S10.4a
New mechanism and detection methods for azole-resistant *Aspergillus fumigatus*

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S10.4 Emerging antifungal resistant fungi, September 24, 2022, 10:30 AM - 12:00 PM

The most studied azole-resistant mechanism of *Aspergillus fumigatus* is decreased affinity of the drug for CYP51A, the drug target molecule, due to its amino acid substitutions. Typically, each azole-resistance caused by the designated amino acid substitution of CYP51A has a specific pattern depending on the substitution site. While uncovering non-cyp51A mechanisms responsible for azole resistance should be essential for developing novel methods for prompt diagnosis and effective drug treatment. In our previous study, we reported results that mutation of hmg1, which codes HMG-CoA reductase, the rate-limiting enzyme in ergosterol biosynthesis, would be the mechanism conferring azole-drugs resistance (EID 2018). On the other hand, different azole susceptibility patterns have been reported even among the strains possessing the same mutation in CYP51A. In this way, the overall picture of molecular mechanisms inducing azole resistance remains unclear.

We performed a comparative genomic analysis among the strains with the same cyp51A mutation isolated from the same patient but with different azole resistance patterns. To investigate the association between the novel mutation and azole resistance, the mutant allele was replaced with the wild-type allele by the CRISPR-Cas9 system. Antifungal susceptibility tests were performed according to the CLSI-M38.

As a result of genome comparison analysis between these two strains using the HiSeq sequencing system (Illumina), another mutation was found in the insulin-induced protein (INSIG) of the multiazole-resistant strain. The INSIG mutation contributes additively to azole resistance in collaboration with the CYP51A mutation but does not alone itself.

We have already reported simple and rapid detection methods for *A. fumigatus* possessing CYP51A mutation using an endonuclease (AAC 2020). Furthermore, using MALDI-TOF-MS, we are developing a discriminant model to detect azole-resistant *A. fumigatus*.

S10.4b
Pathophysiology, diagnosis, and management of chronic pulmonary Aspergillosis

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S10.4 Emerging antifungal resistant fungi, September 24, 2022, 10:30 AM – 12:00 PM

Chronic pulmonary Aspergillosis (CPA) is a complex disease that is difficult to diagnose and resistant to treatment. Many cases are missed and have unfortunate outcomes in clinical settings. Although CPA has long been classified into several types based on pathological findings, it is not always possible to make a pathological diagnosis in all cases, so a clinical diagnosis is often made. In addition, many have an underlying respiratory disease and complications by infections caused by other microorganisms, making treatment further difficult. The diagnosis of CPA requires clinical symptoms and findings, radiological, serological, pathological, and microbiological approaches, and standardization of these diagnostic criteria is challenging. The mainstay of treatment is outpatient oral therapy with azoles, and indications and therapeutic evidence for echinocandins, and polyenes are limited. The development of novel antifungals with different mechanisms of action from conventional agents is also awaited. Azole-resistance of *Aspergillus* caused by long-term treatment is also an issue. It has been reported that resistant strains have been found in CPA patients treated with long-term azole therapy, mutations in the cyp51A and others have also been identified and longer exposure to azoles are correlated with higher MIC values of them. This presentation will focus on epidemiology, pathogenesis, diagnosis, and management including drug resistance in CPA patients.

S10.4c
Successful treatment of mucormycosis in hematological diseases

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The diagnosis of mucormycosis relies upon the identification of organisms in tissue by histopathology with culture confirmation. However, culture often yields no growth, and histopathologic identification of an organism with a structure typical of Mucorales may provide the only evidence of infection. PCR-based technique may contribute to the early diagnosis of mucormycosis. We developed a new antigen test. We searched for secreted or membrane-bound proteins of *Rhizopus oryzae*. Protein RSA (Rhizopus-specific antigen, 23 kDa) was detected at significantly higher concentrations in serum and in lung homogenates of the *R. oryzae*-infected mice as compared to those of uninfected mice. And we will show a case of hematological disease with diagnosis using RSA test and successful treatment with Liposomal amphotericin B. Our study indicates that protein RSA may be a promising biomarker of *R. oryzae* infection.

S10.4d
A unique clinical appearance of *Candida auris* infection in Japan

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S10.4 Emerging antifungal resistant fungi, September 24, 2022, 10:30 AM - 12:00 PM

It has only been 15 years since *Candida auris* was reported isolated from the ear canal of a 70-year-old Japanese woman in Tokyo, and no record of an isolate corresponding to this species has been found prior to 1996. It is a high public health priority concern in several regions of the world. This is because the fungus is multidrug-resistant and can acquire resistance to all three major groups of current antifungal drugs (azoles, echinocandins, and amphotericin B). Outbreaks in healthcare facilities are also a concern. The main reasons for this are as follows: unlike other *Candida* spp. that primarily inhabit the digestive and urinary systems, *C. auris* readily colonizes patient skin and can survive for several weeks on dry, non-living surfaces, contributing to infections and outbreaks in healthcare facilities.

In Japan, *C. auris* was first identified in 2009 in a discharge from the ear canal of a patient admitted to a Japanese hospital, and since then, all isolates have come from the ear canal, with only a few reported strains. For reasons unknown, as of 2022, *C. auris* has not been reported as a cause of invasive disease in Japan, and no nosocomial infections have occurred. Whole genome analysis suggests that all Japanese isolates belong to Clade II, affecting drug resistance and clinical characteristics.

In this symposium, I will present the current status of *C. auris* infection in Japan, the first country where *C. auris* infection originated, together with its unique clinical features and molecular epidemiological analysis.

S10.5a
Fungal respiratory infections in cystic fibrosis patients in the Middle East

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S10.5 Fungal respiratory infections in Cystic Fibrosis, September 24, 2022, 10:30 AM - 12:00 PM

Cystic Fibrosis (CF) is among the most common genetic disorders, which involves multiple organs including the respiratory tract. CF is thought to be an uncommon disease in the Middle East (ME). However, the prevalence is estimated at 1 in 30 000-50 000, while the incidence is estimated at 1 in 2000-5800 live births. Several studies from ME revealed that many children with CF in these populations probably remain undiagnosed due to lack of clinical suspicion and proper diagnostic facilities. According to the experts' idea, CF may be more common in Iran than expected before.

Chronic colonization of the airways of CF patients and infections due to a wide variety of opportunistic fungal pathogens including *Aspergillus*, *Candida*, *Scedosporium* species, *Exophiala dermatitidis*, *Rasamsonia argillacea* complex, and *Lomentospora prolificans* are currently increasing. On the other hand, the resistance of these opportunistic pathogens to commonly available antifungals challenges therapeutic options and consequently endanger the CF patients' life.

Dissimilar to bacterial colonization or infections, the epidemiology and pathogenicity of colonization and fungal respiratory infections in CF are less known. According to our recent study, the prevalence rate of respiratory colonization was reported as 73.3%. Among mold isolates, *Aspergillus* was also the most common genus followed by *Scedosporium* species. In contrast to the reports from western countries, *A. flavus* was also identified as the most prevalent species of *Aspergillus* from ME countries including Iran and India.

In some studies, allergic bronchopulmonary aspergillosis (ABPA) in CF patients from ME was evaluated. In our recent experience from Iran, of 86 patients with CF, 9 (10.5%) cases were met ABPA diagnosis. *A. flavus* was the most common agent followed by *A. fumigatus*, *A. terreus* and *A. tubingensis*.

A significant resistance of *Scedosporium* and *Aspergillus* isolates from CF patients against the main antifungal agents in invasive fungal infections therapy was reported in different studies.

According to these realities, there are a few reports on the Research Topic 'Fungal Respiratory Infections and Colonization in CF' from Iran and some other ME countries. Therefore, in this presentation, we are going to highlight our experiences and other published data from Iran and ME in this field including clinical presentations, fungal species involved, diagnosis strategies, and *in vitro* antifungal susceptibility patterns of fungal isolates from CF patients, and common treatments and prophylactic strategies.

S10.5b
Bacterial and fungal coinfections in cystic fibrosis

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S10.5 Fungal respiratory infections in Cystic Fibrosis, September 24, 2022, 10:30 AM - 12:00 PM

Lung infections due to *Aspergillus fumigatus* are in constant augmentation in cystic fibrosis (CF) patients. Up to 55% of CF patients show respiratory complications and exacerbated inflammation due to *A. fumigatus*. Allergic bronchopulmonary aspergillosis is the major complication found in up to 15% of these CF patients. Moreover, up to 60% of CF patients who are already infected chronically by *Pseudomonas aeruginosa* are also colonized by *A. fumigatus*. The interactions between *A. fumigatus* and *P. aeruginosa* showed a balance between inhibitory and stimulatory effects on fungal growth in mixed *A. fumigatus*-*P. aeruginosa* cultures.

Moreover, the interaction between the bacteria and *A. fumigatus* modulates exposure to fungal patterns and the innate immune response to the pathogens. Indeed, superinfection by *A. fumigatus* of patients already colonized by *P. aeruginosa* leads to hyperinflammation. Therefore, studying the interaction between bacteria and fungus in cystic fibrosis is crucial.

S10.5c
Insight into the role of secondary metabolism in the pathogenesis of *Scedosporium apiospermum*

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S10.5 Fungal respiratory infections in Cystic Fibrosis, September 24, 2022, 10:30 AM - 12:00 PM

Secondary metabolism is a general term defining biosynthetic pathways that occur in plants, bacteria, and fungi and lead to the production of highly diversified molecular structures. Among the diverse functions that were attributed to these molecules, it is now obvious that they are predominantly involved in chemical warfare with competitors in their environments. In fungi, the two main classes of fungal secondary metabolites are polyketides (PKs) and nonribosomal peptides (NRPs). The biosynthesis of such bioactive molecules is performed by large multifunctional enzymes (NRP synthases, NRPS, and polyketide synthases, PKS) encoded by genes usually located within clusters. Conversely to *Aspergillus fumigatus*, only a few data are currently available for other pathogenic fungi. In this context, our research group is interested in delineating the role of secondary metabolism in *Scedosporium apiospermum*, a multi-resistant mold known to colonize chronically the airways of patients with cystic fibrosis (CF).

Taking advantage of the availability of the *S. apiospermum* genome sequence, we first conducted an *in silico* analysis aiming at exploring the PKs and NRPs battery of the fungus. A total of 9 genes encoding PKs, 9 encoding NRPs, and 5 encoding hybrid NRPs/PKs enzymes were identified. All 3 of the PKs gene clusters presented homologies with those involved in the biosynthesis of pseurotin *A. transbergamotene*, and ovalicin, or the tremorgenic toxin b-aflatrem while a fourth one is involved in the biosynthesis of melanin. Among the NRPs encoding genes, 6 exhibited sufficient similarity scores with other fungal NRPs to predict the class of the generated peptide: siderophores (2), epidithiodioxopiperazines (2), and cyclopeptides (2). Nevertheless, substrate prediction methods for NRPs domains failed, thus questioning about the nature of the produced peptides. We thus focused our attention on the characterization of some NRP and PK biosynthetic pathways. Since iron acquisition is known to be crucial for the survival of microorganisms as for the virulence of numerous pathogens, we first investigated clusters predicted to be responsible for the biosynthesis of siderophores in *S. apiospermum*. For instance, we disrupted the SAPIO_CDS2806 gene, an ortholog of sidD which drives the production of the extracellular hydroxamate-type siderophore fusarinin C in *Aspergillus fumigatus*. A comparison of culture supernatants from sidD mutants and their parent strain revealed that *S. apiospermum* secretes a unique extracellular siderophore, namely Nα-methylcoprogen B and that sidD gene was essential for the biosynthesis of this siderophore. sidD mutation resulted in the lack of growth under iron limiting conditions. Interestingly, pyoverdine supported the growth of the parent strain only, suggesting that Nα-methylcoprogen B is required for iron acquisition from this Pseudomonas aeruginosa siderophore. Finally, the deletion of sidD resulted in the loss of virulence in a murine model of scedosporiosis.

Altogether, our results demonstrate that *S. apiospermum* sidD gene drives the synthesis of a unique extracellular siderophore, namely Nα-methylcoprogen B, which is essential for fungal growth and virulence. Above all, we also provide unprecedented data suggesting that this fungal siderophore scavenges iron from pyoverdine, which might explain the antagonism between *S. apiospermum* and *P. aeruginosa* in CF.

S10.5d
Disseminated pulmonary infection due to *Mortierella wolfii* in a 6-year-old patient with X-linked CGD receiving MUD-HSCT

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S10.5 Fungal respiratory infections in Cystic Fibrosis, September 24, 2022, 10:30 AM - 12:00 PM

Objectives: Invasive fungal infections represent one of the major limiting factors for the successful outcome of patients receiving hematopoietic stem cell transplantation (HSCT). The identification and successful treatment of pretransplant fungal colonization/infections may allow for risk modifications before or at the time of HSCT. Here, we report a case of disseminated pulmonary infection due to a hyaline non-septate mold, *Mortierella wolfii* in an X-linked chronic granulomatous disease (X-linked CGD) patient that was successfully treated with a combination of terbinafine and posaconazole antifungal therapy.

Methods: A 6-year-old male with X-linked CGD from Sri Lanka was admitted to NIH Clinical Center, Bethesda, MD, USA to receive a matched unrelated donor (MUD) hematopoietic stem cell transplantation (HSCT). During pre-transplant immunosuppressive conditioning, the patient developed complicated pulmonary signs resulting in diffuse lymphadenitis and meningitis. Upon further radiologic evaluation, a lung biopsy was performed. The lung biopsy sample was submitted to Microbiology Service of Department of Microbiology at NIH Clinical Center for Fungal culture. Antifungal susceptibility testing was conducted in accordance with the Clinical and Laboratory Standards Institute CLSI M38-A3 guidelines.

Results: A pure heavy growth of white mold grew within 2 days on Sabouraud's Dextrose Agar. Microscopic examination showed hyaline (non-pigmented), non-septate branched hyphae. Sporangiophore-like structures were also present. The species-level identification of the isolate was confirmed as *M. wolfii* by PCR-sequencing of the internal transcribed spacer (ITS) region of ribosomal DNA.

Minimum inhibitory/effective concentrations (μg/ml) were as follows in increasing order: terbinafine = 0.25, amphotericin B = 1, isavuconazole = 4, micafungin > 8, itraconazole > 16, voriconazole > 16, and posaconazole > 16. To evaluate the interactions between antifungal drugs, the activity of the posaconazole in combination with terbinafine were also evaluated *M. wolfii* using agar diffusion test. A combination of posaconazole and terbinafine, significantly inhibited the mycelial growth, which indicates synergism. The patient's treatment was started on terbinafine in combination with posaconazole. On several follow-up examinations following treatment on day 30, 90 and 120, the infection had not recurred.

Conclusion: The species of *M. wolfii* a is an environmental mold belongs to the order *Mortierellales* within the subphylum *Mortierellomycotina* of Kingdom Fungi. This fungus has been mostly associated with fungal infections leading to abortion in dairy cows feeding moldy hays and ensilage.

Although posaconazole exhibited high MICs against *M. wolfii*, our *in vitro* combination study demonstrated that posaconazole and terbinafine combined are significantly more potent than either drug alone. As a suggestion, combination therapy could provide an option for the treatment of severe cases of *M. wolfii* in patients with underlying primary immunodeficiencies.

As molecular identification and sequencing techniques continue to develop and become more available, we will likely see more diverse pathogens emerge in patients with underlying primary immunodeficiencies. In this current case. Additional study is warranted to explore insight into human immunity and the efficacy of combination therapy against rare fungal species in CGD patients.

P001

Characteristics and dynamics of azole-resistant *Aspergillus fumigatus* variants emerging over a 28-year period in the Netherlands

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Poster session 1, September 21, 2022, 12:30 PM - 1:30 PM

Background: *Aspergillus fumigatus*, a globally distributed opportunistic pathogen, is the main cause of invasive aspergillosis, especially in immunocompromised patients with high mortality. The emergence of azole-resistant *A. fumigatus* isolates has been a significant concern worldwide and an important clinical problem.

Objectives: We aim to determine the presence of variants in a large collection of clinical *A. fumigatus* isolates from the Netherlands, if the number of variants increased over time and if the presence of additional short nucleotide polymorphisms (SNPs) or tandem repeats (TR) variations impacted on the triazole phenotype.

Methods: The Radboud University Medical Center has collected 11 813 clinical *A. fumigatus* isolates since 1994. The collection includes isolates cultured from patients admitted to our own center, isolates sent from other hospitals for identification and *in vitro* susceptibility testing, and isolates sent from five university medical centers and five teaching hospitals that contribute to the national *Aspergillus* resistance surveillance. The genotypes were detected by Cyp51A Sanger sequencing. All isolates were subjected to *in vitro* susceptibility testing using the EUCAST microdilution reference method. Minimal inhibitory concentrations (MICs) were determined for itraconazole, voriconazole, posaconazole, in all isolates and for isavuconazole in isolates cultured in 2015 and thereafter.

Results: In total, 1826 *A. fumigatus* isolates harbored azole-resistant mutations in the Cyp51A-gene with 92 genotypes. Tandem Repeat-associated resistance genotypes accounted for 55.43% of the variants and were involved in 1728 isolates (94.63%). TR34/L98H and TR46/Y121F/T289A resistance mutations remained dominant, and increasingly additional SNPs in the Cyp51A-gene or changes to the gene promoter were observed. The G448S mutation was relatively common and present in various genetic backgrounds. This SNP was most often found in isolates harboring the TR46 resistance mechanism (8 variants) and was also observed in two variants in the TR34 genetic background. TR34 and TR46 resistance mutations are associated with 1170 (64.07%) isolates that exhibited a pan-azole resistance phenotype, 547 (29.96%) a multi-azole resistance phenotype, and 75 (4.11%) resistance to a single azole. TR34/L98H confers high itraconazole resistance, while T289A confers high voriconazole resistance in the TR46 background. Isolates with a G448S point mutation show high MICs for both voriconazole and itraconazole. The TR34/L98H/T289A/G448S isolate showed low itraconazole MICs but high voriconazole resistance, and mutations in the promoter region, TR34/C-86 G/L98H, and (T-66 G)/TR34/L98H variants, showed increased voriconazole and isavuconazole MIC compared with the parent phenotype. TR46/Y121F/M172I/T289A/G448S variant was observed with an increased itraconazole (GM MIC 16 mg/L, 1→16 mg/l) and decreased voriconazole (GM MIC 18.664 mg/l, 4→16 mg/l) compared with the parent MIC of TR46/Y121F/T289A, while TR92/Y121F/M172I/T289A/G448S and TR46/Y121F/T289A/G448S variants showed the consistent MIC distribution with parent genotype. The variants with more combination mutations showed pan-azole resistance with increased MIC distribution.

Conclusion: Our survey showed a significant increase in resistance genotypes in clinical *A. fumigatus* over a period of 28 years. Azoles resistance phenotypes vary from resistant variants in clinical isolates; it is an implication for clinical *A. fumigatus* infection treatment options and antifungal stewardship.