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 emerging 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 withazole resistance in C. auris environmental isolates.

Materials and Methods: Two sets of environmental isolates including azole-resistant (n = 2) and azole-susceptible (n = 2) 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 ‘clusterProfiler’ function from the ClusterPath package. Only GO categories with a p-value < 0.05 were considered significant.

Results: Our data show significant enrichment of organelle ribosome genes, drug transport, MAPK pathway, as well as chromatin remodeling genes in azole-resistant strains compared to susceptible isolates. A total of 468 and 544 differentially expressed genes were identified in two azole-resistant isolates compared with the susceptible strain. A large number of azole transporters genes (CDR1, MDRI, HGT2, HGT13, HGT17, and NGT1) were differentially expressed between the two sets of isolates. Interestingly, the azole MICs (μg/ml) for all transporters were observed in resistant isolates as compared with susceptible strain. Furthermore, resistant strain has two copies of ERG11 while susceptible strain has single copy of ERG11. Notably, NGT2 genes involved in the ergosterol biosynthesis pathway were found to be induced in azole-resistant isolates. These include MDRI, ERG1, ERG2, ERG4, ERG6, ERG8, ERG13, and ERG5. Furthermore, other multidrug transporters MDRI 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, HGT17 (glucose transporter) and NGT1, (N-acetyl glucosamine transporter) genes associated with azole and polypeptide resistance were found to be upregulated in the resistant isolates as compared with susceptible strain. Additionally, a Glycosphingolipid(synthesized)-anchored protein unique for C. auris, Pan1 was found to be overexpressed in resistant isolate. Importantly, we also identified several secreted aspartic proteases (SAP1, SAP2, SAP3, and SAP4) to be downregulated between the two sets.

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

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 worldwide, where it spreads from the external auditory canal to adjacent anatomical structures, it is classified as Aspergillus otitis externa. Aspergillus otitis externa treatment is initiated through thorough cleaning of the ear canal, accompanied 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 among children of lower socioeconomic status. Treatment of SOM is done depending on the age, sex and the type of tympanic membrane perforation. Commonly, azole and antifungal debridement and systemic antifungal therapy are used 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 resistance. Lactoferrin is currently confirmed for the topical therapy of dermatomycosis. Moreover, it is found that lactoferrin has in vitro activity against some middle and outer ear species. The aim of the present study was to evaluate the efficacy of lactoferrin in comparison to routinely used amphotericin B on clinical isolates of A. niger and A. flavus.

Methods: The study was carried out in the Department of Microbiology, SRIRacha, Chennai. A total of 55 (29 A. niger and 26 A. flavus) strains of Aspergillus otitis externa isolated from clinical otomycosis cases were confirmed based on morphologic and microscopic identification by Lacto Pheno Crystal Blue mount and slide culture techniques. Antifungal susceptibility patterns of all the Aspergillus isolates to itraconazole, voriconazole, posaconazole, and lactoferrin 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): (0.0009 μg/ml) was attributed to lactoferrin followed by posaconazole (0.1409 μg/ml), voriconazole (0.0027 μg/ml) and itraconazole (0.0039 μg/ml). Also, among the azole tested, lactoferrin had the lowest MIC90 and MIC50 values of 0.0009 μg/ml and 0.0078 μg/ml respectively. Among the triazole tested posaconazole had a lower MIC90 and MIC50 values of 0.125 μg/ml and 0.25 μg/ml. Being the drug of choice for invasive aspergillosis voriconazole had a slightly higher MIC90 and MIC50 value of 3 μg/ml and 2 μg/ml. Lactoferrin was found to be more effective even for pan azole-resistant isolates (n = 5) with lower MIC values. Conclusion: The results of these study showed that lactoferrin has an excellent in vitro activity against all Aspergillus isolates with a lower MIC (GM), MIC90, and MIC50 values than the triazole tested. Hence, this novel antifungal antifungal agent can be regarded as appropriate Candida for the treatment of otomycosis caused by A. niger and A. flavus species. Also, lactoferrin showed the most effective MIC values for pan azole resistant isolates, suggesting that it could be a potential antifungal for treating aspergillus caused by pan-azole-resistant isolates.