S70.4a New mechanism and detection methods for azole-resistant Aspergillus fumigatus
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S70.4 Emerging antifungal resistant fungi, September 24, 2022, 10:10 AM - 12:00 PM
The most studied azole-resistant mechanism of Aspergillus fumigatus is dysfunction of the drug for CYP131, the target drug for azole-resistant strains. Typical antifungal resistance caused by the designated azole acid substitution of CYP131 has a specific pattern depending on the substrate. While understanding non-azole CYP131 mechanisms responsible for azole resistance, we screened for novel mechanisms in order to develop novel methods for prompt diagnosis and effective drug treatment. In our previous study, we reported results that mutation of lmg, which induces H3MG-CoA reductase, the rate-limiting enzyme in ergosterol biosynthesis, would be the mechanism conferring azole-drug resistance (EBD 2018). On the other hand, different azole susceptibility patterns have been reported even among the strains possessing the same mutation in CYP131. In this way, the overall picture of molecular mechanisms inducing azole resistance remains unclear.

We have already reported simple and rapid detection methods for A. fumigatus possessing CYP131 mutation using an enzyme-linked assay (ASC 2020). Furthermore, using MALDI-TOF-MS, we are developing a discriminant model to detect azole-resistant A. fumigatus.

S70.4b Pathobiology, diagnosis, and management of chronic pulmonary Aspergillosis
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S70.4 Emerging antifungal resistant fungi, September 24, 2022, 10:10 AM - 12:00 PM
Chronic pulmonary aspergillosis (CPA) is a complex disease that is difficult to diagnose and treat. To improve the accuracy of CPA diagnosis, we have analyzed 324 sputum samples obtained from 120 CPA patients, and have made some findings.

The diagnosis of CPA requires the identification of organisms in patients by histopathology with culture confirmation. However, culture only yields growth, and histopathological identification of an organism with a structure typical of Mucor has proved the only evidence of infection. PCR-based techniques may contribute to the early diagnosis of CPA. We reported a new antigen test. We have searched for murine or human-derived proteins that can be used to detect CPA. This test was performed on our patients and others with a diagnosis of chronic pulmonary disease. In 324 sputum samples obtained from CPA patients, we found that a specific antigen could be detected in CPA patients.

S70.4c Successful treatment of mucormycosis in hematological diseases
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The diagnosis of mucormycosis requires the identification of organisms in tissue by histopathology with culture confirmation. However, culture only yields growth, and histopathological identification of an organism with a structure typical of Mucor may provide the only evidence of infection. PCR-based techniques may contribute to the early diagnosis of CPA. We reported a new antigen test. We have searched for murine or human-derived proteins that can be used to detect CPA. This test was performed on our patients and others with a diagnosis of chronic pulmonary disease. In 324 sputum samples obtained from CPA patients, we found that a specific antigen could be detected in CPA patients.

S70.4d A unique clinical appearance of Candida auris infection in Japan
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S70.4 Emerging antifungal resistant fungi, September 24, 2022, 10:10 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 fungus is multidrug-resistant and can arise resistance to all three major groups of current antifungal drugs (azoles, echinocandins, and amphotericin B). Otitis media in the clinical setting of otomycosis are also common. The main reasons for this are as follows: unlike Candida spp. that primarily inhabit the digestive and urinary systems, C. auris readily colonizes patients who are suffering from cancer or AIDS and have no nosocomial infections heretofore occurred. Whole-genome analysis suggests that all Japanese isolates belong to C. auris, infecting drug resistance and clinical characteristics.

In this symposium, we will present the current status of C. auris infections in Japan, the first country where C. auris infection originated, together with its unique clinical features and molecular epidemiological analysis.
Minimum inhibitory/concetnration (MIC) of azoles were as follows in increasing order: terbinafine = 0.25, amphotericin B = 1, itraconazole = 0.4, voriconazole = 8, posaconazole = 36, and voriconazole = 16. To evaluate the interactions between antifungal drugs, the activity of the posaconazole in combination with terbinafine was also evaluated. M. acu LSU using agar diffusion test. A combination of posaconazole and voriconazole, significantly inhibited the mycelial growth, which indicates synergism. The patient's treatment was started on terbinafine in combination with voriconazole. On several follow-up examinations following treatment on day 59, 90, and 120, the infection had not recurred.

Conclusions: The species M. acu LSU is an environmental mold, belongs to the genus Mortierellales within the phylum Mortierellomycota of Kingdom Fungi. This fungus has been mostly associated with fungal infections leading to abortion in dairy cows causing mild bays and meningitis. Although posaconazole exhibited high MICs against M. acu LSU, 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. acu LSU in patients with underlying 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 the 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.

PO01
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, 12:00 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 single nucleotide polymorphisms (SNPs) or random repeats (RR) varied based on the isolate 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 Aspergillosis resistance surveillance. The genotypes were detected by Cyp51A fingerprinting. All isolates were subjected to an in vitro susceptibility testing using the ECAST antifungal reference method. Minimal inhibitory concentrations (MIC) were determined for voriconazole, posaconazole, and itraconazole, in all isolates and for voriconazole in isolates cultured in 2015 and thereafter.

Results: In total, 1924 A. fumigatus isolates harbored azole-resistant mutations in the Cyp51A gene with 92 genotypes. Tandem Repeat-associated resistance genotypes accounted for 55.4% of the variants and were involved in 3728 isolates (46.63%). TR14/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 G48S 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. TR14 and TR46 resistance mutations are associated with 1170 (6.07%) isolates that exhibited a pan-azole resistance phenotype, 547 (29.96%) a multi-azole resistance phenotype, and 73 (4.11%) resistance to a single azole. TR14/L98H confers high voriconazole resistance, while TR46/Y121F confers high voriconazole resistance in the TR46 background. Isolates with a G48S point mutation show high MICs for both voriconazole and itraconazole. The TR46/Y121F/T289A/G48S isolates showed low resistance MICs but high voriconazole resistance, and trimethoprim in the promoter region, TR46/MIC 8 G39Y and TR46/5G/14M/172/289A/G48S variants showed mixed resistance and voriconazole MIC compared with the parent phenotype. TR46/Y121F/T289A/G48S variant was observed with an increased itraconazole (MIC 36 mg/L, 1–16 mg/L) and decreased voriconazole (MIC 18–64 mg/L). 4–16 mg/L) compared with the parent MIC of TR46/Y121F/T289A, while TR46/Y121F/M172/T289A/G48S and TR46/Y121F/T289A/G48S variants showed the consistent MIC distribution with parent genotype. The variants with more combination mutations showed pan-azole resistance with increased MIC distribution.

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