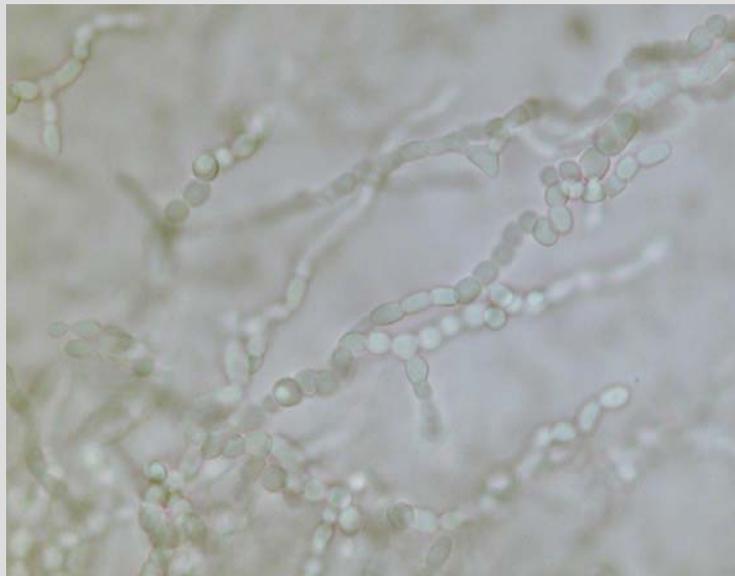# NSMM
Nordic Society for Medical Mycology

3rd Scientific Meeting of the

Nordic Society for Medical Mycology

Program and Abstracts

Nosocomial fungal infection in the ICU.

GSK Konferansesenter, Oslo, Norway

June 7, 2006

Introduction

Dear Friends and Colleagues,

It is with great pleasure that we welcome you to the third scientific meeting of the Nordic Society for Medical Mycology. The meeting this time takes place in Oslo, at the GSK konferensesenter close to the university hospital of Oslo, Norway.

The scientific programme focus on the nosocomial fungal infections in intensive care unit patients, and it has been our goal to cover as many aspects as possible regarding the diagnosis and management of fungal infections in this severely ill patient category. We are especially proud to welcome Jacques Bille from Switzerland as key-note speakers who will provide us with the helicopter view on the topic during his state of the art lecture. However, we are also proud to be are able to welcome a distinguished faculty of speakers from the Nordic countries who are ready to share their most recent data on fungal infections among ICU patients in the Nordic countries. Through the combination of foreign and local speakers and through a combination of lectures and interactive sessions we hope to provide an inspiring day with focus on fungal infections in a Nordic perspective.

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 and discuss important issues within the field of medical mycology.

On behalf of the NSMM board

Peter Gaustad   Maiken Cavling Arendrup
Secretary of NSMM   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

Nosocomial fungal infection in the ICU

Opening Ceremony and Key note lecture

10.00   **Opening remarks and welcome**.
Peter Gaustad (Meetings Secretary) and Maiken Cavling Arendrup (President).

10.10   **Key note lecture: Fungal Infections in Intensive Care Unit Patients – state of the art**.
Jacques Bille CH.

10.55   **The Nordic Perspective: Presentations from ICUs in each Nordic country**.
Kurt Espersen DK, Ole Viborg DK. Fridtjov Riddervold NO. Christina Agvald-Öhman SE.

11.55   Coffee break

Diagnostics and surveillance in a Nordic perspective

12.15   **Mycological Diagnosis in a Nordic perspective**: Results from a Nordic laboratory survey on diagnostic procedures (Jan-March 2006).
Maiken Cavling Arendrup, Peter Gaustad.

12.45   **Surveillance Cultures**: A Pros and Cons session.
Kurt Espersen DK and Malcolm Richardson FI.

13.15   Lunch

14.00   **Workshop on fungal colonisation surveillance and diagnostic procedures**: What, why, when and how? Can we reach an agreement? Moderators: Fridtjov Riddervold NO, Ole Viborg DK.

14.45   **Presentation of workshop conclusions**

Clinical Cases an Interactive session

15.15   **Tooth extraction in a young female**.
Jon Peiter Saunte DK.

15.35   **A liver transplant patient**.
Suheil Andreas Salamon, DK.

15.55   **Left arm paresis in a renal transplanted patient**.
Gorm Atle Hansen, NO.

16.15   **Presentation on Candia krusei**
Timo Hautala, FI.

16.30   Coffee break

Treatment

16.50   **Prophylaxis and empiric therapy, with what, how much, when and to whom**.
Flemming Moesgaard DK.

17.10   **Fungal infections in the critically ill paediatric population**
Tore Abrahamsen NO.

17.30   **Fungal infections in the critically ill adult ICU population**
Arvid Bjørneklett NO.

Free presentations

17.50   **Nordic external quality assessment in medical mycology: results of the 2006 distribution**. Erja Chryssanthou SE

18.05   **Multiplex real-time PCR for detection of Candida infection in blood**. Åsa Innings, Måns Ullberg, Anders Johansson, Carl Johan Rubin, Niklas Noréus, Magnus Isaksson och Björn Herrmann

18.20   **Diversity of moulds species in Norwegian drinking water**. Gunhild Hageskal, Ida Skaar NO

18.35   **Fungal normal flora in immunocompromised hosts**. Karianne Wiger, Truls M Leegaard NO

18.50 Annual General Meeting

19.30 Farewell dinner at the venue

Abstracts

Key-note lecture

Fungal Infections in Intensive Care Unit Patients – state of the art.

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Fungal infections represent an increasing problem, particularly in ICU patients with a mortality higher than bacterial infections. Early diagnosis is challenging, particularly in view of new strategies for recognition and treatment.

The predominant organism involved is Candida spp, but Aspergillus and other moulds are clearly under-recognised in the ICU population. Invasive candidiasis occurs in 0.2-3/1000 hospital admissions, and 10 times more in ICU patients. Up to a third of all episodes of candidemia occur in ICU, usually 3-4 weeks after admission. Risk factors are numerous, with an OR reaching up to 25 for colonisation, broad-spectrum antibiotics administration, and intravascular catheters. Colonisation index is valuable to select a population at higher risk.

Regarding diagnosis, the clinical presentation is variable and non specific. Laboratory diagnosis still relies on conventional methods (blood cultures, cultures of other body sites, as well as histology when available). Non conventional diagnosis is still investigational. Antigen detection tests (mannan or ß-1,3-D glucan) are commercially available, and their main value today is their high negative predictive value. Molecular detection of fungal DNA is still a home-made non standardized approach which holds promise. Probably a combination of both approaches is worth additional studies.

Therapeutic strategies of invasive Candida infections comprise prophylaxis, preemptive, empirical and directed therapy. Prophylaxis has been shown to be effective in a small part of the ICU population, i.e, in some solid organ transplant patients, and after abdominal surgery for gastro-intestinal leak or in necrotizing pancreatitis. Preemptive therapy is tempting, but very few studies are available, often based on the presence of colonization. Simple clinical rules predictive of Candida infections are actively seeked. Empirical therapy is often administered without clear proof of efficacy or guidelines. Many antifungal agents are now available to treat severe Candida infections, and the initial choice should take in consideration the local epidemiology, prior exposure to antifungal agents, presence or not of neutropenia, the severity of clinical presentation, as well as the presence of underlying diseases or organ dysfunction affecting the metabolism or the toxicity of antifungal agents.

Rapid identification to species level and sometimes the availability of antifungal susceptibility test results allow a rapid switch to a more appropriate or less toxic agents. Early and appropriate antifungal treatment has an impact on outcomes.

Fungal infections caused by moulds –in particular by Aspergillus species– are diagnosed in about 3-5% of ICU patients, too often post-mortem. Risk factors in this population comprise glucocorticosteroids and immunosuppressive drugs.

Finally, many oncologic patients often neutropenic are treated in ICU during their hospital stay, adding the classic risk factors for invasive fungal infections.

22.05.06

The Nordic Perspective: Presentations from ICUs in each Nordic country.

Rigshospitalet, University Hospital of Copenhagen, Denmark

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No abstract available

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During a 3 months period (Jan- Mar 2006) we did a survey on antifungal therapy in a 6 bed multidisciplinary mixed ICU at Århus University Hospital, Århus Sygehus, THG.

The ICU treated 114 patients with 123 admissions with a median length of stay (LOS) of 2 days, range 1-60 days and a total of 512 days. 25 patients stayed more than 5 days in the ICU.

In the observation period the ICU treated 70 medical patients of whom 12 had multi-organ dysfunction due to sepsis after chemotherapy for haematological malignancies and 43 of 44 surgical patients with gastrointestinal complications due to abdominal surgery.

A total of 13 patients (11%) were on systemic antifungal therapy (SAT). Six patients had documented fungal infection (DFI) and 7 patients had suspected fungal infection (SFI) according to the study protocol definition.

Out of 25 patients with LOS of more than 5 days 11 patients (44%) on SAT had DFI or SFI.

The cultures reported from our laboratory of microbiology were:

Yeast: 12
Candida albicans: 26
Candida species (Non-albicans): 11
Candida tropicalis: 1
Candida krusei: 1

In the ICU we had no recommendations of surveillance of fungal infection and the only recommendation of antifungal therapy is prophylaxis with fluconazole to haematological patients with neutropenia after chemotherapy.

During the observation period we used a total of 1120 mg of Ambisome, 8500 mg of Fluconazole and 2210 mg of Caspofungin. No other antifungal medication was administered.

The relatively high frequency of DFI and SFI in our patients with LOS of more than 5 days could be explained by a high representation of haematological and abdominal surgical patients 55/114 (48 %).

These observations call for further studies and guidelines for surveillance and treatment of fungal infections in these ICU patients.

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No abstract available.

Karolinska University Hospital Huddinge, Sweden
Candida colonization, colonization index and invasive candidiasis in patients at a multidisciplinary Intensive Care Unit

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Background
Intensive care units (ICU) have emerged as epicentres for fungal infections such as candidaemia and invasive candidiasis. The aim of the study was to investigate candida colonization, colonization index (CI) and its relation to candidaemia and invasive candidiasis in patients with a length of ICU stay (LOS) ≥7 days. Furthermore, to assess the impact of patient's immunological status on the risk to gain invasive candidiasis or candidaemia, repeated measure of histocompatibility leukocyte antigen-DR (HLA-DR) was performed.

Material and methods
ICU patients with a LOS ≥ 7 days were consecutively included for sampling during Mars 2004 to July 2005. The study was approved by the Ethical Committee in Stockholm. A total of 59 patients, 38 men and 21 women, mean age 59 years (range 19-81) were included. Mean LOS was 19.8 days (range 7-77) and mean days of ventilation was 16 (range 0-74). A majority of the 59 patients were exposed to antimycotic drugs during their ICU stay, 22 received fluconazole, 17 liposomal amphotericin, 3 voriconazole and 3 caspofungin. Samples were collected at day seven and then weekly as long as the patient was admitted to the ICU. Sampling sites were oral cavity, lower airways, urine, blood and rectum from all included patients and from drainage and wounds when this was an option. The non-blood samples from each patient were cultured and the colonisation index (CI) for each sampling occasion was calculated, i.e. the ratio of all positive non-blood samples with all negative non-blood samples taken at the same occasion.
In addition, one blood sample to determine the immunological status measuring histocompatibility leukocyte antigen-DR (HLA-DR) was also taken at each sampling occasion and analysed with three colour flow cytometric analysis (FCM).

Results
Microbiological analysis

Fourteen patients were not colonized by candida at all (CI=0). *C. albicans* was isolated from 35 patients, *C. glabrata* from 10, and 12 had other non-albicans species. Seven patients were colonized by ≥ 2 species of whom two by ≥ 3 species. At the first sampling occasion 42 % of the patients (25/59) had a CI ≥ 0.5 of whom eight had CI 1.0, while at day 14, 32 % (10/31) had an index ≥ 0.5 and two had CI 1.0. Only 10 patients or fewer were sampled at days 21, 28, 35, 42 and 49, range of mean CI were 0.4-0.7.

Ten patients developed invasive candidiasis, of which six had candidaemia. Mean CI for these 10 patients were 0.8 (range: 0.25-1.0) and all invasive species were also colonizing species. Infections were caused by *C. albicans* (6), *C. glabrata* (3), *C. tropicalis* (1) and *C. dubliniensis* (1). All patients with invasive candidiasis were treated with at least one antimycotic drug during the study period, the majority with liposomal amphotericin (7/10) and fluconazole (6/10). The three months mortality among these patients was 60% compared to 49% (29/59) among all included patients.

HLA-DR analysis

The expression of HLA-DR was not significantly decreased in our study population. At day seven 29/59 patients had an HLA-DR expression of ≤ 85 % and only 9/59 had an HLA-DR expression of ≤ 70 %. None of the patients had an HLA-DR expression of ≤ 30 %. Of the 16 patients that died in the ICU only three patients had a HLA-DR expression of ≤ 70%. Six of the 29 patients that died within three months had an HLA-DR expression of ≤ 70%.

Discussion

The ICU in the present study is a tertiary unit at a university hospital and 66% of the patients were treated with immunosuppressive drugs (17/59) and/or cortisone (39/59). Despite the fact that the majority of patients (17/25 were treated with antimycotic drugs as soon as CI exceeded 0.5), invasive candidiasis was diagnosed during the ICU stay in 17 % of included patients, mean CI among these ten patients were 0.8.

The three months mortality among these ten patients was 60% compared to 49% (29/59) among all included patients. Only two of the patients with invasive candidiasis was transplanted (bonemarrow transplantation and liver-and bonemarrow respectively), this is probably due to the prophylactic/preemptive treatment that is early initiated and broadly used in these patients.

Seven out of ten patients (70%) had undergone major surgical operations and (60%) had been reoperated at least once in discrepancy to 18/59 (30%) and 12/59 (20%) for all patients studied. Two patients (20%) had diabetes and five patients (50%) had acute renal failure and were treated with continuous renal replacement treatment (CRRT) compared to 12/59 (21%) that had diabetes and 20/59 (34%) that were treated with CRRT among all included patients. Seven out of ten patients were treated with low-dose cortisone and two patients (transplanted) had other immunosuppressive treatment as well.

The patients that were not treated with antimycotic drugs despite a CI ≥ 0.5 had been exposed to less antibiotic drugs compared to the whole subgroup of 25 patients (mean number 2,6 compared to 3,9). Mean CI for these eight patients were 0.59 and none developed an invasive candidiasis during the study period.

At our unit we use a multimodality evaluation of the patients daily and in close cooperation with both the microbiology laboratory and infectious disease specialists. Still it seems that a sustained part of the ICU patients will gain an invasive candidiasis despite our efforts to prevent this.

We could not demonstrate a significantly decreased HLA-DR expression for these patients. We also compared mortality both at the ICU and the three months mortality without detecting any use of HLA-DR expression as a prognostic marker.

Conclusion

ICU patients are a vulnerable group of patients. In the present study a high CI was correlated to a high incidence of invasive fungal infection but seems to be only one of the risk factors and needs to be evaluated together with other known risk factors to prevent fungal infections.

Diagnostics and surveillance in a Nordic perspective

Mycological Diagnosis in a Nordic perspective: Results from a Nordic laboratory survey on diagnostic procedures (Jan-March 2006).

Maiken Cavling Arendrup and Peter Gaustad.
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A questionnaire focusing on the procedures and recommendations concerning handling of specimens and treatment of patients in the ICU was sent to all departments of clinical microbiology in Denmark, Norway and Sweden. A total of 33 of 65 laboratories returned the questionnaires (DK: 9/15 including 4 university laboratories, NO: 17/20 including 4 university laboratories and SE: 7/30 included 4 university laboratories). Surveillance cultures were regularly taken at 10 of the 33 laboratories 1 – 3 times a week. The number and location of sites investigated varied widely as did the degree of species identification (full species identification on all isolates, on some isolates or just albicans versus non-albicans discrimination). Five of the laboratories performing surveillance cultures performed susceptibility testing either always (1 lab) or sometimes (4 labs).

Species identification for Isolates in blood cultures were provided from 25/33 laboratories and from 22/33 laboratories when the specimen was from other sterile sites. 20-24 laboratories provided susceptibility testing in such cases. The number of laboratories providing species identification and susceptibility testing was lower for specimens from airways, abdomen and other sites.

Half of the departments of clinical microbiology 14/28 recommended fluconazole as first line treatment of candidemia before species identification even though the number of *C.* glabrata infections accounts for 10 – 20%. Otherwise the recommendations were quite different both within and between labs from the different countries.

In conclusion the survey revealed a lack of consensus among the practices in the Nordic departments of clinical microbiology and that this topic was an obvious subject for further discussion! We were somewhat surprised by the fact that

- ID (and Susceptibility testing) of isolates involved in invasive infections is still not provided by all laboratories (in house or via reference laboratory)
- Treatment recommendations: That fluconazole widely used also before ID and caspofungin rarely recommended. And that Amphotericin B is recommended more often than Voriconazole for invasive *Aspergillus* infections.

Surveillance Cultures: A Pros and Cons session.

Pros: Surveillance cultures

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Many of the patients in an ICU will become immune suppressed due to their underlining sickness and thereby in a great risk of getting nosocominal infections. These patients also have many of the risk factors for getting a fungal infection.
Fungal infections are very common in the ICU and the incidence is increasing. The mortality due to fungal infections is increasing as well. Invasive fungal infections have the highest mortality.
Early start of treatment of fungal infections improves outcome.
Patients with candida species grown from more than 2 foci are more likely to have an invasive fungal infection.
Resistance to antifungal drugs is becoming a more important aspect in the treatment of fungal infections.

In our ICU we recommend routine microbiological surveillance cultures for many reasons
- early start of antifungal therapy
- to prevent colonisation becoming invasive infections
- look for treatment response
- detect resistance

Cons: The diagnostic value of fungal surveillance cultures in critically ill patients.

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Heavy fungal colonization is a known risk factor for fungal infection, yet the value of fungal surveillance cultures is uncertain. It is quite clear from the literature, and from our own experience that surveillance cultures for predicting invasive pulmonary aspergillosis are of limited value. Surveillance cultures for evaluating yeast colonization and carriage are helpful in determining colonization but do not have a high positive predictive value for fungal infection in a broad population of intensive unit patients. However, fungal infection is more likely in heavily colonized patients and surveillance cultures show that fungal infection is extremely unlikely in patients without fungal colonization. Not all studies confirm the association between fungal colonization and infection. Evaluation of surveillance culture results is far from a perfect test and should not be used as a sole diagnostic criterion for fungal infection. Routine prospective surveillance cultures have cost implications and are laborious to perform and analyse.

Clinical Cases an Interactive session

Tooth extraction in a young female.

Jon Peiter Saunte

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Purpose: To describe a patient with endogenous *Candida Albicans (CA)* endophthalmitis, which occurred 11 days after tooth extraction.

Design: Single interventional case report.

Methods: A 28-year-old caucasian healthy woman presented with a two day history of pain and floaters in her right eye 11 days after extraction of a tooth, which was fractured six months earlier. She received oral penicillin on the day of the dental operation. Ophthalmologic examination revealed endophthalmitis with a white, lobular parafoveal infiltrate in the right eye. Microbiologic and cytologic studies of the vitreous body aspiration were performed. A full medical work up and radiology investigation was initiated. Cultures from cornea, blood, urine, skin, nose, mouth, vagina and cervix and her intra uterine device were collected. Investigation for immunodefiency was performed.

Results: The best visual acuity in the right eye improved from 1/20 to 20/20 and floaters and pain disappeared during seven weeks of therapy. Four out of four cultures of the vitreous body in the right eye revealed *CA*, and one cervix culture was positive for *Actinomyces*. All other cultures were negative. Magnetic resonance imaging of cerebrum and orbit, computed tomography of thorax and abdomen, ultrasound of heart and abdomen, were all without signs of abscesses or other pathology. Examinations by Dentists, Ear –Nose –Throat specialist, Cardiologist, Haemotologist and Gynaecologist resulted all normal. The blood count, lymphocyte marker, lymphocyte stimulation, total haemolytic complement, immunoglobuline, mannan-binding lectin, and flowcytomtric analysis of peripheral blood analysis were all normal. The HIV test was negative. Investigation for somatic hypermutation of immunoglobulin kappa light-weight genes was abnormally low in the first blood sample, but the ratio had normalised spontaneously when the test was repeated 6 months later.

Treatment profile: Immediate vitrectomy of the right eye with intravitreal amfotericin B, ceftazidim and vancomycin. Intravenous vancomycin and ceftazidim was tapered after five days. Intravenous 14 days of amfotericin B 200 mg once daily and 5-flucytocin 250 mg three times daily, followed by five weeks of oral fluconasol 200 mg once daily.

Conclusion: Endogenous endophthalmitis is a rare finding in healthy persons. It is a sight threatening as well as potential life threatening finding. We describe a case of successful treatment with vitrectomy of an eye with *Candida albicans* endolphthalmitis due to prior tooth extraction in a 28-year-old healthy woman. When vision loss is found in healthy individuals fungal endophthalmitis should be considered a differential diagnosis and treatment initiated without delay and handled as a multi-speciality challenge.

A liver transplant patient.
Pulmonic valve candida endocarditis: relapse after 3 months of therapy.

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Isolated pulmonic valve infective endocarditis is very rare and is usually associated with tricuspid valve endocarditis. Although it has been reported with increased frequancy in the last years, Candida is still an uncommon cause of endocarditis. The most common predisposing factors are pre-existing heart diseases, immunosuppressive treatment and intravenous drug abuse.

A case is reported of native pulmonic valve Candida albicans endocarditis in a lever transplanted young woman with immunosuppressive therapy. She underwent treatment with, at first liposomal amphotericin B, and then fluconazol and caspofungin for 3 months. After 2 months without any therapy, the pulmonic valve C. albicans endocarditis relapsed with right pulmonary embolism as a complication. The patient underwent replacement of the infected pulmonic valve, under treatment with flucoanzol and caspofungin.

It has been decided that intravenous treatment with caspofungin and fluconazol must continue for at least 8 weeks and then shift to fluconazol as oral monotherapy for at least 2 years.

Left arm paresis in a renal transplanted patient.
Recurrent Candida albicans infections: Treated candidemia followed by late recurrent vertebral osteomylitis.

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Background: *Candida albicans* vertebral osteomyelitis is rare. We report one patient with candidemia who after antifungal treatment several months later presented vertebral infection.

Case: 68-y-old man who underwent renal transplantation in March 2005, was admitted to hospital June 2005 because of fever, chills and fatigue. Blood cultures and urine culture yielded *Candida albicans* for which he was treated with fluconazole 200 mg (1.day), 100 mg q 24h for 3 weeks. (serum creatinine: 462 µmol/l). He was readmitted April 2006 with pain in the neck and shoulders, fever and fatigue, and paresis in his left arm. Spondylodiscitis was demonstrated by magnetic resonance (MR) scan at level C4/C5. He underwent surgical extirpation of C4 and debridement of pus. Blood culture and pus culture from C4/C5 yielded *Candida albicans* . He was treated with caspofungin 50 mg q 24h and was transferred back to the local hospital after three days.

 Conclusion: By phenotypic characterization the two isolates were identical and there was no emergence of antifungal drug resistance between the initial and recurrent episode. (Both initial and recurrent isolates had similar MIC for fluconazole (0.5 mg/l). A possible cause of the recurrent infection is inadequate treatment by low dosage of fluconazole.

Treatment

Antifungal agents for preventing fungal infections in ill defined surgical patients.

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Fungi have emerged worldwide as an increasingly frequent cause of nosocomial infections. The most frequent clinical manifestations of invasive candidiasis in surgical and ICU patients include candidemia, intra-abdominal candidiasis and disseminated candidiasis. Other manifestations such as *Candida* endophthalmitis, pulmonary candidiasis, and *Candida* endocarditis occur less frequently.

C. albicans has historically been the most frequent cause of candidemia. However, the frequency of candidemia due to non-*albicans* species of *Candida* rose during the 1990s. The species that have grown in frequency have been *C. tropicalis*, *C. glabrata*, *C. parapsilosis* and *C. krusei.* The clinical manifestations of candidemia range from nothing more than fever to overt and life-threatening sepsis.

The gastrointestinal tract is a major reservoir of *Candida* species and an important portal for intra-abdominal infections. Therefore, patients with perforation of a viscus or anastomotic leak have an increased risk of fungal dissemination to the peritoneal cavity and extra-abdominal tissues and organs. Under most circumstances, *Candida* will be cleared quickly from peritoneal cavity, but in some patients, peritoneal seeding result in the development of an intra-abdominal *Candida* infection, with a risk of dissemination to the bloodstream and to extra-abdominal tissues and organs.

Invasive candidiasis is associated with substantial morbidity, high crude and attributable mortality (40%-60% and 30%-40%, respectively), prolonged hospital stay, and increased health care cost.
Therefore, prompt initiation of antifungal therapy is essential for control of infection and outcome.
No single antifungal agent can cover the spectrum of all intraabdominal fungal infections.

The value of antifungal prophylaxis for preventing fungal infections in surgical patients has been evaluated in randomized controlled trials. In a recent meta-analysis twelve unique trials involving 1606 patients. All trials but two were restricted to ICU patients and fluconazole/ketoconazole were compared with no antifungal agents. The meta-analysis demonstrated, if the trials were combined, that antifungal prophylaxis reduced proven invasive fungal infections and mortality significantly.

However, potential ecological effects of widespread use of antifungal agents, are of particular concern because of selection and spread of resistant fungal strains and species. Certain *Candida* species, such as *C. glabrata* and *C. krusei*, including *Aspergillus species*, are intrinsically or relatively fluconazole-resistant.

The selection of surgical patients for antifungal prophylaxis should include critically ill surgical patients with multiple clinical risk factors, e.g. patients undergoing reoperations for intraabdomial infections (intraabdominal abscess, peritonitis, anastomotic leak) and patients with severe peritonitis. Antifungal prophylaxis with fluconazole is evidence-based with a safe toxicity profile. Fluconazole 800 mg/day initially, then 400 mg/day. Duration for prophylaxis is about 12 days or untill ICU discharge.

Playford EG, Webster AC, Sorrell TC, Craig JC. Antifungal agents for preventing fungal infections in non-neutropenic critically ill and surgical patients: systemic review and meta-analysis of randomized clinical trials. *J Antimicrob Chemother* 2006; **57**: 628-38.

Fungal infections in the critically ill paediatric population

Tore Abrahamsen
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There is a correlation between the number of immunocompromised patients and the number of fungal infections. Many of the newborns treated in the neonatal intensive care unit (NICU) have risk factors for contracting systemic yeast infections. The most frequent fungal pathogen is still *Candida albicans* also in our hospital, but we have seen *Candida parapsilosis* as well. In the NICU we still use amphotericin B deoxycholate as the first choice in systemic yeast infections while some of the very low birth weight infants receive fluconazole prophylaxis.

In paediatric ICUs the frequency of fungal infections is low, but, of course, depending on the diagnosis of the patients being treated. In a PICU in Birmingham Children's Hospital 143 microorganisms were isolated from bloodcultures, seven of them were *Candida* spp. Liposomal amphotericin is our treatment alternative in these patients that often have received other toxic drugs and/or will have renal problems in addition.

Among the molds *aspergillus* spp such as *Aspergillus fumigatus*, are usually found. However, these filamentous fungi are not common as a cause of nosocomial infections in immunocompromised children. In our department invasive aspergillosis is found in children who have undergone allogeneic bone marrow transplantation or being treated for acute myelogenous leukaemia. These children may end up being treated in a PICU. Voriconazole has improved the treatment results for these patients.

In all these three clinical settings empiric treatment with antifungals is being more and more common. This underscores the need to improve the accuracy of the fungal diagnosis to decrease the use of these drugs.

Fungal infections in the critically ill adult ICU population

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Patient populations cared for in ICU´s varies greatly between institutions and so do the risk of acquiring invasive fungal infections (IFI). The populations may also differ over time with changing surgical procedures, like more extensive organ transplantations being performed in ever more high risk patients. The patients may also be more heavily immuno-suppressed. This may to some extent be the case in the ICU for adults at Rikshospitalet in Oslo. We have, however, not observed a significant increase neither in proven IFI´s nor in clinical significant fungal isolates during the last 15 years. We have, however, had a five-fold increase in consumption of systemic antifungals. Some of this is accounted for by an increase in daily dosage. More than 90% of the treatments are empirical or "preemptive". The threshold for starting antifungal treatment has definitely been lowered and one may ask if it has become too low. During the last years newer, broader spectrum and also more expensive agents has become increasingly used although we have until now no indications of a more resistant fungal flora in our institution. During a three months period in 2005 every treatment with an antiinfective was recorded by the prescriber who at the same time wrote down his reason for starting the treatment. We have reviewed these recordings and scored them as appropriate or not. Possible causes for the increasing use of antifungals is discussed.

Nordic external quality assessment in medical mycology: results of the 2006 distribution

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Introduction: In 2004 Nordic Reference Group on Methods in Medical Mycology (NRMM) was established. The main task of NRMM is to harmonise methods used for species identification and antifungal susceptibility testing in Nordic clinical mycology. It also organises Nordic external quality assessment programs focusing on clinical mycology issues. The second Nordic EQA was organised in March 2006. Medical microbiology laboratories in the five Nordic countries were invited to participate at no cost and 59 laboratories accepted this offer (Denmark: 12, Finland: 8, Norway: 19 and Sweden 20 laboratories). The EQA shipment consisted of five simulated specimens. The laboratories were asked to handle the specimens as ordinary, routine specimens and to report clinical important micro organisms (both fungi and bacteria) as in the normal routine work.

Results: A selective medium such as Sabouraud agar or a chromogenic medium is recommended for the cultivation of fungi from clinical specimens. Compared with the Nordic EQA distribution last year the use of chromogenic agar has increased to ~ 30-75% of the laboratories. For identification of yeasts most laboratories used a combination of chromogenic yeast agar and ID32C or Vitek identification system. Rapid identification of *C. glabrata* and *C. krusei* by the commercially available tests were only used by few laboratories in Norway and Sweden.

Specimen 1: Blood culture with *C. albicans, C. glabrata.* All laboratories correctly reported the presence of the *C. albicans* isolate. Fourteen laboratories also reported the second isolate as *C. glabrata* (7 labs), *Candida* non-*albicans* (5 labs) or yeast (1 lab). Six laboratories reported species but failed to correctly identify it. However, 66% (39/59) of the laboratories failed to detect the presence of a second yeast. *C. glabrata* grows more slowly than *C. albicans* and growth may be inhibited by the presence of faster growing species especially if the plate is heavily inoculated. Therefore the use of chromogenic media, a proper spreading of the specimen allowing single colonies and careful observation of these for at least 3 days is recommended.

Specimen 2: Blood culture with *Cryptococcus neoformans.* Most laboratories (81%) correctly identified this specimen. Five laboratories did not identify the isolate to genus level and 5 laboratories reported *Candida* species. *Cryptococcus* forms smooth and some times slimy colonies within 2-4 days. The identification may be aided by a positive urease test combined with the typical micro morphology of round, encapsulated budding yeast cells.

Specimen 3 : Bal with *Aspergillus fumigatus, Enterobacter cloacae, C. albicans. A. fumigatus* was reported by 54% of the laboratories and additionally 12 laboratories reported presence of *Aspergillus* sp, mould or *A. niger*. Fourteen laboratories failed to detect the mould. Since many laboratories did not detect *A. fumigatus* in a BAL specimen from a febrile neutropenic patient requiring intensive care treatment illustrates a need of a strengthened awareness of detection of human pathogenic filamentous fungi among high risk patients. Observation of cultures of BAL fluid for 5-7 days and use of selective fungal media in the primary culture are recommended.

Specimen 4: Peritoneal fluid obtained during surgery with *C. tropicalis, Klebsiella pneumoniae, Enterococcus faecium. C. tropicalis* isolate was reported by 78% of the laboratories and 8 laboratories reported presence of *Candida* sp. or yeast. Five laboratories detected but misidentified the isolate. Only the Danish laboratories reported the presence of bacteria, which indicates that they followed the instruction to handle these specimens as routine samples. All laboratories detected the yeast isolate in this mixed sample, though the growth of yeast may be suppressed and difficult to recognise in samples containing bacteria, if selective yeast media are not used.

Specimen 5: Sterile urine. All laboratories except 2 correctly reported this sample as negative.

Susceptibility testing was reported by 5/12 laboratories in Denmark, 15/19 in Norway and 18/28 in Sweden and Finland. All together 629 susceptibility results were reported. In general, the overall agreement between the results as regards to S, I/SDD and R group was high and the majority of the results were correct. This was especially true for amphotericin B and caspofungin, while the results for the azoles showed more variation. Many laboratories (34%) failed to identify the _C. neoformans_ isolate as fluconazole resistant. Several laboratories also failed to detect the reduced azole susceptibility in the _C. glabrata_ isolate and in the _C. albicans_ isolate which had acquired resistance to fluconazole and itraconazole.

Multiplex real-time PCR for detection of Candida infection in blood.

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Background: Invasive candidiasis is a leading cause of morbidity and mortality among critically ill and immunocompromised patients. Given the high mortality rates, there is need for improved diagnostic methods. For this purpose a real-time PCR based on the target gene RPR1 was designed. Our aim was to develop a multiplex real-time PCR method for detection of the seven most common Candida species causing septicaemia: C. albicans, C. glabrata C. guilliermondii, C. krusei, C. parapsilosis and C. tropicalis and C. dubliniensis.

Materials and Methods: A multiplex four channel real-time PCR assay was developed using the target gene RPR1, coding for the RNA subunit of ribonuclease P. The RPR1 sequences of the seven Candida species were determined. The multiplex PCR included four primers and four TaqMan probes labeled with fluorophores of different wavelengths. Three of the probes enabled specific detection of C. albicans, C. glabrata and C. krusei and the fourth allowed general detection of the remaining species. Candida DNA was extracted from 3 ml human blood using lyticase and the QIAamp tissue kit according to Loeffler et al.

Results: The multiplex PCR was able to detect 2-10 genome copies when the detection limit was tested repeatedly for the four species C. albicans, C. glabrata, C. krusei and C. guillermondi. No significant difference in detection limit was seen when the multiplex format was compared with single species PCR, i.e. two primers and one probe. When the Candida DNA was extracted from spiked human blood a detection limit of <10 cells per ml was reached. The multiplex system has a built-in specificity control as only one channel should be positive for a specific species and this was achieved in all runs performed. The sensitivity of the Candida PCR was also compared with blood culture (BacT/ALERT FA). Four patients with known C. albicans septicaemia were monitored by repeated sampling. All positive PCR samples were accompanied by a positive blood culture collected the same day.

Diversity of moulds species in Norwegian drinking water

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Background:
Fungi are receiving growing attention as agents of human allergies and infections. Studies of potable water in hospitals and private dwellings indicate the public drinking water system as transmission route for allergenic, toxigenic and opportunistic fungi. In Norway, it is largely unknown which fungi are resident and capable to survive and contaminate the drinking water. Therefore data on the frequency of individual species throughout the water system is needed.

Materials and Methods:
In order to determine the occurrence and significance of moulds in public drinking water systems in Norway, water sampling from 14 water supplies, both with surface and ground water source was performed. Frequencies (cfu/100 ml) of the most common fungal species and the species diversity in samples from raw water, treated water, and from home and hospital installations were determined on the basis of incubation of membrane-filtered samples on DG18 media. The moulds were phenotypically identified to species level. In addition, a few non-identifiable isolates that were molecularly identified by ITS sequencing.

Results:
Moulds were recovered from 70% of the surface samples, while 42% of the ground water samples were positive. The risk to recover moulds from surface water is three times higher compared to ground water, and it is more likely to detect moulds in cold water than in showers and hot taps. A total of 94 different mould species, belonging to 30 genera were identified. The mycobiota was dominated by species of *Penicillium*, *Trichoderma* and *Aspergillus.* Some of the species identified occurred throughout the entire drinking water system.

Discussion:
Several of the species identified are known to be able to cause allergic reactions or disease in humans; others are common contaminators in food and beverage industry, and some may have sensoric or technical concern in water distribution systems. Our results indicate that the mycobiota should be considered in terms of assessing microbiological safety and quality in drinking water.

Fungal normal flora in immunocompromised hosts

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Background:
Severely immunocompromised patients receive antimicrobial therapy, including antifungals, more often than other patient groups. This raises the question whether they are prone to

infections with drug resistant organisms or if drug resistance is induced in the normal bacterial and yeast flora. In two consecutive studies we investigated these problems in immunocompromised patients. The first group were adults with HIV-infection, the second group were children (0-16 yrs) with either cancer or cystic fibrosis (CF). In addition a control group consisting of healthy children were investigated. This last study is still ongoing.

Materials and Methods:

During the period 1998-2006 serial throat swabs and faecal samples were collected from 107 patients with HIV, 45 children with cancer, 33 children with cystic fibrosis and 46 healthy children. The samples were plated out on sabouraud agar and incubated at 28°C for 5 days. Candida isolates were identified to species level and tested for susceptibility to antifungal drugs.

Results:

Of the 107 patients with HIV, 64 had at least one positive *Candida*-culture: 56 *C. albicans*, 11 *C. glabrata*, five *C. tropicalis* and one to two each of *C. parapsilosis*, *C. krusei*, *C. famata* and

C.lambica. Of the 45 children with cancer, 33 had at least one positive candida-culture: 24 *C. albicans*, 13 *C. parapsilosis*, and one to two each of *C. dubliniensis, C. magnolia, C. guillermondii, C. tropicalis, C. famata, C. rugosa* and *C. lusitaneae.* Preliminary results for the 33 children with CF have shown that 19 have at least one positive *Candida*-culture: 18 *C. albicans* and one each *C. krusei* and *C. tropicalis.* Preliminary results for the 47 healthy children have shown that 22 have at least one positive *Candida*-culture: 15 *C. albicans, six C. parapsilosis,* two *C. lusitaniae,* and one *C. sphaerica*. Several of the patients and healthy controls were colonized with two or three different *Candida* species. In addition to the *Candida* strains, other yeasts such as *Saccharomyces cerviciae* and *Rodotorula* sp. were found.

All *C. albicans* strains isolated from the children were susceptible to antifungal drugs. In patients with HIV, however, reduced susceptibility to fluconazole could be detected in a subset   of *C. albicans* isolates (8,5%). In HIV-patients there was also a significant relationship between carrying fluconazole resistant *Candida* sp. other than *C. albicans* and previous fluconazole use. Antibiotic use also proved to promote carriage of *C. albicans*.

Discussion:

Candida carriage seems to be very common, both in the adult and paediatric population. *C. albicans* is by far the most dominating strain, and for the most part completely susceptible to fluconazole. Many HIV patients receive multiple courses of antifungal treatment and/or prophylaxis. This has in some cases given rise to reduced fluconazole susceptibility. We have not found the same in our pediatric cancer population which is probably due to much less use of antifungal prophylaxis and treatment. Although this has not yet been thoroughly analyzed.

POSTERS

Study on the effect of Mycograb in combination with Fluconazole and Caspofungin on *Cryptococcus neoformans* by population study.

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Background:
Hsp90 has been shown to potentiate the evolution of new drug resistance in many ways in fungi by allowing new mutations to have immediate phenotypic consequences. Mycograb is a recombinant antibody derived against the LKVIRKNIV epitope of heat shock protein 90. Here we asked whether Mycograb, an inhibitor of hsp90, could prevent the improvement of resistance to caspofungin and fluconazole in *Cryptococcus neoformans*.

Materials and Methods:
Three isolates of *C. neoformans* were used in this study; fluconazole-sensitive strain, fluconazole-resistance strain and caspofungin-resistance strain. A population analysis was carried out by plating serial dilutions of 72h cultures (0.5 McFarland standards, equal to $\geq 10^{10}$ colony-forming unit (cfu/ml)) of the isolates, on Sabouraud Dextrose plates. Each plate contained a single antifungal concentration of fluconazole or caspofungin ranging from 0.0625 to 512µg/ml. In addition, each plate contained different concentrations of Mycograb in the range of 4 - 6 µg/ml. Plates were incubated at 30ºC for 72h. Colony counts were made from Sabouraud plates and the results were expressed as CFU/mL $-Log^{10}$. A control plate was containing only Mycograb was included in each assay.

Results:
Addition of 16µg/mL of Mycograb to 16 µg/mL of fluconazole and either 4 or 8 µg/mL of Mycograb to 32 µg/mL of fluconazole altered a fungistatic effect to a fungicidal effect of fluconazole in fluconazole-sensitive strain. With fluconazole-resistance strain, the combination between 16 µg/mL of Mycograb with 64 µg/mL of fluconazole or 8 µg/mL of Mycograb with 128 µg/mL of fluconazole led to change the static effect of antifungals to fungicidal effect.
The present study found that adding Mycograb to caspofungin did not improve the antifungal effect of caspofungin, although there was slightly decrease in the colony counts (CFU/ml log_{10}).

Discussion:
The present study demonstrated that Mycograb plus fluconazole is better than fluconazole alone and that the addition of Mycograb did not improve the antifungal effect of caspofungin. These results suggest that the combination of Mycograb and fluconazole would be worth exploring in the treatment of cryptococcosis. Additionally, Mycograb abrogated resistance to fluconazole. The lack of activity between Mycograb and caspofungin may reflect the intrinsic lack of activity of caspofungin against *C. neoformans* due to the reduce activity against *C. neoformans* glucan synthase.

ALIS-FLP (Amplified ligation selected fragment lengtpolymorphism), a new method for distinguishing of Candida species.

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Background: ALIS-FLP is a new method for genotyping, developed at DTU Denmark. In many ways the method is similar to the known SE-AFLP, but it only uses one specific

restriction enzyme. This method is utilizing one restriction enzyme *Tsp*RI, which recognises the sequence NNCASTGNN^ and creates 9 nucleotides cohesive 3' ends. The basis of the method is application of two types of oligonucletides, a long specific one and short degenerated one. Both oligonucleotides are ligated to complementary *Tsp*RI generated ends. Only few *Tsp*RI generated restriction fragments carrying two complementary cohesive ends to the specific long oligonucleotide, which is carrying the target sequence for the specific primer. Therefore, only these DNA fragments are selectively amplified in the PCR and can be analysed by standard agarose gel electrophoresis. The method has all the advantages of AFLP technique like robustness and reliability, but it is also more rapid, universal, simple and cheap. Presented method was successfully applied in distinguishing of several species of the fungi Candida.

Materials and Methods: Isolated, from the homogenous culture, chromosomal DNA was digested with *Tsp*RI endonuclease for 1 h at 65°C. Digested DNA was purified using QIAquick PCR Purification kit (QIAGEN). A set of oligonucleotides (Uni1TspAA and CompAA) was added. After a short incubation at 70°C, the mixture was cooled down and T4 DNA ligase was added, and incubated for 1 h at 37°C. The ligation mixture was dilluted in TE buffer, and used as a template for the PCR amplification. The reaction was performed using the mixture of *Taq* polymerase, PCR buffer, dNTP mix, MgCl$_2$, Uni2 primer and ligated DNA. Obtained reaction products were analysed on a 2% agarose gel with ethidium bromide.

Results: The method was tested on different isolates of 6 different species of *Candida* (*albicans, glabrata, krusei, dublinensis, holmii* and *parapsilosis*). The chromosomal DNA of an each species was independently isolated 3 times and used for ALIS-FLP genotyping. Resulted paterns of PCR products separated on 2% agarose gel for the each *Candida* species was unique, but identical for all 3 DNA extractions from the same species.

Discussion: The method was found as a very robust technique of distinguishing of *Candida* species. We observed that the method had some limitation, good quality DNA of high concentration was necessary to apply this technique. We found that the method could be developed and used as a diagnostic tool for identification of *Candida* species difficult to identify by routine techniques.

Resistance testing of 100 mycological isolates at St Olavs hospital.

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Background: 100 E-tests were offered to test and compare the new substance caspofungin with fluconazole, caspofungin, voriconazole and amphotericin B on clinically significant yeast isolates.

Materials and Methods: Period of testing: 03.09.04-21.12.05. The tested isolates were from the respiratory tract (n=37), blood cultures (n=30), tissues (n=23), urine (n=4) and wounds (n=4). Candida spp. were suspended in 0,85% NaCl to 0,5 Mc Farland turbidity from Sabouraud agar after 24 hrs, and C. neoformans to 1 Mc Farland turbidity from colonies incubated for 48 hrs. 400 μl. were swabbed to 150 mm RPMI agar plates and absorbed for at least 15 min until dry. E-tests for C. spp. were applied and incubated for 24-48 hrs until growth was clearly seen. Amphotericin B was read at complete inhibition of all growth. Caspofungin and azoles at 80 % inhibition. C. tropicalis and C. glabrata were read at 48 hrs to detect heteroresistance. CLSI breakpoints were applied and tentative sensitivity values for voriconazole/caspofungin </=1 (ref. 1). Identification was managed through use of growth on Saboraud agar, germ tube test, ID32C Biomérieux, CHROMagar Candida (BD/Smith). Rice agar was used for morphology, incubation at 42°C, and Glabrata R.T.T. (Fumouze diagnostics) at suspected C. glabrata.

Results: 58,4% (n=89) of the yeast isolates were C. albicans, 2,2 % C. dubliniensis, 15,7% C. glabrata, 3,4% C. guillermondii, 1,1% C. kefyr, 4,5% C. krusei, 3,4% C.

norvegiensis, 1.1% C. parapsilosis, 6,7% C. tropicalis, 1,1% C. neoformans and 2,2% Saccharomyces cerevisiae.

All C. albicans isolates were sensitive to all the tested drugs. 4 of 14 C. glabrata were resistant to fluconazol, 1/14 had increased MIC for voriconazol, 1/14 had increased MIC of 1 for amfotericin B. All C. tropicalis/kefyr/S. cerevisiae were sensitive to all of the tested antifungals.

1/14 C. glabrata was resistant to voriconazole and 4/14 had reduced sensitivity to fluconazole. 2/3 C. guillermondii were resistant to caspofungin. All C. krusei had elevated MIC to fluconazole. Both C. norvegiensis isolates showed reduced sensitivity for fluconazole. There was resistance to caspofungin for the C. neoformans isolate. C. parapsilosis had a MIC of 3 for caspofungin.

Discussion: All C. albicans isolates were sensitive to all tested drugs even though 19,2% received fluconazole prophylaxis. The C. glabrata resistant to voriconazole was from bloodculture of a 78 year old woman with respiratory failure. The patient was treated with fluconazole 8 days prior to voriconazole treatment. All intravasal catheters were changed when growth of yeast was discovered. The voriconazole treatment was not changed due to clinical effect. Cross-resistance to triazoles has recently been demonstrated for C. glabrata (ref. 2). One C. glabrata isolate had intermediate sensitivity to amphotericin B, and was found in urine of a 95 year old patient with BPH and permanent urinary catheter. Almost 1/3 of the C. glabrata isolates were resistant to fluconazole, and this has been demonstrated in other studies. All these patients were treated at our ICU.

We found no heteroresistant C. tropicalis. There was found no link between the isolates, and all patients had multimorbidity. Our two C. dubliniensis isolates were susceptible to fluconazole. C. dubliniensis resistance to azoles is described (ref. 3).

The observed resistance of C. guillermondii to caspofungin was here found in severely immunodeficient patiens (leukemia/neutropenia), and has been demonstrated earlier (ref 4). The C. krusei/norvegiensis reduced sensitivity for fluconazole is due to intrinsic resistance. Our C. parapsilosis isolate was resistant to caspofungin but even though less activity in vitro is observed, clinical effect is shown (ref 5). S. cerevisiae was susceptible to all tested drugs as shown earlier (ref 6). The C. neoformans resistance to caspofungin was seen as described before (ref 7).

Reference:
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Rapid identification of yeasts

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Background:

In recent years we have observed an increase in serious yeast infections. Since the yeasts have a predictable susceptibility pattern (*C. krusei* is resistant to fluconazole, *C. glabrata* has lower susceptibility to fluconazole and *C. albicans* is susceptible), a rapid identification is very important.

Materials and Methods:

Methods : Study one
112 isolates were tested. First the isolates were tested to yield out *C. albicans* (formation of "feet", germ tube, Bichro-latex Albicans Fumouze). The remaining isolates not identified as *C. albicans* were tested for further identification (Glabrata RTT Fumouze, Rapid Trehalose test and Krusei-color Fumouze). All the results were individually evaluated by 3 technicians.

Methods : Study two
The investigation only included samples with high incidence of yeasts. 159 samples were cultivated on chromagar in addition to Sabouraudagar (SA). SA was examined as usual in

the routine laboratory. Results from the chromogenic media were compared with the results from the routine laboratory. The plates were incubated for a minimum of 48 hours. On the chromogenic plates, the yeasts were identified as far as possible from the colours. Plates with a plentiful amount of yeast, not identified by the chromogenic media, were further identified by Vitek2 or rapid methods. Only samples with findings on both media were considered in the evaluation.

Results:
All the tests in the studies have a high specificity. Except the formation of "feet", they all have high sensitivity.

Discussion:

The *C. albicans*-methods will in addition detect *C. dublinienseis*. *C.dubliniensis* is susceptible to fluconazole, and will therefore not influence the treatment. Since 70% of all yeasts are *C. albicans*, these will be detected by the "*C. albicans*-tests". The remaining isolates should be tested with Glabrata RTT Fumouze, Rapid Trehalose test and Krusei-color Fumouze. And in more than 80% of the cases, species identification is obtained within 24 hours. This gives valuable information for treatment. When using regular bacterial media, it's only possible to detect *Candida* species. If chromagar is used, it will be possible to identify 3-8 *Candida* species, depending on experience. In addition it will detect far more mixed cultures. Mixed cultures not detected on SA, can lead to wrong identification. Species in a mixed culture that are resistant to fluconazole, may therefore sometimes not be detected. If chromagar is not used in addition to the other rapid tests, it can lead to wrong treatment of the patient.