PRYOSEQUENCING™; A NEW RAPID AND RELIABLE DNA-SEQUENCING TECHNOLOGY FOR SPECIFIC IDENTIFICATION OF FUNGI, VIRUSES AND BACTERIA

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Invasive fungal infections are increasingly observed life threatening complications and important causes of morbidity and mortality in immunocompromised hosts. Early detection of infection permits timely initiation of antifungal therapy with a greater possibility for improved survival and reduced morbidity. Pyrosequencing™ technology was used for identification of different clinically relevant fungi. Pyrosequencing is a non-electrophoretic DNA sequencing method based on sequencing-by-synthesis. The method employs a four-enzyme mixture to sequentially determine the sequence of a target DNA in real-time. The tests were performed on amplicons derived from the 18S rRNA gene using PCR universal primers for amplification. Sequencing was performed up to 40 bases in a variable region with a designed general sequencing primer and the pyrosequence data were analysed by BLAST sequence homology search in the GenBank database. DNA from fungal specimens, including strains of clinically relevant fungi and clinical specimens from patients suffering from proven invasive fungal infections were PCR-amplified and analysed by gel electrophoresis, PCR-ELISA and pyrosequencing. All data obtained by pyrosequencing were identical with the results obtained by PCR-ELISA using species/genus-specific oligonucleotides and were as well in accordance with the culture results. The results demonstrate that pyrosequencing is a rapid, reproducible, and highly specific technique for identification and typing of fungal pathogens. The method is inexpensive, robust and well suited for large-scale programs. Furthermore we have applied pyrosequencing for detection and genotyping of Human Papillomaviruses (which are known to be important causative agents for cervical cancer) and detection and identification of bacteria in children with sepsis. These results will be discussed in detail along with other future applications.

ONYCHOMYCOSIS IN CHILDREN

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Onychomycosis in children is an uncommon occurrence compared to adults. Regarding epidemiologic data from the literature its prevalence is estimated to be 0 and 0.44%, repectively. In childhood onychomycosis there are different similarities with adults according the clinical picture and the causative pathogen. As in adults childhood onychomycosis is often accompanied by tinea pedis and a family history of onychomycosis. The clinical presentations of fungal nailplate infection in children reveals the same pattern as in adults with distal and lateral subungual onychomycosis (DLSO) being the most common type. The etiologic agent most commonly isolated is *Trichophyton rubrum*. Nevertheless oral antifungal treatment is required to achieve cure, especially with forms of moderate to severe onychomycosis with nail matrix involvement.

Unlike in adults there is only little experience with the use of the three new oral antifungals – itraconazole, terbinafine, and fluconazole – in the treatment of onychomycosis in childhood population as these drugs are not specifically approved in this age group so far. In accordance to the literature and our own experience these drugs appear to be safe and effective with the advantage of short duration of therapy and availability of liquid formulation for better compliance.

Meanwhile we can report on our experience with treatment of childhood onychomycosis in 24 children aged 4 to 17 years. In all children toenails were affected mainly presenting the DLSO type. 19 children received itraconazole and 5 terbinafine therapy in a continuous treatment and weight depending dosage schedule as recommended. In all but two cure could be achieved. Only in one child a clinical side effect (fatigue) was reported.

Conclusion: To our experience with these new antifungals in childhood onychomycosis cure seems to be achieved in a shorter time than in adults, in addition severe dystrophic nail changes cleared in all. All cases were followed and no recurrences were detected.

INVASIVE FUNGAL INFECTIONS IN INTENSIVE CARE UNIT

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Purpose: Invasive fungal infections (IFI) is associated with a high mortality rate, specially in patients with delayed beginning of antifungal treatment. To examine clinical features of IFI, the presence of individuals risk factors, the active list in spread of fungal infection by healthcare staff's hands or by contaminated medical equipment.

Patients and methods: During 2001, 2 cases of invasive candidosis and 1 case of invasive aspergillosis were observed in intensive care unit (ICU). Patients were evaluated in respect on diagnostic criteria. Patients included 1 man, (aged 66 years), dialyzed since '85, with otomycosis developed a CNS infection by *C. glabrata*; 1 woman (aged 25 years) after an aortoplastic surgery to treat aortic coartation, developed a pleural empyema by *C. albicans*; 1 child with left heart hypoplasic syndrome

and developed a mediastinitis by *Aspergillus*. All patients received lipid complexed amphotericin B (5 mg/Kg/die for 4 weeks).
Results: Adults responded to antifungal therapy, the child died because of an ongoing infection in spite of therapy.
Conclusions: Clinical diagnosis is difficult due non-specific signs. Dialysis, superficial colonization and *Aspergillus* spores in the air should be taken in consideration for risk factors. Preventive treatments and full understanding of epidemiology is required. Prompt antifungal therapy could warrant to save patient's life.

BUCCAL APPLICATION OF ITRCONAZOLE SOLUTION – TOPICAL EFFECT AND PLASMA LEVEL

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Introduction: The incidence of deep fungal infections is increasing worldwide and the mortality associated with a systemic mycosis is still high. The therapeutic use of antifungal drugs is often limited by important disadvantages like inadequate antimicrobial spectrum, route of administration or side effects. Thus, identification of relevant risk factors for systemic mycoses is gaining importance. Fungal colonization is an independent risk factor for developing invasive candidosis including candidemia.
Methods: In a prospective study, we analysed the topical effect of itraconazole and its plasma level in 4 non-hematological intensive-care patients. In each of these 4 patients, a pharyngeal colonization with at least one species of *Candida* was detected. Treatment consisted of local application of 25 mg of itraconazole solution into each buccal pouch every 6 hours (200 mg/d). Plasma levels of itraconazole and hydroxyitraconazole were measured daily by HPLC. Pharyngeal swabs were cultured for fungal growth initially and several times for follow-up.
Results: The fungal cultures grew *Candida glabrata* in one case and *Candida parapsilosis* in two cases. In the fourth case, a mixed culture of *Candida albicans* and *Candida glabrata* was detected. In all subjects, upon follow-up cultural examination, a reduction in germ counts, but no eradication was detected. Median plasma levels were 110 ng/ml (range 0 to 267) for itraconazole, and 157 ng/ml (range < 100 to 639) for hydroxyitraconazole.
Conclusions: Buccal application of itraconazole solution in a dose of 200 mg/d was not effective to eradicate pharyngeal colonization of *Candida* species as a risk factor for systemic candidosis in non-haematological ICU-patients. A reduction of germ count seems possible, though. The lack of efficacy is probably due to incomplete distribution of the antifungal drug in the oral cavity and throat. The measured plasma concentrations of itraconazole and its metabolite hydroxyitraconazole were markedly below known therapeutic levels. This may be explained by ineffective swallowing in these sedated, mechanically ventilated patients. Furthermore, pharmacokinetic interactions leading to diminished resorption of itraconazole must be considered.

MYCOLOGICAL FLORA IN THE ENVIRONMENT OF A SOCIAL WELFARE HOME

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The objective of the study was an evaluation of fungi in the environment of a social welfare home and on the skin of its inhabitants in four seasons

The mycological evaluation comprised specimens sampled from the indoor air (using a MAS 100 device), the walls of the rooms (a Count-Tact method) and swabs from the skin and skin lesions.

During four seasons, 104 indoor air samples, 40 imprints from the walls, and 83 clinical materials from the inhabitants were collected. The same fungal species were isolated from the air, the walls and the inhabitants' skin. The lowest number of colony forming units in one cubic metre of air (CFU/m^3) were found in winter, while the highest in autumn (up to 6533 CFU/m^3). Not only the quantities of fungi were different in various seasons but also their composition. Out of the moulds, *Penicillium* and *Alternaria* dominated in spring, additionally *Cladosporium* in summer and in autumn, and *Aspergillus* in winter. The most frequent yeast-like fungi were *Candida.*

ADHERENCE STUDIES ON *TRICHOPHYTON MENTAGROPHYTES* VAR. *GRANULOSUM* SPORES IN DERMATOMYCOSES

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Dermatophytes colonize in stratum corneum and adhere to epithelial cells which helps the development of infections.

In the present study, the adherence capacity of different types of dermatomycoses was examined applying Faergemann's method. Spore suspension (10^7 cells × ml^{-1}) was isolated from skin scrapings of 93 patients of dermatomycoses, as well as 47 controls (eczema, seborrhoeic dermatitis, psoriasis, atopic dermatitis).

In patients with mycosis, the average spore adherence was 4.8, whereas was 4.3 in controls meaning no significant difference between the two groups. According to the type and regional distribution of mycosis only slight differences were found in the adherence capacity.