

Invited

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Antifungal Therapy - State of the Art in View of Changing Epidemiological Patterns

Andrew Ullmann

Julius-Maximilians-University Department of Internal Medicine II, Infectious Diseases Würzburg, Würzburg, Germany

It is well-known that *Aspergillus terreus* harbors higher MIC for amphotericin B and in the past couple of years the body of evidence showing azole-resistant *Aspergillus* spp. is increasing. Clinicians are treating more high-risk populations, and prophylactic therapy in clinical practice has a growing impact influencing the epidemiological data. In *Candida* disease there seems to be shift from *Candida albicans* to non-*albicans* *Candida* species. In ICUs, there is evidence that patients with non-*albicans* infection have a nearly seven-fold mortality compared with those with *C. albicans*. The global epidemiology is variable, with *C. glabrata* most common in North America and northern Europe (16%-24%), *C. tropicalis* most frequent in southeast Asia and South America (20%-35%), and *C. parapsilosis* seen most in Spain, South America, Turkey, and Australia (20%-32%). In invasive *Aspergillus* disease voriconazole is highly recommended by many scientific societies including ESCMID. The new azole isavuconazole received high recommendations as well, followed by a weaker recommendation for liposomal amphotericin B due to its higher incidence of nephrotoxicity. Since diagnostic procedures remain challenging, antifungal prophylaxis appears a worthy alternative. From an infectious diseases standpoint, prophylaxis is always second best to targeted treatment, however posaconazole prophylaxis has been shown to provide a better chance of survival for patients in AML induction/remission and allogeneic HSCT with GVHD. All three available echinocandins are strongly recommended for targeted initial treatment of candidaemia, while liposomal amphotericin B and voriconazole are supported with moderate, and fluconazole with marginal strength by ESCMID. Further studies demonstrated that delayed start of caspofungin treatment delivers a worse outcome. Acquired resistance appears rare, however there is ongoing discussion whether echinocandins are appropriate for treating *Candida parapsilosis*.

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Azole Resistant *Aspergillus fumigatus* – Global Epidemiology of Clinical and Environmental Resistance

 Jacques F. Meis^{1,2}
¹Department of Medical Microbiology and Infectious Diseases, Canisius Wilhelmina Hospital, Nijmegen, the Netherlands and

²Department of Medical Microbiology, Radboud University Medical Center, Nijmegen, the Netherlands

Aspergillus fumigatus, a ubiquitously distributed opportunistic pathogen, is the global leading cause of aspergillosis. Azole antifungals play an important role in the management of aspergillosis. However, over a decade azole resistance in *A. fumigatus* isolates has been increasingly reported especially in Europe and is potentially challenging the effective management of aspergillosis. The high mortality rates observed in patients with invasive aspergillosis caused by azole resistant *A. fumigatus* isolates pose serious challenges to the clinical microbiologist for timely identification of resistance and appropriate therapeutic interventions. The 'TR34/L98H' mutation in the *cyp51A* gene of *Aspergillus fumigatus* is responsible for most multi-azole resistance seen in many European countries, the Middle East, China, Turkey, India, Australia and the Americas. Azole-resistant isolates carrying this mutation have been reported from both patients

and the environment. In addition, a new resistance mechanism, TR46/Y121F/T289A, conferring high voriconazole and variable itraconazole MICs in *A. fumigatus* was lately described from clinical and environmental sources in the Netherlands, Denmark, Belgium, Germany, France, Spain, Tanzania, China, India, Colombia and USA. Azole resistance is now reported from all continents except Antarctica. Considering that azole antifungals are the mainstay of therapy, especially for chronic invasive and allergic aspergillosis, emergence of resistance especially in resource limited countries will have profound impact on healthcare. This presentation highlights the emerging development of azole resistance in *A. fumigatus* and the possible relation with environmental fungicide use.

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Biodiversity and Ecology of *Cryptococcus*: Relevance for the Clinic?

 Teun Boekhout,^{1,2,3} Massimo Cogliati,⁴ Kika Colom,⁵ Kantarawee Khayhan,^{1,6} Chen Min,³ Retno Wahyuningsih,^{7,8} Robiatul Adawiyah,⁷ Bart Theelen¹ and Ferry Hagen⁹
¹CBS Fungal Biodiversity Centre, Utrecht, The Netherlands;

²Shanghai Key Laboratory of Molecular Medical Mycology, Department of Dermatology, Changhai Hospital, Second Military Medical University, Shanghai, China;

³Institute of Microbiology, Chinese Academy of Sciences, Beijing, China;

⁴Laboratorio Micologia Medica, Dipartimento di Scienze Biomediche per la Salute, Università degli Studi di Milano, Milano, Italy;

⁵Laboratorio de Micologia, Facultad de Medicina, University Miguel Hernández, Alicante, Spain;

⁶Department of Microbiology and Parasitology, Faculty of Medical Sciences, University of Phayao, Phayao, Thailand;

⁷Department of Parasitology, Division of Mycology, Faculty of Medicine, University of Indonesia Jakarta, Jakarta, Indonesia;

⁸Department of Parasitology, Faculty of Medicine, Indonesian Christian University Jakarta, Jakarta, Indonesia and

⁹Department of Medical Microbiology and Infectious Diseases, Canisius-Wilhelmina Hospital, Nijmegen, The Netherlands

Species of the *Cryptococcus neoformans/C. gattii* complex can cause serious infection, mainly in immunocompromised patients, but also in immunocompetent humans. The species complex currently comprises seven species that cause, to some extent, disease in different patient groups. The small basidiospores are considered to be the infectious propagules but the sexual state that produces these spores has not yet been identified in nature. Desiccated yeast cells may be another source of infection. Yeasts of the complex occur widely in the environment, in e.g. soil, on trees, but also in bird guano. In the so-called Vancouver Island outbreak it has been suggested that the spore density may correlate with the number of diseased humans and animals. Furthermore, in various parts of the world a relationship has been observed between the presence of the yeasts and some climatological parameters. Several observations suggest zoonotic transmission from certain animals, such as parrots, magpies, but also ferrets to humans. Thus there may be a link between the occurrence of the pathogen in the environment and the infection. Here we present data from various parts of the world on the genetic diversity of members of the complex both from nature and from patients as assessed by AFLP and MLST. Most notable, we will present results obtained by the ISHAM *Cryptococcus* working group on the occurrence of the yeast in the European environment. The survey drew a first picture of species, molecular types and mating type distribution, as well as tree colonization in Europe.

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Environmental Reservoirs of the *Scedosporium apiospermum* species complex

Wieland Meyer,¹ Sandrine Giraud,² Amandine Rougeron,² Tram Pham,² Azian Harun^{1,3} and Jean-Philippe Bouchara^{2,4}

¹Molecular Mycology Research Laboratory, Centre for Infectious Diseases and Microbiology, Sydney Medical School – Westmead Hospital, Marie Bashir Institute for Infectious Diseases and Biosecurity, The University of Sydney, The Westmead Institute for Medical Research, Westmead, NSW, Australia; ²L'UNAM Université, Université d'Angers, Groupe d'Etude des Interactions Hôte-Pathogène, EA, Angers, France; ³School of Medical Sciences, Universiti Sains Malaysia, Kota Bharu, Kelantan, Malaysia and ⁴Laboratoire de Parasitologie-Mycologie, Centre Hospitalier Universitaire, Angers, France

Scedosporium apiospermum (formerly anamorph of *Pseudallescheria boydii*) is now recognized as a species complex comprising of five distinct species (*S. apiospermum sensu stricto*, *S. boydii*, *S. aurantiacum*, *S. minutisporum* and *S. dehoogii*). These fungi have gained interest due to their world-wide recognition as emerging pathogens in cystic fibrosis (CF) patients, ranking the second amongst filamentous fungi colonizing CF airways. Due to the propensity of these fungi to disseminate in case of immunodeficiency, particularly after lung transplantation, ultimate treatment for CF patients, all attempts should be made to eradicate these fungi. To eliminate colonization/disease either new antifungal targets need to be identified as they exhibit limited *in vitro* susceptibility to current antifungals, or alternatively, prophylactic measures may arise from a better knowledge of the ecology of these fungi and the identification of potential sources of contamination. Environmental surveys conducted at the homes of CF patients colonized, analyzing air, surfaces in the living room, bathroom and bedroom, water from bathtub/shower, soil of potted plants, revealed a unique reservoir of these fungi - soil from potted plants - after cultivation on semi-selective culture media (DRBC + benomyl, and YPDA + cycloheximide). However, due to the numerous other fungi recovered and a lack of selectivity of the culture media used, the presence of other potential sources of contamination in the indoor environment should not be disregarded. To overcome these difficulties a highly selective culture medium called Scedo-Select III was used to clarify the ecology of the *S. apiospermum* species complex. The highest densities of *Scedosporium* isolates were found in human-impacted areas in Austria, Australia and in western France. Some discrepancies between the species distribution within the complex in the outdoor environment and from patients with CF was recognized. *Scedosporium boydii* predominant especially in French CF patients is followed by *S. apiospermum*, *S. aurantiacum* and *S. minutisporum*. In contrast *S. dehoogii* was the most abundant species in the environment but has never been reported from sputum samples from CF patients. *Scedosporium minutisporum* was recovered only once, while the three other species were equally represented. Ecological preferences may partly explain the differences in their respective frequency in the CF context, with *S. boydii* and to a lesser extent *S. apiospermum* exhibiting ubiquitous features whereas *S. aurantiacum* is mostly found in agricultural areas. Together these studies demonstrated that potted plants constitute a potential source of contamination of the patients, and further studies using more selective culture media are needed to determine if there are other reservoirs in the indoor environment. In addition, human activities largely influence the ecology of these fungi, but questions remain regarding the natural habitat of these fungi and the discrepancy between the high frequency of *S. dehoogii* in the environment and its lack of pathogenicity for CF patients.

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The 'Sick Building Syndrome': are Indoor Moulds a Contributory Factor?

Malcolm Richardson

Mycology Reference Centre, University Hospital of South Manchester and University of Manchester, Manchester, UK

'Sick building syndrome' (SBS) is a term coined for a set of clinically recognizable symptoms and ailments without a clear cause reported by occupants of a building. Alternative terms include: 'Building-Related Occupant Complaint syndrome' and 'Abused Building Syndrome'. Environmental fungi may be partially responsible. Some buildings, especially those that are water damaged, cause respiratory symptoms when people live or work in them. Symptoms disappear when people live or work elsewhere. Symptoms return when people return to the home or workplace. Convincing evidence that the building is 'sick' is the presence of symptoms in many individuals. Microscopic fungi are important biological pollutants in the indoor environment, they are spread generally: on building materials, carpets, ceiling tiles, insulating materials, any surfaces, wallpapers, or in heating, ventilation, and air conditioning systems. Moulds are able to grow on any materials, as long as moisture and oxygen are available. Exposure to fungi in indoor environments (especially in water-damaged buildings) can exacerbate existing respiratory problems such as allergy, asthma, hypersensitivity pneumonitis or cause disease, for example, chronic pulmonary aspergillosis and very rarely, invasive sinusitis or pulmonary disease in immunocompromised patients. Serious adverse health effects could be caused antifungal prevention is an absolute need. Work carried out in several laboratories showed that indoor fungi can release groups of spores, individual spores and fungal fragments, and produce volatile organic compounds and mycotoxins. Integrating all findings a holistic interpretation emerged for the sick building syndrome. Healthy houses and buildings, with low indoor humidity, display no appreciable indoor fungal growth, and outdoor moulds such as Basidiomycetes, *Alternaria*, *Aspergillus* and *Cladosporium* dominate. On the contrary, in damp, sick houses and buildings, high indoor humidity allows fungal growth (mainly *Penicillium* and *Trichoderma*), with concomitant release of conidia, fragments and allergens into the atmosphere. Heavily contaminated indoor environments can be investigated using conventional mycological sampling methods. Cleaner atmospheres are more difficult to study and require molecular tools to detect interstitial mould. It is concluded that fungi can be useful indicators of indoor air quality and that it is important to deepen the studies of indoor atmospheres in order to promote air quality, the health and well-being of all, and a better understanding of the biology of indoor fungi.

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Microbiology of Sands and Its Impact on Human Health

João Brandão

Reference Unit for Parasitic and Fungal Infections - Department of Infectious Diseases, National Institute of Health Dr. Ricardo Jorge, Lisboa, Portugal

Recent studies suggest that sand can serve as a vehicle for exposure of humans to potential pathogenic microorganisms at beach sites, sandboxes and recreational areas. Recreational water quality, worldwide, focuses on monitoring bacterial indicators of possible faecal contamination by pathogens that cause Gastro-intestinal illness. The most recent bathing water directive in Europe hints on recreational water surrounding areas as a possible contamination source in itself. Yet, it leaves behind a clear message that sand is a relevant source of microorganisms, despite WHO's recommendation of sand monitoring in 2003; especially in regions where beach users stay mainly on

the sand due to low temperatures of the water. This recommendation has been backed up recently by an epidemiological study conducted by Heaney *et al.* in 2012 and the information collected during a 5 year beach sand monitoring program of the whole of the coast of Portugal. Given the diversity of microbes found in sand, studies are urgently needed to identify the most significant aetiologic agents of disease that may be conveyed through sand, and to relate microbial measurements to human health risk. Currently monitoring in sand-boxes is limited to measurements of *Toxocara* eggs, although other microbes have been documented. A newly emerging group of fungi of concern include the black yeast-like fungi and in non-coastal settings, *Cryptococcus gattii* has been gaining significance already given to endemic and fungi resistant to antimicrobials, especially in Children and immune-impaired individuals. Sampling for microorganisms in sand should therefore be considered for inclusion in regulatory programs aimed at protecting recreational users from infectious disease. Overall, environmental and epidemiological studies to support the link between fungi exposure in sand and human health impacts are recommended. Also, sand analysis and sampling procedures need to be reviewed and asserted in order to ensure that representativity of samples and other potential pathogens are covered, including GI tract viruses; known to be the biggest cause of GI illness in beach environments.

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Data from TerrNet – *Aspergillus terreus*: a Special Pathogen in View of Changing Patterns of Aspergillosis

Cornelia Lass-Flörl

Division of Hygiene and Medical Microbiology, Medical University of Innsbruck, Innsbruck, Austria

Invasive aspergillosis (IA) has emerged worldwide as an important cause of infections among patients undergoing cancer chemotherapy, haematopoietic stem-cell transplantation, or solid organ transplantation. Aspergilli are ubiquitous fungi and the major pathogens are *Aspergillus fumigatus*, *Aspergillus flavus* and *Aspergillus terreus*. Among the various species *A. terreus* takes an exceptional position: most isolates are in vitro and in vivo resistant to amphotericin B (AMB) (3). *A. terreus* is regarded intrinsically resistant to AMB, which is one of the broadest antifungal drugs and widely used for life threatening fungal infections. AMB MICs (48 h / 37 °C) are significantly higher (≥ 4 µg/ml broth dilution, > 8 µg/ml Etest) than for *A. fumigatus* (0.5 - 1 µg/ml). Drug resistant fungal infections are becoming more prevalent and are major health issues facing us today. *A. terreus* is a common cause of IA in some geographically disparate institutions, such as University of Texas M.D. Anderson Cancer Center in Houston, TX, and The University Hospital of Innsbruck, Austria. The aim of TerrNet is to determine the global prevalence of *A. terreus* in mould infections, and to broaden the knowledge on epidemiology, on clinical courses of infections and to investigate mechanisms behind differences in amphotericin B and azole susceptibility. The European Confederation of Medical Mycology (ECMM) and the International Society for Human and Animal Mycology (ISHAM) supported TerrNet.

Main objectives

- to determine worldwide distribution of *A. terreus*
- to identify global prevalence
- to evaluate global epidemiology and strain diversity
- to identify new species within the section terreii
- to collect amphotericin B susceptible strains (if indicated also azole resistance)
- to identify patient population at risk
- to determine the clinical pattern of disease
- to characterize in vitro susceptibility
- to check in vitro susceptibility and antifungal-drug combinations
- to check experimental antifungal drugs in vitro and in vivo in animal models.

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Mucormycosis – State-of-Art

Oliver A. Cornely

Department I of Internal Medicine, University of Cologne, Cologne, Germany

The European Society for Clinical Microbiology and Infectious Diseases (ESCMID) and European Confederation of Medical Mycology (ECMM) issued Joint Clinical Guidelines in 2014 that comprise diagnosis and management of mucormycosis. Key recommendations are as follows. To diagnose mucormycosis, direct microscopy preferably using optical brighteners, histopathology and culture are strongly recommended. Pathogen identification to species level by molecular methods and susceptibility testing are strongly recommended, but to establish epidemiological knowledge only. Guiding treatment based on MICs is supported only marginally. Imaging is strongly recommended to determine the extent of disease. To differentiate mucormycosis from aspergillosis in haematological malignancy and stem cell transplantation recipients, identification of the reverse halo sign on computed tomography is advised with moderate strength. For adults and children surgical debridement in addition to immediate first-line antifungal treatment with liposomal or lipid-complex amphotericin B with a minimum dose of 5 mg/kg/day is strongly recommended. Amphotericin B deoxycholate should be avoided because of severe adverse effects. For salvage treatment posaconazole 4 x 200 mg/day is strongly recommended. Reversal of predisposing conditions is strongly recommended, i.e. using granulocyte colony-stimulating factor in haematological patients with ongoing neutropenia, controlling hyperglycaemia and ketoacidosis in diabetic patients, and limiting glucocorticosteroids to the minimum dose required. Use of deferasirox is discouraged in haematological patients, and marginally supported in diabetic patients. Hyperbaric oxygen is supported with marginal strength only. Finally, it is strongly recommended to continue treatment until complete response demonstrated on imaging and permanent reversal of predisposing factors. New developments since guideline publication are experimental T-cell diagnostics and broadening treatment options, such as high-dose liposomal amphotericin B, posaconazole tablets and intravenous formulations and the licensing of isavuconazole on the basis of successful first-line treatment as compared with matched cases from the ECMM Fungiscope™ registry.

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The Effect of Environmental Changes on Fungi Affecting CF Patients

Frank-Michael Müller

Pediatric Pulmonology, Allergy & Infectious Diseases, Pediatricum Heidelberg, Heidelberg, Germany

Mutations in CFTR genes result in a defective mucociliary clearance with production of viscous and sticky bronchial mucus that facilitates the colonization with airborne bacteria and fungal spores. *Candida* spp. are the most common isolated yeasts, whereas *Aspergillus* spp., *Scedosporium apiospermum*, as well as *Exophiala dermatitidis* in some countries, are the most frequent moulds recovered from respiratory specimens. Other *Aspergillus* spp., e.g. *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus nidulans* as well as *Pneumocystis jirovecii*, *Rasamsonia argillacea*, *Trichosporon mycotoxinivorans* and *Lomentospora prolificans* are transient colonizers of the airways. The CF airways are a highly complex and dynamic ecosystem in which bacteria and fungi must adapt to multiple environmental challenges that fluctuate in both time and location (upper vs. lower airways). For example, the heterogeneous distribution of oxygen, nutrient availability and interactions with other microorganisms, as well as antibiotic treatments, biofilm formation and attacks from the immune system, are all factors that contribute to environmental complexity. Moreover, the lung

pathology changes over time as infection progresses. The CF respiratory tract is an open system into which microbes from the environment or from other patients may continuously invade and challenge the existing population structure. In addition, the whole system goes through controlled perturbations (e.g. anti-microbial therapy), random perturbations (e.g. deep coughing that increases the possibilities for mixing) and dissections (e.g. lung transplant and sinus surgery). **Conclusion** Changes in the lung mycobiota by environmental changes over time and/or during therapies may contribute to the decline of the lung function and the CF lung disease progression.

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Update on the *Malassezia* Associated Skin Diseases. The Impact of Climate Change

Aristea Velegraki

Department of Microbiology, Medical School National and Kapodistrian University of Athens, Athens, Greece

Malassezia yeasts are members of the human skin microbiome. It is from within their niche in the microbiome that they can cause disease under specific host predisposing factors and under currently investigated environmental conditions. In humans they cause pityriasis versicolor, a condition involving the function of skin melanocytes, resulting in hypo- or hyperpigmented plaques with characteristic absence of inflammation and mild alterations in the epidermal barrier function. *Malassezia* yeasts also aggravate persistent and common inflammatory conditions as is seborrheic dermatitis and the head and neck variant of atopic dermatitis whereas invasion of the hair follicle under topical favourable factors cause the appearance of *Malassezia* folliculitis. Little is known on the pathophysiology of these conditions, due to the complexity of the interaction of *Malassezia* with skin, an organ that has been on extreme selection pressure during evolution. The commensal *Malassezia* yeasts are in constant interaction with the skin receiving essential nutrients, such as lipids and amino-acids. When this balance is disturbed, *Malassezia* yeasts modify the expression of enzymes involved in the acquirement of energy and structural materials (lipases, phospholipases). At the same time, and when they use human sweat L-tryptophan as a sole source of nitrogen, they synthesize bioactive indoles as indolo[3,2-b]carbazole (ICZ), its alkaloid nanomolecular photosensitizer derivative 6-formylindolo [3,2-b]carbazole (6-FICZ) and indirubin, that that are aryl-hydrocarbon (AhR) receptor (dioxin receptor) ligands acting on almost all cell types found in the epidermis. Of note, is that 6-FICZ has the highest affinity with the AhR receptor (Kd=0.07nM), highest even of 2,3,7,8-tetrachlorodibenzodioxin (Kd=0.48). The 6-FICZ association in skin carcinogenesis, in particular the basal cell carcinoma (BCC), is an exciting topic for investigation. Equally exciting is the finding that these *Malassezia* metabolites exhibit broad spectrum antifungal properties at clinically significant concentrations, as reached in skin. The ongoing climate change and the consequence of increasing UV radiation, in conjunction with the nanomolecular photodynamic action of 6-FICZ, can play a primary role in causing photooxidative stress of skin cells, especially to skin cells with inflammatory conditions. As a result photoaging (dermatoheliosis) and skin carcinogenesis is aggravated. In evidence of this, recent data show that more potent ligands, as 6-FICZ, bind to the AhR under inflammatory skin conditions even at small UVA dosimetry (5J per cm²). It is a major challenge to interpret the current prolific multidisciplinary data that contribute in elucidating the biochemical interaction pathways of *Malassezia* yeasts as commensals and as pathogens involved in non-inflammatory, and importantly, in inflammatory skin conditions.

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Antifungal Susceptibility Testing in View of Changing Patterns in Systemic Fungal Infections

Malcolm Richardson

Mycology Reference Centre, University Hospital of South Manchester and University of Manchester, Manchester, UK

Fungi are increasingly recognised as major pathogens in immunocompromised patients and in those who are critically ill. *Candida* species are yeasts most frequently isolated in clinical practice. In recent years, a number of yeast species that had previously been thought to represent contamination or harmless colonisation have been recognised as significant pathogens. The genera most likely to cause invasive infection include *Malassezia* and *Trichosporon*, but *Blastoschizomyces* (*Magnusiomyces*, *Saprochaete*), *Saccharomyces* and *Rhodotorula* have also been reported to cause fungaemia with or without organ invasion. Establishing the diagnosis is difficult and depends on the detection of the organism in tissue sections or smears of clinical material as well as isolation in culture. Antifungal susceptibility testing is crucial for successful management. The most frequent filamentous fungi (moulds) isolated are *Aspergillus* species, but *Fusarium* species, *Scedosporium* species, and agents of mucormycosis are increasingly seen. Several reasons have been proposed for the increase in invasive fungal infections, including the use of antineoplastic and immunosuppressive agents, broad-spectrum antibiotics, and prosthetic devices and grafts, and more aggressive surgery. Patients with burns, neutropenia, HIV infection and pancreatitis are also predisposed to fungal infection. Many of these 'new' fungal pathogens appear to be less responsive to first-line antifungal treatments. The use of molecular tools has led to the description of new cryptic species among different *Aspergillus* species complexes. Their frequency in the clinical setting has been reported to be between 10 and 15%. The susceptibility to azoles and amphotericin B of many of these species is low, and some of them, such as *Aspergillus calidoustus* or *Aspergillus lentulus*, are considered multi-resistant. The changing epidemiology, the frequency of cryptic species, and the different susceptibility profiles make antifungal susceptibility testing an important tool to identify the optimal antifungal agent to treat the infections caused by these species. Rare *Candida* species are emerging as significant pathogens, for example, *Candida rugosa* and *C. guilliermondii*. Several recommendations for routine use of AFST for these *Candida* species in the clinical microbiology laboratory have been developed. Selective application of susceptibility testing, together with a precise identification of *Candida* to the species level should be useful in selecting agents for primary therapy as well as in a de-escalating strategy, especially in difficult-to-manage cases of invasive candidosis.

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Fighting Against Fungi Outside the Host – a Multifaceted Issue

Mihai Mares

Ion Ionescu de la Brad University, Department of Public Health, Laboratory of Antimicrobial Chemotherapy, Iasi, Romania

Fungi are ubiquitous organisms that can easily adapt to different ecological niches. Most of them are vegetal decay degraders widely spread in soil (saprobic fungi). Their spores are both outdoor and indoor airborne allowing the contamination of various habitats. Other group of fungi consists in commensal microorganisms that are able to survive and multiply in different hosts as part of their normal microbiota. Medically important fungi are opportunistic organisms belonging to both groups that promptly turn into pathogenic elements when host barriers and immune defense are impaired. The lecture will be focused on resistance of fungal spores in the environment, their behavior to various physical and chemical agents

used for microbial control and different methods of air decontamination (air filtration, UV radiations, high temperature, non-thermal plasma discharge and plasma-activated water). Their usefulness and efficiency in prevention of invasive fungal infections in immunocompromised patients will also be discussed.

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Molecular Approaches for the Investigation of Nosocomial/Outbreaks Due to Human Pathogenic Fungi

Wieland Meyer

Molecular Mycology Research Laboratory, Centre for Infectious Diseases and Microbiology, Sydney Medical School – Westmead Hospital, Marie Bashir Institute for Infectious Diseases and Biosecurity, The University of Sydney, Westmead Millennium Institute, Sydney, Australia

Globally infections due to human/animal pathogenic fungi are on the rise. It is now estimated that they exceed the number of Malaria and Tuberculosis world-wide. Due to the larger number of fungal infections and a constant change of the potential host population and ongoing drastic changes of the environment fungi are also more and more implemented in nosocomial infections and disease outbreaks. The most well known one is the outbreak of cryptococcosis on Vancouver Island due to *Cryptococcus gattii* VGII in 1999, which has subsequently spread to the Pacific Northwest of the USA. On the other site nosocomial infections are also on the rise, such as the case clusters of *Pneumocystis jirovecii* infections in kidney transplants at the east coast of Australia, or the emergence of *Aspergillus* or *Scedosporium* infections associated with building constructions near major hospitals. Advances in molecular biology have transformed our possibilities to investigate such outbreaks. Methods applicable to nosocomial/outbreak investigations reach from simple PCR-fingerprinting/RAPD technologies via Multilocus Microsatellite Typing (MLMT) and Multilocus Sequence Typing to whole genome SNP analysis. The discriminatory potential and applicability of the available molecular techniques is constantly increasing. Characteristics as well as advantages and disadvantages will be discussed and examples will be given.

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Pathogenesis of Invasive Aspergillosis: from Survival in the Environment to Host Invasion

Ronen Ben-Ami

Infectious Diseases Unit, Tel Aviv Medical Center, Sackler School of Medicine, Tel-Aviv, Israel

Aspergillus species are highly successful scavengers of decaying organic matter. To survive and thrive in highly dynamic environments, *Aspergilli* have developed sensory and regulatory systems that detect altered environmental conditions and induce adaptive responses in gene expression. The same properties govern the pathogenicity of *Aspergillus* spp. and facilitate tolerance to hypoxia, resistance to oxidative stress, nutrient scavenging and angiogenesis. Understanding the mechanisms underlying the growth of *Aspergillus* in the environment allows us to gain new perspectives about the pathogenesis of invasive aspergillosis. Ultimately, such insights may help spawn novel treatments and preventive strategies for this often devastating infection.

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Cluster of *Fusarium solani* Isolations in a Bronchoscopy Unit

Shmuel Benenson,¹ Liran Levy,² Colin Block,¹ Carmela Schwartz,¹ Ilana Gross,¹ Matan Cohen,¹ Zvi G Fridlender,² Allon E Moses¹ and Neville Berkman²

¹Department of Clinical Microbiology and Infectious Diseases, Hadassah-Hebrew University Medical Center, Jerusalem, Israel and ²Institute of Pulmonary Medicine, Hadassah-Hebrew University Medical Center, Jerusalem, Israel

Background During May 2012, tissue cultures from trans-bronchial lung biopsy in five patients grew the fungus *Fusarium solani*. An epidemiological investigation was initiated.

Materials and Methods Environmental samples were obtained for culture from various items and surfaces in the bronchoscopy unit, including bronchoscopes, biopsy forceps, feed water and the bronchoscope washer-disinfector. Organisms were identified using routine and molecular methods.

Results No clinical manifestations were associated with the presence of *F. solani* in cultures. A culture taken from a biopsy forceps which was used in all cases was positive for *F. solani*. It was found that biopsy forceps were not routinely sterilized according to guidelines but were disinfected in the washer disinfector used for bronchoscope re-processing. Additionally, reverse osmosis water used in the washer disinfector was supplied from a non-recirculating tank.

Conclusions We showed that a cluster of *F. solani* isolates in a bronchoscopy unit was associated with a contaminated biopsy forceps. This pseudo-outbreak resulted from inadequate decontamination of an instrument that requires sterilization according to the manufacturer's instructions. In addition improper operation of the reverse osmosis water supply was documented. No further positive cultures were encountered after correcting these errors. Our experience emphasizes the importance of following infection control guidelines in all invasive procedures.

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Impact of Environmental Factors on the Epidemiology of Dermatophytoses

Esther Segal

Department of Clinical Microbiology and Immunology, Sackler School of Medicine, Tel-Aviv University, Tel-Aviv, Israel

Fungal dermal diseases caused by the molds of the Dermatophyte family are among the most frequent human infectious diseases affecting quality of life. There are 3 attributed sources of infection by Dermatophytes: 1) humans; 2) animals and 3) soil. Dermatophytes possess the ability to utilize keratin from human and animal tissues, or debris from dead animal sources found in soil, such as feathers, skin or nails. Hence, Dermatophytes are abundant in different ecological niches. All 3 groups can infect humans via direct or indirect contact, causing dermatophytoses manifested in different clinical entities involving skin, hair or nails. Dermatophytes are found universally, however the prevalence of dermatophytoses caused by different Dermatophytes may vary in different geographic areas according to climatic conditions or lifestyle. Studies in different geographic areas assessing the specific fungal etiology involved are of epidemiological relevance and may possibly serve as baseline information for management of dermatophytoses at the local level. This presentation will focus, mostly, on epidemiological data from published surveys conducted in different geographic/climatic areas analyzing the prevalence of specific Dermatophyte species in regard to gender, age, type of infection in context of environmental factors. Specifically, it will concentrate on the association of Dermatophytes and dermatophytoses with pets, beach sand and sea water, swimming pools, gyms

and other recreational activities. Association of Dermatophyte infections with work related occupation, such as farming, nail-care salon activities or army service will be also covered. Awareness of epidemiological aspects of Dermatophytes and dermatophytoses might lead to recommendations of significance for public health.

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Molds and Mycotoxins in Agricultural Products and Food – Strategies to Overcome them

Edward Sionov

Institute for Postharvest and Food Sciences, Department of Food Quality and Safety, Agricultural Research Organization, Bet Dagan, Israel

Mycotoxins are low-molecular weight natural products produced as secondary metabolites by toxigenic filamentous fungi that contaminate food, the food chain, and represent a risk to human and animal health. The major mycotoxins that occur in food and animal feed-stuffs are produced by *Fusarium* (deoxynivalenol, trichothecenes, fumonisins and zearalenone), *Aspergillus* and/or *Penicillium* (aflatoxins, ochratoxin A, patulin). When present in foods at sufficiently high levels, these fungal metabolites can have a toxic effect that ranges from acute (for example, liver or kidney deterioration), to chronic (for example, liver cancer). Mycotoxins can enter the food chain either in the field, during storage, or at a later point. The mycotoxin problem is exacerbated whenever shipping, handling, and storage practices are conducive to mold growth. This contributes to mycotoxins being commonly found in foods. Moreover, the presence of mycotoxins in agricultural products is also an economic concern. A quarter of the world's crops are estimated to be contaminated to some extent with mycotoxins. The increasing consumer demand for safe food is accompanied by the need for early, rapid and cost-effective analytical and molecular techniques capable of screening for the presence of mycotoxins and/or mycotoxigenic fungi. Our laboratory is focusing on implementation of molecular biology techniques and analytical methods for rapid detection and identification of mycotoxigenic fungi and mycotoxins in wide range of agricultural commodities. This would allow prevention of mycotoxin contamination of the human food supply and have a significant effect on public health.

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From Pond to Fish: the Eco-Epidemiology of *Saprolegnia* Infections

Rama Falk¹ and Itzhack Polacheck²

¹Central Fish Health laboratory, Department of Fisheries and Aquaculture, Ministry of Agriculture and Rural Development, Nir-David, Israel and ²Hadassah-Hebrew University Medical Center, Department of Clinical Microbiology and Infectious Diseases, Jerusalem, Israel

In recent years, saprolegniasis outbreaks, mostly in farmed *Tilapia*, have become common in Israel. These outbreaks have been especially severe in winter, when the fish are exposed to harsh conditions of handling and crowding. The problem was exacerbated following a ban imposed on the use of the effective agent, malachite green, due to its toxicity. The main objectives of this study were to identify and characterize the infective agent causing saprolegniasis in Israel and to find an alternative treatment for this infection. All the molds isolated from fish were identified by molecular diagnosis (based on ITS 1 and ITS2 sequences) as *Saprolegnia parasitica*. Physiological studies indicated that fungi isolated from fish exhibited a relatively higher growth rate at a low temperature (4 °C), whereas those isolated from water had relatively higher growth rates at an elevated temperature

(30 °C). Samplings of fish ponds over the course of one year, in various farms and natural water sources, failed to detect *Saprolegnia* from April to October, while from November till March relatively constant levels of zoospores (~100 zoospores l⁻¹) were found. The main factors initiating *Saprolegnia* infection in *Tilapia* were: low temperature, physical stress of handling, a critical zoospore concentration in water, and the duration of exposure of fish to the zoospores. A novel screening assay system was developed for evaluating potential anti-saprolegnia agents. Several compounds were tested by this assay for in-vitro susceptibility, fish toxicity, environmental impact assessment and therapeutic efficacy in a tilapia-saprolegniasis model. A stilbene derivative was the most effective anti-saprolegnia agent in this system, possibly due to its interaction with cellulose microfibrils and the resulting disruption of cellulose in *Saprolegnia* cell wall.

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Presence of Different Species of Fungi in Various Sites Around Swimming Pools in Israel

Avner Shemer

Sheba Medical Center, Tel HaShomer and Sackler School of Medicine, Tel-Aviv University, Tel-Aviv, Israel

Background Tinea pedis is a fungal infection of the plantar aspect of the feet and the interdigital spaces which is caused by dermatophytes. Swimming pools play an important role in human contamination by different kinds of fungi. The common hypothesis in medical literature is that people exposed to these fungi can be infected by them but there is no unequivocal evidence for this.

Objectives The objective of this paper was to examine the correlation between the prevalence of different types of fungi in the vicinity of swimming pools and the morbidity of Tinea pedis among swimming pools employees exposed to them.

Methods Twenty-one swimming pools were tested in the Netanya area in Israel. Samples were taken from sites surrounding the swimming pools and from swimming pool employees. The identification of the specific fungi in the samples taken from the employees was attained by direct smear with KOH and culture on Sabouraud Dextrose Agar plates. The identification of the specific fungi in the samples taken from the surfaces of sites surrounding the swimming pools was attained by culture alone. The fungi type was then determined by the appearance of the micro and macro-conidia together with the general appearance of the fungal growth on the culture plates.

Results The prevalence of Tinea pedis among pool employees was 29.5% 95%CI (23.1-36.9%). The prevalence of employees with findings of Onychomycosis and a single positive culture with dermatophytes was 24.6% 95%CI (18.6-31.6%) while any fungi was 32.9% 95%CI (26.3-40.4%).

Conclusions We found a higher incidence rate of Tinea pedis among the swimming pool employees in comparison to the general population. But we did not find a correlation between the prevalence of fungi in the vicinity of swimming pools and the morbidity of Tinea pedis among employees.

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From Man to Environment, to Animal, and Back to Man

Daniel Elad

Department of Clinical Bacteriology and Mycology, The Kimron Veterinary Institute, Israel

Environmental changes have been shown to influence the epidemiology of infectious diseases by, for instance, increasing the area suitable for vector multiplication or niche encroaching. Here, a broader perspective of the impact on human wellbeing, caused by of four

recently emerged fungal diseases in animals is presented. White Nose Disease (WND), caused by *Pseudogymnoascus destructans*, spreading in the US and Canada, causes mass mortality in insectivorous bats, due to energy depletion during hibernation and was recently reported from China. Seemingly the fungus is a recent introduction since in Europe it has no deleterious effects on bat population. Chytridiomycosis, caused by the oomycete *Batrachomyces dendrobatidis*, infects the skin (a respiratory organ) of frogs. Whole populations have been annihilated. International transport of carrier frogs and their release into susceptible populations has been suggested to have caused the fungus' spread. Snake Fungal Disease (SFD) is a lethal cutaneous

mycosis caused by *Ophidiomyces ophidiicola*. Its spread is similar to that of WND, and it has been suggested that snakes weakened by anthropogenic activities may be susceptible to infection. Bee nose-matosis is caused by microsporidia *Nosema apis* and *N. ceranae*. The latter, recently introduced from the East, negatively impacts the bees' navigation capabilities and may be involved in the Honey Bee Colony Collapse syndrome. Thus, the increase of risk of arthropod borne diseases, insect damage of crops (WNS) and decrease of plant productivity due to lack of pollination (nosematosis) indirectly impacts human wellbeing, this, in addition to the reduction of wildlife biodiversity.