Case 1 and the Etest method in Case 2.

- National Committee for Clinical Laboratory Standards.

Discussion: *C. albicans* usually develops green colonies on CHROMagar plates. The isolates presented here developed pink colonies instead. By classical phenotypic criteria, biochemical assimilation pattern and molecular characterisation the isolates were however identified as belonging to the *C. albicans* species.

We therefore conclude, that the development of pink colonies on a CHROMagar plate, does not exclude the possibility of the isolate being a *C. albicans* isolate.