Nordic Society for Medical Mycology

Finnish Society for Medical Mycology

4th Scientific Meeting of the Nordic Society for Medical Mycology

Biomedicum Helsinki 30th May 2007, 11.00-17.30

Superficial Fungal Infections

Programme and Abstracts

Welcome

Dear Friends,

We want warmly welcome all participants and speakers to the fourth scientific meeting of Nordic Society for Medical Mycology, which is now held here in Biomedicum, Helsinki Finland. The meeting is arranged together with Finnish Society for Medical Mycology.

The scientific programme is focused on superficial fungal infections and we begin together with our distinguished keynote lecturer Jack Sobel with vaginal infections and go on with eminent speakers from Nordic countries handling other areas of superficial fungal infections. Altogether these infections are among the most common ones in human populations and almost everyone will at some time of his/her life come to contact with today's topic.

This fourth meeting ends the first four-year chain of Nordic scientific meetings of NSMM and we hope that it will give you a rewarding day and inspire the work of the young society. We finally want to acknowledge gratefully the role of our sponsors.

On behalf of the organisers

Maiken Cavling Arendrup
President of NSMM

Risto Visakorpi
President of FSMM

Programme

11.00:	Welcome: Finn Soc Med Mycol President/NSMM President
11.05:	Key-Note address: Mycological Aspects of Recurrent Vulvovaginal Candidiasis. Jack Sobel MD (USA).
12.00:	Lunch/Posters
13.00:	Non-dermatophytes as agents of onychomycosis. Jouni Issakainen (Finland).
13.30:	Update on recurrent vulvovaginal candidosis: Nordic perspective. J Paavonen (Finland).
14.00:	The use of probiotics in controlling oral candidosis: M. Richardson (Finland).
14.30:	Diagnostic and treatment challenges in skin and mucosal fungal infections: Three clinical cases. Else L Svejgaard and Merete Hædersdal (Denmark).
15.00:	Onychomycosis: New Treatment Options. Jan Faergemann (Sweden)
15.30	Coffee/Tea Break/Posters
16.00	Skin and mucosal manifestations of systemic fungal infections. Veli-Jukka Anttila (Finland)
16.30	Problems in antifungal therapy for superficial fungal infections Asko Järvinen (Finland)
17.00	Free/offered papers (3)
17.30	End of meeting
17.45	Annual General Meeting, followed by dinner

Recurrent Vulvovaginal Candidiasis Advances in Pathogenesis and Treatment

JD Sobel, M.D.

Recurrent vulvovaginal candidiasis (RVVC) continues to involve approximately five to eight percent of women in their reproductive years. There are multiple causes for RVVC and accordingly therapy should be specifically directed at causation. In North America, more than 90% of women with RVVC are caused by *Candida albicans*, highly susceptible to fluconazole. Epidemiologic studies in several other areas reveals that up to 20-30% of cases are due to non-albicans *Candida* species. The availability of the azole class of oral antifungals has, together with the experience gained by a strategy of long-term maintenance suppressive therapy, provided an opportunity for excellent control but not cure of RVVC. Accordingly women can be told that their symptomatology can be effectively controlled today in virtually all cases. Therapeutic challenges, nevertheless exist in relation to control of non-*albicans Candida* infections as well as when suppressive azole therapy is not feasible. Little progress has been made in understanding causation of idiopathic RVVC per se. Issues in relation to yeast genetic factors that predispose to vaginal persistence of yeast will be discussed together with host factors that may facilitate recurrent episodes of *Candida* vaginitis. Unfortunately, there are no new major therapeutic advances likely in the near future and the latest generation azoles offer no clear therapeutic advantage and many other disadvantages. The potential for topical echinocandins as therapeutic agents will further be discussed. Studies are just emerging of host polymorphism as a critical factor in susceptibility to RVVC.

Non-dermatophytes as agents of onychomycosis

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In addition to Dermatophytes (Arthrodermataceae), more than ten unrelated genera of fungi are known to infect nails. These Non-Dermatophytic Nail Pathogens (NoDNaP fungi, below) are usually widespread saprotrophs wich also possess some enzymes for digestion of keratin or other nail components. Local, general or temporary defects of the host defence may initiate or support the infection. Mould infections comprise about 1 to 10 % of fungal nail infections, depending on study population, geographic area and diagnostic criteria. Yeasts colonize disturbed surfaces more readily. Accordingly, the number of yeast cases greatly depends on how the limit between infection and colonization is drawn.

Regularly encountered mitosporic moulds include *Scopulariopsis, Scytalidium, Aspergillus, Fusarium, Acremonium* and *Onychocola* species. NoDNaP yeasts include various *Candida* species and *Trichosporon*. Yeast infections often occur secondary to occupational irritation or genetic defects.

Diagnosis of a NoDNaP infection requires careful exclusion of Dermatophytes and other common causes, as well as good documentation of long-term fungal growth in the nail. Symptom-causing infection can seldom be separated *a priori* from saprotrophic colonization, however. Final proof of the role of the fungus is obtained by clinical response during relevant antifungal treatment.

By careful diagnosis and treatment, most NoDNaP infections can be cured. A majority of mold cases have responded to long systemic treatments based on itraconazole or terbinafine. Many treatment failures and cases of obscure etiology remain, however, partly due to hasty diagnosis and treatment.

Interesting future prospects include the development of rapid molecular tests which cover rare causative agents and their enzyme armamentarium. New methods are not free of many old limitations, however, such as sampling error and contamination. More data is needed about the interaction of fungi and different types of nail tissue.

Update on recurrent vulvovaginal candidiasis: Nordic perspective

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Vulvovaginal candidiasis (VVC) is a mucosal infection caused by Candida species. These Candida species include Candida albicans and non-Candida albicans species, the most common of which are C. glabrata and C. tropicalis. Most women experience one or more episodes of candida vaginitis. Recurrent VVC is a common clinical problem accounting for large number of gynecologic outpatient visits and costs to health care system. Recurrent VVC is defined as four or more episodes of proven infection with candida during a 12-month period. Management of recurrent VVC is problematic and causes frustration to patients and health care providers alike. Many factors may contribute to increased susceptibility to recurrent or chronic colonization with Candida. These factors include immunologic host factors, hormonal factors, co-infections and complex virulence factors. Thus, recurrent VVC is a condition which causes considerable suffering and has a negative effect on sexual relations. Patients with recurrent VVC often use over-the-counter antimycotics which are not highly effective or are inconvenient. Therefore, maintenance oral fluconazole therapy for recurrent VVC has become increasingly popular. Studies have shown that prolonged weekly treatment with fluconazole is effective and reduces the rate of recurrent episodes. Such long-term therapy is safe and well tolerated. Antifungal drug resistance in Candida to azoles appears to be relatively rare and not a common problem in clinical practice. However, on the basis of what is known about antibiotic resistance in bacterial pathogens, low doses of drugs administered over long periods of time either continuously or intermittently is likely to accelerate the development of drug resistance. Recurrent VVC is often misdiagnosed based on symptoms only. Symptoms may relate to hypersensitivity reactions or chemical or allergic reaction to topical antimycotics which result in symptoms falsely thought to be caused by Candida. Other treatment modalities often recommended to women with recurrent VVC include topical boric acid, other antimycotics including polyene class of antifungal drugs such as nystatin, probiotics such as vaginal lactobacilli preparations, and dietary counseling. Immunopathogenesis of recurrent VVC is complex and poorly understood, and little is known about specific vaginal immune mechanisms. Development of prophylactic or therapeutic anti-Candida vaccine is a major challenge in the future.

The use of probiotics in controlling oral candidosis

Malcolm Richardson, Department of Bacteriology & Immunology, University of Helsinki, Finland.

Probiotics are defined as live microorganisms which when administered in adequate amounts confer a health benefit on the host. There is strong evidence that *Lactobacillus rhamnosus GG* (LGG) is effective for the treatment of acute rotavirus diarrhoea in children, causing a significant reduction of its duration. In addition, their usefulness for the prevention and/or treatment of many other diseases, such as antibiotic-associated diarrhoea (*Saccharomyces boulardii), Helicobacter pylori* infections, inflammatory bowel diseases, allergy, cancer, urinary tract infections and vagonosis, is under research.

Colonisation of mucosal surfaces by *Candida albicans* is a major cause of disease, with vulvovaginal and oral-pharyngeal infection being particulary prevalent. Mucosal infection in the upper gastrointestinal tract is a precedent for systemic spread in subjects with compromised immunity although the mechanisms of protection that contain mucosal colonization, on the one hand, and systemic invasion on the other hand, appear to differ. *Candida* infection of the oral mucosa in mice triggers an inflammatory response and stimulates cellular immunity. Feeding nude mice probiotics prolongs their survival following intestinal challenge with live *C. albicans* by enhancing both antibody and cell-mediated immunity. Reports of protection against clinical mucosal infection following ingestion of yoghurt containing *Lactobacillus acidophilus* are encouraging. More recently, we have shown that probiotic cheese containing a combination of LGG and bifidobacteria in elderly subjects reduced the prevalence of oral Candida. Because antifungal drug resistance in *Candida* spp. is increasing this observation is interesting and it opens future visions for eventually controlling oral yeast infection by use of probiotics as an adjunct therapy.

References:

Elahi S, Pang G, Ashman R, Clancy R. Enhanced clearance of *Candida albicans* from the oral cavities of mice following oral administration of *Lactobacillus acidophilus*. Clin Experi Immunol 2005; 141: 29-36.

Hatakka K, Ahola AJ, Yli-Knuuttila H, Richardson M, Poussa T, Meurman JH, Korpela R. Probiotics reduce the prevalence of oral *Candida* in the elderly – a randomized controlled trial. J Dent Res 2007; 86: 125-130.

Meurman JH. Probiotics: do they have a role in oral Medicine and dentistry? Eur J Oral Sci 2005; 113: 188-196.

Onychomycosis: New Treatment Options

Jan Faergemann, Department of Dermatology, Sahlgrenska University Hospital, Gothenburg, Sweden

Onychomycosis is the most prevalent nail disease responsible for 50 % of all nail disturbances. Dermatophytes are the dominant fungi (85-90 %) in toenail onychomycosis and *Candida* sp. are the dominant fungi in fingernail onychomycosis. However, *Candida* onychomycosis is almost always secondary to paranychia or onycholysis. With matrix involvement only oral treatment is effective. Terbinafine is the most effective therapy for dermatophyte onychomycosis and itraconazole for *Candida* onychomycosis. However, even with terbinafine only around 50-55 % of the patients are completely cured. Recently combination therapy with oral terbinafine and topical therapy with amorolfine was more effective than oral therapy alone with cure rates of 70 to 75 %. In this study terbinafine was given once daily for 12 weeks and amorolfine nail lacquer once weekly for 12 months or shorter if the nail was normal. Combination therapy may be a new option in difficult cases of onychomycosis.

Skin and mucosal manifestations of systemic fungal infections

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Consultant in Infectious Diseases, Helsinki University Central Hospital

Systemic fungal infections are usually problems in immunocompromised patients. These infections occur especially in leukaemia patients, after stem cell or organ transplantation, or after prolonged treatment at intensive care unit or in very complicated surgery patients. The diagnosis of deep fungal infections can be very difficult to verify. Fungal lesions in deep tissues are not easily detected by imaging methods, like CT, MRI or ultrasound. Cultures of blood and tissue samples often remain negative. For the definite microbiological confirmation of diagnosis an invasive procedure, such as US or CT guided fine needle biopsy, laparoscopy or open lung biopsy is required. In addition to the difficulties with diagnosis, the mortality from deep fungal infections is still high. The mortality has varied from 20- to over 80 per cent.

Invasive skin and mucosal lesions are not a common feature of deep fungal infection. When they occur, they are signs of dissemination (candidosis, fusariosis) of infection or are local invasive lesions (Mucor). The origin of disseminated yeast infection is usually superficial mucosal infection or colonisation. Most often disseminated candidosis are due to the mucosal damages of the gastrointestinal tract caused by cytotoxic treatment of haematological malignancy. In a study by Bae et al (International Journal of Dermatology 2005; 44: 550–555.) skin lesions were seen in 35 per cent of patients with systemic candidiasis. Most often the causative species was *C. tropicalis*. The skin lesions were a maculopapular or nodular rash and plaques. In the mentioned study most patients had haematological malignancy and the mortality was high, over 84 per cent.

In mold infections skin lesions can occur especially in fusariosis and mucor infections. In the most common mould infection, aspergillosis, the skin or mucosal lesions are usually a sign of heavy dissemination and it means poor prognosis for a patient. *Fusarium* skin lesions are often tender, especially subcutaneous nodules, and can involve any skin site, although they appear predominantly in the extremities. In opposite to the Aspergillosis, *Fusarium* can be cultured usually from blood. Mucor infections can originate by the

dissemination of fungus or they can be local invasive and aggressive, and cause ulcerous skin damage. In the mouth they can cause an ulceration or a fungal mass. Most often mould infections of skin or mucosal area occur in patients with haematological malignancies. The cornerstone of therapy of single lesion is radical surgery concomitantly with systemic antifungal treatment.

Amphotericin B has been for decades the most effective antifungal. It is a broad spectrum antifungal, but it is quite toxic, especially for the kidneys. Amphotericin B lipid formulations are better tolerated but more expensive alternatives to the conventional amphotercin B deoxycholate. Recently, new systemic antifungals (voriconazole, posaconazole and caspofungin in Europe) have approved for the therapy of deep fungal infections. Caspofungin and other echinocandins (anidulafungin, micafungin) are effective against most candida species and aspergillosis. Voriconazole has efficacy against most fluconazole resistant Candida species (C.krusei, C.glabrata) and has good activity against aspergillosis and fusariosis. Posaconazole is the only azole effective against Mucor infections.

Detection of mucosal or skin manifestations of deep fungal infections in immunocompromised patients are important to detect, because they are a great help for fungal diagnostics. Skin biopsy is less invasive and easy to perform than biopsy from deep organs.

PROBLEMS IN ANTIFUNGAL THERAPY FOR SUPERFICIAL FUNGAL INFECTIONS

Asko Järvinen

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Drug related problems in antifungal therapy are mostly related to systemic therapy with azoles. They may be involved in interactions with other drugs. Some of them are also poorly and unreliably absorbed. Furthermore, they can potentially be involved in cardiac arrythmias leading to sudden death.

Absorption

Ketoconazole and itraconazole are poorly and variably absorbed. Absorption can be enhanced by dosing the drug with acidic liquids or together with a meal and by avoiding drugs that diminish gastric acidity. Although, the average bioavailability of itraconazole is around 55 % it varies widely between individuals. In some patients, only a minimal amount of the drug may be absorbed from oral itraconazole capsules. Oral solution of itraconazole is somewhat better and more reliably absorbed counteracting partly these problems but with a significant extra cost. High inter-individual variation may also make drug interactions more likely in some patients and this phenomenon is shared with many newer azoles as well. Absorption of itraconazole is most probably also variable between the doses within the same patient – a phenomenon usually observed with drugs with high inter-individual variability. This might be thought to cause treatment failures in dosing schemes where the drug is taken seldom or even provoke resistance development although no evidence of these hypothetical risks is available. Fluconazole is, however, well and reliably absorbed also in oral dosing.

Interactions

The pharmacologic basis for antifungal effect of all azoles is inhibition of a CYP-family enzyme in fungal membrane. These CYP's (cytochromal enzymes) are, however, found throughout the phylogenetic spectrum and they are the most important drug metabolising system in human body. They are concentrated in liver and there are 12 different isoforms of CYP's that are mainly responsible for drug metabolism. Agents that use the same metabolic

pathway may interact with each other if one of the agents is capable of binding more tightly to a specific CYP isoform (enzyme inhibition) or inducing production of a CYP enzyme and thus increasing the metabolic capacity (enzyme inducers).

Those azoles that are metabolised through CYP's in human body (keto-, itra-, vori- and posaconazole) are also affected by enzyme inducers. Common enzyme inducers are e.g. many anticonvulsants like phenytoin, carbamazepine and rifampicin and an increasingly used diuretic agent, spironolactone. When used simultaneously with enzyme inducers the concentrations of these azoles are much decreased.

Azoles are potent inhibitors of main drug metabolising CYP's but they do not share this property equally. While ketoconazole and miconazole as well as the newer azoles itra-, vori and posaconazole are all potent CYP 3A4 inhibitors fluconazole inhibits CYP 3A4 only when given in high doses, usually at least 200 mg /day. CYP3A4 inhibition leads to elevated drug levels of many other drugs when given at the same time with these azoles. In particular, drug interactions may occur with many anticonvulsants, benzodiazepines, calcium channel blockers, digoxin, lipid lowering statins, warfarin, opiates, HIV protease inhibitors etc. The effect of azoles on corticosteroids seem to be variable. While dexamethasone and methylprednisolone levels are significantly elevated, only minor changes can be seen in prednisone levels during concomitant azole therapy.

Fluconazole and voriconazole and to a lesser extent ketoconazole are also inhibitors of CYP 2C9 and 2C19, a route used by much less drugs than CYP 3A4. These azoles may increase levels of e.x. some tricyclic antidepressants, cyclosporine, tacrolimus, warfarin, oral diabetes drugs and phenytoin. It is to be noted that the lists above are not complete but when azoles are used at the same time with other drugs the product summary of both drugs must be read carefully to avoid interactions. Even local treatment may cause interactions as is the case with oral trush treatment with miconazole which has been described to significantly potentiate oral warfarin anticoagulation.

Terbinafin is not totally free of interaction risk but it inhibits CYP 2D6. The target drugs with potential concentration increase during terbinafin

therapy include at least some tricyclic antidepressants, some newer antipsychotics and antidepressants and some analgesics as well as antiarrythmic agents and some beta-blockers.

OTc-prolongation

Prolonged cardiac recovery may be behind an arrythmia leading to sudden death. The risk is observed as prolonged QTc-time in electrocardiography. QTc-time prolongation may be familial and in these patients all drugs that may have an effect on QTc-time should be avoided. Azoles should not be used in these patients as they may slightly prolong QTc-time. They effect is so small that problems in healthy people can not be expected unless they have other drugs that also prolong QTc-time.

Right dosing of fluconazole

Fluconazole resistant Candida species have become more prevalent. Experience with antibacterials suggest that risk for resistance development could be smaller if the drug is dosed correctly. Recent pharmacokinetic data suggests that for maximal efficacy against Candida species fluconazole serum concentration should exceed MIC at least 50 % of the dosing interval. This means that fluconazole should be dosed frequently for best treatment results.

POSTERS

Glans penis and prepuce colonization of yeast fungi in a pediatric population: pre-and post- circumcision results

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Background: The members of the genera *Candida* and *Malassezia* comprises opportunistic yeasts with a natural habitat on the skin of humans and warm-blooded animals. This study aimed to compare the prevalence of these yeast fungi in samples from the glans penis and prepuce 3-5 minutes prior to circumcision and after one-month follow-up by mycological examination.
Materials and Methods: A total of 77 children aged between 0.01-13.0 were included in the study. Impression preparations were made on modified Dixon and Leeming-Notman agar without cycloheximide. The isolates were identified by morphological, biochemical and physiological characteristics.
Results: The frequency of yeast colonization was found to be significantly decreased after circumcision (from 9/77 to 1/77) as detected at one-month follow-up by mycological examination. Both study media presented identical power of isolation. The glans penis and prepuce were colonized with especially *Candida albicans* (50%) followed by, *Malassezia furfur* (40%) and *Malassezia sympodialis* (10%). However, the colonization were decreased significantly after circumcision in the pediatric population (McNemar $p = 0.008$).
Discussion: This sudy highlighted the potential medical benefits of circumcision as a significant factor decreasing the colonization rate of yeast fungi. We suggest that circumcision, rather than age, plays an important part in the reduction of yeast fungi in genitalia. Since literature concerning colonization of yeast fungi including pre- and post-circumcision results is almost non-existent, this study may be regarded as first of its kind, dealing with genital colonization prior and following circumcision.

Indoor air of St.Petersburg metro: a possible source of potential human pathogenic fungi?

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Background: During the last decades a great number of saprotrophic fungi have been reported as new opportunistic human pathogens. It is due to many reasons, among which there are human susceptibility induced by various diseases, and specific ecological conditions in man-made environments. One of such environments is metro, which consists of a network of tunnels with constant conditions, and stations which are typically overcrowded by people. In the metro of St.Petersburg the average number of trips is more than 4 million / day. At the same time, technical conditions of tunnels sometimes are far from ideal. Many building constructions in the metro biodeteriorated by fungi and other microorganisms. So, the main aim of this study was to reveal possible fungal human pathogens in the indoor air of four metro stations in St.Petersburg and to assess the extent of potential risk for metro passengers.

Materials and Methods: Samples of metro air microbiota were taken by passive sedimentation on Petri dishes at 4 metro stations of different type (open-type underground, open-type ground, closed-type underground and transfer node). Czapek agar and meat-peptone agar were used for fungi and bacteria, respectively. Then fungal and bacterial colonies were counted, microfungi isolated into pure culture and identified. To evaluate potential virulence of isolated strains we determined their phospholipase and protease activities using common test media with egg yolk and bovine serum albumin, respectively. These enzymes are known as virulence factors which allow fungi to invade human tissues. Active strains (those which have shown high values of one or two virulence factors) were treated as potentially pathogenic for humans.

Results: In the indoor air of St.Petersburg metro there were found 38 fungal species, the main genera by their occurrence were *Acremonium, Aspergillus, Cladosporium, Penicillium*. CFU number of fungi varied at different stations from $0.44 \cdot 10^2$ to $3.13 \cdot 10^2$, bacterial CFU number varied from $0.3 \cdot 10^4$ to $2.1 \cdot 10^4$. 75 fungal isolates were tested for their protease and phospholipases activities. Protease activity was found in 40 isolates (53%), phospholipase activity – in 56 isolates (75%). Both activities were found in 32 isolates (43%), among which 9 (12%) may be regarded as dangerous for humans due to high values of both enzymes activities.

Discussion: Fungal CFU number was about the sanitary level, but bacterial CFU number sometimes significantly exceeded normal sanitary levels, accepted for public buildings. High percentage of enzymatically active fungal strains may be explained by natural selection of fungi with biodeteriorative properties in such specific environment as metro. At the same time, number of potentially dangerous fungi is not so high (12%), but still considerable. Taking into account the situation with metro overcrowding (which may highly increase fungal number in the air), especially in peak hours, it may be concluded that the risk of mycotic "mould" diseases for metro passengers in St.Petersburg does exist.

Activity of posaconazole against clinical isolates of Candida albicans with decreased sensitivity to fluconazole from APECED patients

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Objectives: Most patients with APECED (autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy) suffer from chronic oral candidosis from early childhood. Thus most patients receive repeated treatment and maintenance courses of azole antifungals, principally ketoconazole and fluconazole, throughout their lives. This has resulted in both mycological and clinical resistance. Our aim was to determine the susceptibility of patient isolates from Finnish patients with APECED to the new triazole antifungal posaconazole, recently approved for the treatment of oropharyngeal candidosis, including infections refractory to fluconazole. In previous studies posaconazole has been shown to be active against most fluconazole-resistant C. albicans isolates.
Methods: The antifungal susceptibility profiles and antifungal usage of all 56 APECED patients followed in our centre were reviewed for the period 1994-2004. C. albicans isolates of 11 patients reported to have decreased fluconazole sensitivity (n=27, MIC range: 8-32 mg/L) were tested for their sensitivity to posaconazole using a broth dilution technique as detailed in the CLSI document M23-A2. C. albicans strains of 11 patients APECED patients reported to be sensitive to azoles (n=16, MIC range: 0.12-2 mg/L) were tested in a similar manner.

Results: C. albicans isolates previously shown to be of decreased susceptibility to fluconazole were uniformly sensitive to posaconazole (MIC range: 0.03-1 mg/L). Isolates previously scored as fluconazole sensitive were equally sensitive to posaconazole (MIC range: 0.12-1 mg/L). The upper limit of posaconazole sensitivity has been tentatively been set at ≤1 mg/L.

Conclusions: Decrease in the susceptibility of the colonizing C. albicans strains to antifungals was noted in the mid-1990s. Currently, symptomatic patients are prescribed topical polyenes. In some patients eradication treatment of the fluconazole resistant strains with iv caspofungin or liposomal amphotericin B has been successful in combination with topical polyenes and professional oral hygiene procedures. The present data, which highlights the fungistatic and fungicidal activity of posaconazole against strains with decreased fluconazole sensitivity, suggests that oral posaconazole would be effective for treatment of candidosis caused by strains of C. albicans with decreased sensitivity to fluconazole in APECED patients. Furthermore, posaconazole could be used as a first-line drug for eradication treatment.

Analysis of DNA signatures in fungal infections – a feasibility study for the diagnostic laboratory

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Background: Microbiological diagnosis of systemic fungal infections can be challenging, and often the etiologic agent(s) remains unknown. In this study, we evaluated the usefulness of Broad-range fungal PCR amplification in identification of fungal isolates, as well as in detection and identification of fungal pathogens directly from clinical samples. **Methods:** PCR primers targeting conserved sequences of the fungal 18S rDNA (SSU) gene and the internal transcribed spacer (ITS) were used. In all, 12 different yeast and nine dermatophyte strains were studied by PCR, followed by DNA sequencing. Additionally, 11 cryopreserved fungal culture positive clinical tissue samples were analyzed. **Results:** The sensitivity of the assay was up to 4fg and 4pg of purified *Candida albicans* DNA per assay using primers targeting the SSU and ITS regions, respectively. When *Aspergillus flavus* (ATCC 10124) DNA was used as template, the corresponding assay-sensitivities were 600fg and 6ng. All twenty fungal isolates exhibited reactivity in the PCR assays, whereas no cross reactivity from four human or two bacterial DNA isolates were observed. Six of the 11 culture-positive clinical tissue samples were positive with the SSU PCR, and based on DNA identification five of these results correlated with the classical culture findings. In one case genotypic identification was not possible due to a double infection that was revealed by fungal culture. Only one of the samples was correctly identified with the ITS assay, nine other samples remaining PCR negative and one containing a mix of more than one sequence. Of note is that all broad range PCR positive clinical samples contained fungal elements as visualized by native microscopy. By contrast, all four culture positive, microscopy-negative samples were also PCR negative. **Conclusions:** Broad range fungal PCR may be advantageous as a supplemental tool for determining the fungal etiology of certain fungal infections, particularly from culture isolates. However, the use of the current ITS methodology for

direct analysis of clinical samples is limited by its low sensitivity. Moreover, the use of the SSU gene for fungal identification is hampered by the lack of interspecies sequence variation. More experiments are needed to validate whether these broad range PCR assays are beneficial for microbial identification of culture-negative, microscopy-positive fungal infections.

Key words: broad range fungal 18S rDNA PCR, fungal infection, molecular

Sponsors

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