P021

Comparative transcriptomic analysis of environmental *Candida auris* showing variable azole susceptibility

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

Objective: *Candida auris* is a multidrug-resistant pathogen that presents a serious global threat to human health. The U.S. Centers for Disease Control and Prevention has classified *C. auris* as an urgent threat to public health due to its clinical and economic impact and future projections of new infections over the next 10 years. *Candida auris* infections are difficult to treat since many isolates display high levels of resistance to fluconazole and exhibit variable resistance to amphotericin B and echinocandins. In this study, we performed comparative transcriptomics to understand the molecular mechanisms associated with azole-resistance in *C. auris* environmental isolates.

Material and Methods: Two sets of environmental isolates including azole-resistant ($n = 2$) and azole susceptible ($n = 1$) isolates were used for RNA-Seq analysis. Pair-wise comparisons in edgeR were used for comparing the number of differentially expressed genes (DEGs) between the azole susceptible and resistant isolates. GO term enrichment analysis was performed using the 'enrichGO' function from the cluster Profiler package. Only GO categories with a q-value <0.05 were considered significant.

Results: Our data show significant enrichment of ergosterol biosynthesis genes, drug transport, MAPK pathway as well as chromatin remodeling genes in azole-resistant strains compared to susceptible isolates. A total of 468 and 564 differentially expressed genes were identified in two azole-resistant isolates compared with the susceptible strain. A large number of multidrug transporter genes (CDR1, MDR1, HGT2, HGT7, HGT13, HGT17, and NGT1) were differentially expressed between the two sets of strains. Interestingly, the overexpression of ERG11 (azole target gene), and CDR1 (drug transporter) genes was observed in resistant isolates as compared with susceptible strain. Furthermore, resistant strain has two copies of ERG11 while susceptible isolate has single copy of ERG11. Notably, 8/21 genes involved in the ergosterol biosynthesis pathway were found to be induced in azole resistant isolates. These include HMG1, ERG1, ERG2, ERG3, ERG6, ERG10, ERG13, and ERG25. Furthermore, other multidrug transporters MDR1 and SNQ2 responsible for azole resistance in other *Candida* species like *C. glabrata* also showed significant expression changes between the two sets of isolates. Furthermore, HGT7 (glucose transporter) and NGT1, (N-acetyl glucosamine transporter) genes associated with azole and polyene resistance were found to be upregulated in the resistant isolate as compared with susceptible strain. Additionally, a Glycophosphatidylinositol (GPI)-anchored protein unique for *C. auris*, PGA7 was found to be overexpressed in resistant isolate. Importantly, we also identified several secreted aspartic proteases (SAP3, SAP5, SAP8, and SAP9) to be downregulated between the two sets.

Conclusion: The present study identifies several gene families that are differentially expressed in azole resistant vs susceptible *C. auris* strains. These findings suggest that azole-resistance in *C. auris* environmental isolates is influenced by changes in cell wall, lipid, and ergosterol biosynthesis. Overall, these data provide a framework for the mechanistic understanding of azole resistance mechanisms in *C. auris* environmental isolates.

P022

Luliconazole—a novel potent imidazole activity against *Aspergillus niger* and *Aspergillus flavus* causing otomycosis

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Background and Objectives: Suppurative otitis media (SOM) is characterized by the inflammation of the middle ear and mastoid, tympanic membrane perforation as well as discharge. The tympanic membrane perforation may result in increased exposure of the middle ear to pathogens. *Aspergillus niger* and *Aspergillus flavus*, are the most common causative agents of otomycosis with worldwide distribution, when it spreads from the external auditory canal to adjacent anatomical structures, it is classified as *Aspergillus* invasive otitis externa. *Aspergillus* otomycosis treatment is initiated by thorough cleaning of the ear canal, accomplished with suction, and drying with cotton swabs. In developing countries, SOM is a major cause of preventable hearing loss, its incidence ranges from 7% to 46% and is common amongst children of lower socioeconomic status. Treatment of SOM is directed at debridement and drying the ear with topical antifungal agents. Extensive surgical debridement and systemic antifungal therapy are needed in cases of refractory otomycosis or *Aspergillus* invasive otitis externa. Despite this management, treatment failure may result from suboptimal therapeutic management caused by antifungal agent toxicity. Luliconazole is currently confirmed for the topical therapy of dermatophytosis. Moreover, it is found that luliconazole has *in vitro* activity against some molds and yeast species. The aim of the present study was to evaluate the efficacy of luliconazole in comparison to routinely used antifungals on clinical isolates of *A. niger* and *A. flavus*.

Methods: The study was carried out in the Department of Microbiology, SRIHER, Chennai. A total of 55 (29 *A. niger* and 26 *A. flavus*) strains of *Aspergillus* isolates obtained from clinical otomycosis cases were confirmed based on macroscopic and microscopic identification by Lacto Phenol Cotton Blue mount and slide culture technique. Antifungal susceptibility patterns of all the *Aspergillus* isolates to itraconazole, voriconazole, posaconazole, and luliconazole were determined by broth microdilution method as per Clinical Laboratory Standards Institute (CLSI) M38-A2 guidelines.

Results: The lowest minimum inhibitory concentration (MIC) geometric mean (GM) (0.00309 μg/ml) was attributed to luliconazole followed by posaconazole (0.18409 μg/ml), voriconazole (1.02727 μg/ml) and itraconazole (11.0091 μg/ml). Also, among the azoles tested, luliconazole had the lowest MIC50 and MIC90 values of 0.00098 μg/ml and 0.00781 μg/ml respectively. Among the triazoles tested posaconazole had a lower MIC50 and MIC90 values of 0.125 μg/ml and 0.25 μg/ml. Being the drug of choice for invasive aspergillosis voriconazole had a slightly higher MIC50 and MIC90 value of 1 μg/ml and 2 μg/ml. Luliconazole was found to be more effective even for pan azole-resistant isolates ($n = 3$) with lower MIC values.

Conclusion: The results of this study showed that luliconazole had an excellent *in vitro* activity against all *Aspergillus* isolates with a lower MIC GM, MIC50, and MIC90 values than the triazoles tested. Hence, this novel imidazole antifungal agent can be regarded as appropriate Candidate for the treatment of otomycosis caused by *A. niger* and *A. flavus* strains. Also, luliconazole showed better efficacy with lower MIC values for pan azole resistant isolates, suggesting that it could be a potential antifungal for treating aspergillosis caused by pan azole-resistant isolates.

P023

Evaluation of Beta-D-glucan assay as a tool for antifungal stewardship at a hospital in Mumbai, India

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Objectives: Clinical evidence suggests that the Beta-D glucan (BDG) test is useful as a tool for antifungal stewardship by helping in discontinuing empiric antifungal therapy. This study was hence initiated with the following objectives: (1) to calculate the percentage of echinocandin prescriptions in patients with a subsequent negative BDG test and the compliance to recommendations for stopping echinocandins for the above prescriptions, (2) to study outcomes in patients where echinocandins were stopped, and (3) to study the total cost savings.

Methods: The study was conducted for a 1-year period from January 2021 to December 2021 in a tertiary care hospital in Mumbai. The antimicrobial stewardship committee recommends sending a serum sample for BDG along with paired blood culture for all patients before starting empirical antifungal therapy. The choice of empiric antifungal therapy at our hospital is echinocandins (caspofungin, micafungin, and anidulafungin). The BDG test was performed using Fungitell® assay (Associates of Cape Cod, Massachusetts) that quantitatively measures 1, 3-β-D-glucan levels which is run twice a week on Wednesday and Saturday. The cut-offs for a negative, indeterminate and positive result are 60 pg/ml, 60-79 pg/ml, and >80 pg/ml respectively. The result of the BDG test and blood culture was promptly informed to the consultant in charge and recommendations were made to discontinue the echinocandin if both tests were negative. The compliance with these recommendations was monitored. The patients in whom the echinocandin was stopped were monitored during their hospital stay or on day 28 after stopping the echinocandin whichever was earlier. The total cost savings [in Indian Rupees (INR) and converted into US Dollars (USD)] were calculated based on an average of 10 days extra therapy with echinocandins.

Results: A total of 337 echinocandins were prescribed in 294 patients and reviewed during the study period; anidulafungin (170, 50%) was the most commonly prescribed echinocandin followed by caspofungin (131, 39%), and micafungin (36, 11%). The BDG result as well as blood fungal cultures were negative for 53 prescriptions in 49 patients (15.7%). The compliance to recommendations for stopping echinocandin in these prescriptions was 100% (53/53). None of these 49 patients had a sterile culture positive for *Candida* during the follow-up period. A total of 17 patients died, 1 was discharged against medical advice, 19 were discharged and 12 were still in hospital at the end of the follow-up period. No deaths could be attributed to invasive fungal infections at 28-day follow-up. Total cost savings were 6794 440 INR, corresponding to 89 000 USD during the study year.

Conclusion: BDG test-based stewardship strategy helped in reducing the use of echinocandins with cost savings and no increased risk of invasive fungal infections related to adverse outcomes in patients where echinocandins were discontinued. The universal compliance to recommendations to deescalate could be achieved by constant dialogue between the departments of Clinical Microbiology and Clinical Medicine.

P024

Genetic determinants of antifungal drug resistance in *Fusarium solani* species complex

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Due to its challenging diagnosis and treatment, fungal keratitis is one of the most serious kinds of corneal infection. The *Fusarium solani* species complex is responsible for nearly half of all fungal keratitis cases. *Fusarium* infection is difficult to treat because of the increased antifungal resistance of *Fusarium* species.

Objective: To check the antifungal susceptibility in keratitis-causing isolates of F. *solani* species complex.

To find the genetic determinants of resistance using whole genome sequencing.

Methodology: Prior to AFST and according to CLSI, clinical isolates of *Fusarium* species ($n = 5$) were cultured on potato dextrose agar for 7 days at room temperature. Fungal spores were harvested using 0.8% NaCl and antifungal drug susceptibility testing of 6 different antifungal drugs were tested using CLSI standards broth microdilution method and minimum inhibitory concentrations were obtained. After 24 and 48 h, broth microdilution plates were manually examined. The wells for growth control were also examined. The minimum inhibitory concentration of anti-fungal drugs was the lowest dose that inhibits growth completely (compared to the growth control well). Minimal effective concentration (MEC) was also determined against antifungal drugs. Whole genome sequencing was done using Illumina Hiseqx10. Bioinformatics analysis was done using different bioinformatics tools and software (FASTQC, SPAdes, OmicsBox, AUGUSTUS, etc.).

Results: In this study the antifungal drug susceptibility from average MIC value were found as fluconazole (512 μg/ml) > itraconazole (32 μg/ml) > amphotericin B (8 μg/ml) > natamycin (8 μg/ml) > posaconazole (2 μg/ml) > voriconazole (1 μg/ml). Fluconazole had a higher MIC value (512 g/ml) in all isolates, however, voriconazole was shown to be more sensitive, with a lower MIC range (0.25-4 g/ml). Glyoxalase/Bleomycin resistance protein, mfs—multidrug resistance transporter, fusaric acid resistance protein, efflux pump antibiotic resistance protein, and copper resistance protein were found by genome-based analysis.

Conclusion: As a conclusion of this study, we observed antifungal drug resistance in *Fusarium* spp., which is often used to treat keratitis in patients, employing fluconazole, itraconazole, and amphotericin B. Resistance to first-line azoles was as a gene variant which contributed to antifungal drug resistance. This study using the phenotypic and genotypic characterization of drug resistance patterns will help to combat antifungal drug resistance.