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ABSTRACTS

SESSION VIII

Pathogenic fungi and mycoses of humans and animals

The occurrence of intestinal microsporidia in patients in Poland

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Microsporidial infections may be asymptomatic in immunocompetent hosts, but can develop into severe or disseminated form in HIV/AIDS patients, children, elderly or immunocompromised individuals, including those with primary or medically-induced immunodeficiencies.

Nine hundred and sixty-three faecal samples were collected from 347 clinical patients, immunodeficient or immunocompetent, with or without abdominal symptoms, and tested for the presence of the parasites. Microsporidia were screened using two methods: trichrome staining of faecal smears and/ or PCR technique by amplification of 16S rRNA gene fragment.

Microsporidia infections were the most abundant in the group of adult transplant recipients (with medically-induced immunosuppression), 17% of them tested positive. The first case of *Enterocytozoon bieneusi* infection in Poland was identified in the liver transplant patient. *Enterocytozoon bieneusi* sequence from the case was deposited in GenBank, under accession no. JN107808. The prevalence of intestinal microsporidia infections in the other groups of patients (children with primary immunodeficiency, adults and children with chronic diarrhea) was markedly lower.

Detection of microsporidia in patients in Poland suggests that these pathogens should be taken into account when other etiological agents cannot be found in diarrheic patients or in those undergoing immunosuppressive treatment before or after transplantation.

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Leeches (Hirudinida) as vectors of fungi potentially pathogenic to birds

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Fungi occur in water reservoirs of different kinds. They often colonize water plants as well as vertebrate and invertebrate animals. For this reason they can cause mycoses of water animals. In present study the leeches of the species *Theromyzon maculosum* (Rathke, 1862), that feed on blood of aquatic birds, were examined in terms of presence of fungi.

The research was conducted from spring to winter 2008. The experimental material consisted of microfungi isolated from tegument and digestive system of 10 specimens of *T. maculosum*, as well as water sampled in the Czarna Hańcza river in the Suwalski Landscape Park, northeast region of Poland. A membranous filtration method as well as methods of isolation, culture and identification specific for mycological diagnostics were applied. Moreover, ability to growth and development of fungi in temperatures 4°C, 25°C, 37°C and 42°C was estimated.

Analyses conducted in the study demonstrated the presence of 22 yeast-like fungi that belong to 8 genera: *Candida*, *Citeromyces*, *Debaryomyces*, *Kluyveromyces*, *Lodderomyces*, *Pichia*, *Saccharomyces* and *Saccharomycodes.* In the water from the Czarna Hańcza river 9 species of yeast-like fungi were isolated. Most frequently recorded fungi were *Candida* and *Kluyveromyces*. In total, 18 fungal species were isolated from leeches *T. maculosum*: 7 from the tegument and 11 from the digestive system. Among fungi isolated from the tegument the most frequently occurring were species of the genus *Candida*, however, in the digestive system the genus *Saccharomyces* was dominant. In 19% of fungal species growth of pseudomycelium was recorded independently on temperature. It might indicate that isolated fungi are potentially pathogenic to birds and they can form a pseudomycelium directly in water. Finding of the same fungal species on the leech tegument and in the Czarna Hańcza river suggests that the leech *T. maculosum* can be a vector of transmission of yeast-like fungi potentially pathogenic to animals, especially aquatic birds.

Prevalence of fungi in patients with geographic and plicated tongue

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Geographic tongue is a benign inflammatory condition characterized by the presence of irregular red spots on the dorsal surface of the tongue; plicated tongue is a malformation associated with abnormal superficial muscle structure of tongue, which is accompanied by a variety of fissures, slots and grooves of irregular shape. The pathological changes of dorsal surface of the tongue create favorable conditions for different living microorganisms and can cause inflammation in the oral cavity.

The aim of the study was to evaluate the prevalence of fungi in cases of geographic and plicated tongue, the determination of species and their susceptibility to antifungal drugs.

The study involved 104 patients with changes on the tongue: in 45 (43.3%) cases the plicated tongue was diagnosed, in 28 (26.9%) geographic tongue, and in 31 (20.8%), both of these changes simultaneously. Fungi were detected in 73 (70.2%) patients, including 37 (82.2%) with plicated tongue, 19 (67.9%) with geographic tongue, and 17 (54.8%) with concomitant changes. The most frequently *Candida albicans* – 48 strains (65.8%) was detected, much less *Candida tropicalis* – 11 strains (15.1%). In single cases non-*Candida* species such as *C. glabrata, C. kefyr, C. guillermondii, C. parapsilosis, C. famata, C. inconspicua, C. lusitaniae, C. humicol* and fungi of the genus *Saccharomyces – S. cerevisiae* were recognized. The sensitivity of the fungal strains was higher for Nystatin then for Miconazole.

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Fusarium solani and *Fusarium oxysporum* – phytopathogens potentially pathogenic to man

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The fungi of the genus *Fusarium* belong to the group of polyphagic phytopathogens, the reservoir of which is soil of different climatic zones. Their genetic and physiological variability make them extremely flexible and expansive, and with the potential to attack human organisms. Different species of these fungi exhibit different degrees of affinity to various tissues and organs (often to blood vessels and keratinized tissues). Two terms are usually used to describe mycoses induced by fungi of the genus *Fusarium*: hyalohyphomycoses (from "hyalyne hyphae") or fusarioses. However, they are hardly ever noted in Poland and worldwide.

The goal of this study was to analyze a few cases of hyalohyphomycosis induced by *Fusarium solani* and *F. oxysporum* and to highlight that sporadic records of fungi from the genus *Fusarium* may result from mistakes in standard diagnostics. The experimental material included swabs and fragments of necrotic tissue from an inflammatory focus of a wound at the shank resulting from cutting a dry stalk of dill, and those of hallux nailfolds from which *Candida albicans* was earlier isolated. Mycological analyses were carried out in direct specimens and in macrocultures incubated on Sabouraud and Czapek-Dox medium at a temperature of 37°C for 72 hours. When wooly, thick and creamy mycelium with agglomerations resembling sporodochia full of crescent spores appeared on the third day, the material was sieved on a PDA medium. After 48 hours, a very clear aerial mycelium producing an orange-red pigment and mycelium with pink-purple hue was developed. The species isolated from the wound on shank was *Fusarium solani*, whereas those isolated from the nailfolds were *F. solani* in one case and *F. oxysporum* in the second case. Being facultative phytoparasites, they lead part of their life cycle on a dead organic substratum – which in the discussed cases was necrotic human tissue. Isolation of these fungi from the culture and their correct identification to species was possible owing to the use of the PDA medium, being the basic medium in phytopathological diagnostics but very seldom applied in medical Mycology.

Remarks on the culture and identification of fungi of the genus *Penicillium* with various ecologies

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Species diversity within the genus *Penicillium*, as well as morphological variability within the species, pose significant problems in the identification of these fungi. *Penicillium* spores are abundant in the air of cold climatic zones and permanently present in the bioaerosol of school buildings in this area. In the heating season, the spores of *Penicillium* may even predominate in damp rooms. Their relatively high metabolic flexibility allows some species/strains to colonize different ecological niches and under some conditions, also to colonize a human body. Adaptation to new conditions may require morphological changes of reproductive structures, e.g. through modification of conidiophores or their significant simplification/retardation, which impairs rapid diagnostics.

The aim of this study was to facilitate the diagnostic procedure for fungi of the genus *Penicillium* isolated from different materials, including those from human sources. The experimental material included isolates of fungi originating from the air of public facilities (*Penicillium citrinum, P. ekspansum, P. meleagrinum, P. commune, P. nigricans, P. canescens, P. digitatum, P. stecki, P. cyaneovulvum),* and soil (*P. roseopurpureum*), as well as fungi isolated from human sputum (*P. nalgiovense*), pleural effusion (*P. roseopurpureum*) and nasal cavities (*P. simplicissimum* s. *P. janthinellum*). Differences were observed in the morphology of diagnostic traits of the analyzed isolates cultured on Sabouraud medium, with and without antibiotics, Czapek-Dox medium and Columbia medium with blood, incubated under various conditions.

Fungi of the genus *Penicillium* usually grow well on Columbia medium with blood, a medium typical of pathogenic microorganisms, though their thick aerial mycelium sporulates more slowly or becomes sterile. The use of Sabouraud medium with antibiotics and incubation at 37°C, which is common in clinical laboratories, proves ineffective in the identification of *Penicillium* genus fungi. The diagnostic procedure may be facilitied only as a result of simultaneous incubation run at 25 and 37°C. Although fungi of the genus *Penicillium* usually grow better on Sabouraud medium without antibiotics, it is advisable to run observations also on the Czapek-Dox medium. Differences in the morphology of colonies observed on both culture media are helpful in the accurate identification of the genus *Penicillium*.

Occurrence of dermatophytes in the soil of recreation places inhabitants of Łódź

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Fungal infections of the skin and its appendages affects people with a high frequency, which may be with contact with animals (zoophilic dermatophytes) and soil (geophilic dermatophytes).

Animals, especially dogs, often are present in human recreation areas, so soil of playgrounds and parks can be a source of dermatophyte infections. Physical activity of children and youth in playgrounds and sports fields can promote skin contamination with soil, which facilitates fungi invade the human body.

The aim of the study was to evaluate the prevalence of dermatophytes in the soil of recreational areas.

The studied material consisted of 104 soil samples collected from the surface layer and the depth of 10 cm below the surface of 26 recreation places in Lodz: playgrounds, and parks. Soil samples were collected at two research seasons autumn and spring. The hair bait tests were prepared from the collected soil using a sterile children hair, and were evaluated after 4–8 weeks of incubation. Positive cultures were passaged onto the Mycoline medium. Dermatophytes were identified in accordance with accepted principles of mycological examination.

Dermatophytes were found in the soil of 23 localities (88.5%), and have been classified into 6 species from 4 genera. The most frequently was isolated *Trichophyton ajelloi* (39.4% – 41 isolates from 19 samples). The second was *Microsporum gypseum* (21.2% – 21 isolates from 11 localities). Other fungi *Nannizzia cajetana* (formerly *M. cookei*) – 5.77% (6 isolates from 4 localities) and *Keratinomyces ceretanicus* – 5.77% (6 isolates from 5 localities) were noted less frequently. In single cases *T. terrestre* – 2.88% (3 times) and *M. nanum* – only 1.92% (2 times) were identified.

Isolated species are classified as common geophilic dermatophytes, potentially pathogenic, especially three of them: *M. gypseum*, *T. ajelloi* and *T. terrestre* are quoted frequently in cases of superficial fungal infections of humans and domestic animals.

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The identification of Microsporidia in patients with immunosupression

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Microsporidia are eukaryotic, obligate intracellular, opportunistic parasites that can infect most species of vertebrates and invertebrates. In humans, four species are most commonly reported: *Enterocytozoon bieneusi* and three species of the genus *Encephalitozoon*, which include *E. cuniculi*, *E. intestinalis* and *E. hellem*. Microsporidia infections are seen mainly in immunocompromised people, especially in patients infected with HIV and/or with AIDS, as well as in patients treated with immunosuppressants after organ transplantation or other forms of immunosuppression. In particular, these parasites infect enterocytes of the small intestine, causing their destruction and leading to chronic diarrhea, malabsorption, weight loss, dehydration and even death. Since microsporidia have been found in almost all organs, it is obvious that these parasites can be also the cause of disseminated microsporidiosis.

The aim of the study was to analyse the frequency of *E. bieneusi* and Microsporidia belonging to the genus *Encephalitozoon* in different samples from immunocompromised patients after organ transplantation and after oncological treatment. As a result, a high prevalence of microsporidiosis was shown in the analyzed group. A sequence analysis of identified parasites revealed that the most common species was *E. cuniculi* while *E. bieneusi* was seen less frequently.

The study was supported by the National Science Centre grant (no. 2012/05/D/NZ6/00615).

Indoor fungal analysis as a bioindicator of the potential risk to human health

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The negative influence of biologically polluted air on health and the economy is commonly discussed in the context of quality of life and its measurements. Hence, one very important factor is the quality of indoor air because nearly 90% of daily life takes place in closed rooms. Among the variety of health effects of air pollution caused by biological agents (microorganisms, mites, pollens) hazard fungi was evaluated in own research (2009–2012 years).

Mycological comparative analysis was carried out in a number of Wrocław public libraries and bookshops, private and halls of residence as well as in two poultry firms located near the Wrocław area (Lower Silesia, Poland). Taking indoor air samples using a MAS-100 air sampler (Merck), based on the principle of the Andersen air sampler, proved to be simple and convenient method. The speed of air flow through the sampler was about 11 m/s, air volumes were 5–500 liters (depending on expected contamination level) and the sampling rate was 100 l/min. Mould fungi were determined using Sabouraud medium cultured for 5 days at 26°C. Quantitative results expressed in CFU/m^3, i.e. colony forming units in 1 m^3 of the examined inside air, revealed seasonal variations in the concentration of the number of fungi. The largest number was reported in the spring, when conditions for fungal growth are best, but were almost ten times less in the winter. Inside the housing area, CFU values ranged from 10 CFU/m^3 to 9.2×10^2 CFU/m^3 and did not exceed the reference value of 5.0×10^3 CFU/m^3, according to Górny and Dutkiewicz (2002). The number of moulds in the atmospheric air of the poultry houses ranged between 2.0×10^1–1.3×10^4 CFU/m^3.

Out of 19 identified genera, the most common were representatives of *Cladosporium, Penicillium*, *Aspergillus*, *Alternaria*, and *Fusarium*; the last frequently were diagnosed fungi of the genera *Geotrichum* and *Peacilomyces*. The majority of these fungi are known to be potential respiratory allergens. Yeast species were often the dominant fungi in the poultry houses.

Comparison of proteolytic activity of *Candida* sp. strains depending on their origin

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For the invasion of host cells by fungi several virulence factors are responsible, like e.g. proteolytic enzymes, which due to the broad substrate specificity provide adaptation of fungi to different environments. Also, their activity over a wide pH range (2.0–7.0) allows the survival of fungi in various host's organs and infection of many tissues. In addition, proteases enhance the development of other virulence features and weaken the immune response of the host, increasing the pathogenicity of strains.

The objectives of the research were to determine proteolytic activity of *Candida* sp. strains isolated from the oral cavity of outpatients, hospitalized persons and individuals without clinical symptoms and to demonstrate the presence of aspartyl protease genes (SAP) in those strains. The study included 134 strains of fungi – 30 isolated from the persons without changes in the oral cavity (control), 50 – from the subjects with leukoplakia and prosthetic stomatitis (dental patients), and 54 – from the hospitalized patients undergoing radiation therapy of head and neck tumors (oncological patients).The fungi exhibited the largest protein lysis zone at pH 3.5, smaller – at pH 4.5, while they did not secrete proteases into the medium at pH 6.5. The proteolysis were the strongest for strains isolated from dental patients and the weakest from persons without changes in the oral cavity.

In all studied groups, 61.9% of the strains showed the presence of at least one of the aspartyl protease SAP1-3 genes. SAP1 was the most common, SAP3 was slightly less frequent followed by SAP2; none of the strains was positive for SAP4-6 genes. All genes were more frequent in the strains isolated from the dental patients than from the cancer patients and the control group. Based on this research it was noted that the weakest activity of proteolytic enzymes and the lowest number of aspartyl proteases genes are observed among strains isolated from individuals without clinical symptoms of infection. Moreover, a group of SAP1-3 genes is most frequently detected in the strains isolated from the oral cavity.

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Small rodents as reservoirs of *Enterocytozoon bieneusi* in the area of south-western Poland

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Microsporidia are interacellular eukaryotic parasitic organisms now considered to be fungi. *Enterocytozoon bieneusi* is the most common species pathogenic for humans. In addition, *E. bieneusi* has been commonly identified in a wide range of animals and water, due to zoonotic and waterborne transmission of microsporidia. *E. bieneusi* is a complex species with multiple genotypes. Hence, typing of this species relies on PCR analysis of ITS of the rRNA gene.

The aim of our study was to determine of the occurrence of *E. bieneusi* in small rodents (*A. agrarius*, *A. flavicollis, M. glareolus* and *M. musculus*). Molecular studies were conducted to elucidate the genotypes of this microsporidium. Stool (n=193) and spleen samples (n=252) from rodents were subjected to analyses. The overall prevalence was 38.9%. Values of prevalence were different for stool and spleen DNA isolates: 51.2% and 14.3% respectively. During our research we were able to identify the presence both the host-adapted and zoonotic *E. bieneusi* genotypes.

Occurrence of *Encephalitozoon intestinalis* in oncology patients in Poland

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Encephalitozoon intestinalis and *Enterocytozoon bieneusi* are the most common species associated in opportunistic infections observed in immunosuppressed patients and occasionally in immunocompetent individuals.

The aim of our study was to examine cancer patients hospitalized in Szczecin for the presence of microsporidian spores.

A total of 73 stool samples obtained from cancer patients were examined using conventional staining (chromotrope-2R) and PCR. Microsporidial spores were detected in one case by PCR and staining, whereas two samples were positive only by staining. Molecular analyses showed presence of *E. intestinalis*. The results indicate that oncologists need to know about these dangerous pathogens.

Microsporidiosis in HIV-positive patients in Poland

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Microsporidia are opportunistic pathogens in humans which usually cause self-limited infection in immunocompetent persons, but can cause severe diseases which can lead to death in immunodeficient patients. The first human microsporidiosis was reported in 1959, but interest in this group of parasites has increased since the pandemic of HIV-infection/AIDS.

The phylum Microsporidia consists of nearly 150 genera, but only seven genera (*Enterocytozoon, Encephalitozoon, Pleistophora, Trachipleistophora, Brachiola, Vittaforma* and *Nosema*) have been described as pathogens of humans.

The purpose of this study was to establish the prevalence of microsporidia in HIV/AIDS infected patients. Specimens were diagnosed using molecular method and stained with modified trichrom stain. The microsporidia were identified as *E. bieneusi* and *E. cuniculi*.

In conclusion, immunocompromised individuals such as HIV/AIDS infected patients should be screened for microsporidia. This study indicates the need for the development and standardization of laboratory methods for precise detection of microsporidia in human samples.

Potentially pathogenic yeasts and yeast-like fungi from sandpits of the Łódź area (Poland)

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Yeasts and yeast like fungi classified as Ascomycota or Basidiomycota may become potential human and animal pathogens, particularly for individuals with a depressed immune system. Their presence in the soil, may favour their spread into human ontocenoses. Children playing in the sand of sandpits may be exposed to diversity of microorganisms, not only helminths developmental stadia, also bacteria and fungi. Thus, we performed mycological evaluation of sand taken from several sandpits from children' playgrounds in Lodz, Poland. The surveys were carried out in in autumn 2010 and 2011, and in spring 2011 and 2012. The sand samples were taken from 17 localities in 5 districts of Lodz: 5 from sandpits situated in kindergartens, 4 – from sandpits of school` playgrounds, 6 – from sandpits located in housing estates of the town and 2 samples – from sandpits of two Lodz` parks.

A total of 68 samples were collected: 24 samples during autumn 2010 and spring 2011 from the same 6 localities and 44 samples during autumn 2011 and spring 2012 from the same 11 localities. Yeasts were isolated by classical microbiological methods and identified on the base of morphological and biochemical features. The fungi were found in 73.5% and in 58.8% of the examined samples taken in autumn and spring, respectively. There were 40 isolates from autumn samples and 29 – from spring samples belonging to 12 species: *Candida famata, C. guilliermondii, C. lusitaniae, Cryptococcus albidus, C. laurentii, C. neoformans, Kloeckera japonica, Geotrichum candidum, G. penicillatum, Rhodotorula mucilaginosa, R. glutinis* and *Trichosporon cutaneum.* The most frequently were isolated fungi from species: *T. cutaneum* (22 isolates), *C. neoformans* (10) and *Rhodotorula glutinis* (9). The *Candida* species were not identified from kindergartens samples, but *C. neoformans*, an etiological factor of cryptococcal meningitis, was present in the 2 of them. The concentration of fungal species in particular samples characterized great variability, but in the majority of samples, fungi were present in concentration of up to 1×10^2 CFU/1g of soil. Potentially pathogenic yeasts present in the sand of sandpits should be regarded as a fact of potential public health concern.

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