O225
1-hour Oral Session
From antifungal susceptibility to resistance

Prevalence of azole resistance in clinical Aspergillus flavus isolates: presence of novel S196F, A324P, N423D and V465M substitutions in the cyp51C gene

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Background: *Aspergillus flavus* is the second leading cause of invasive aspergillosis in immunocompromised patients and is also an important causative agent of fungal rhinosinusitis and keratitis. Voriconazole (VRC) is being used as first line and empiric therapy for the treatment of invasive aspergillosis. Prior exposure to azoles in *A.fumigatus* results in acquired resistance. Although, the mechanism of azole resistance in *A.fumigatus* is well studied and is mainly attributed to hotspot mutation in *Cyp51A* gene but in *A. flavus* the resistant mechanism is not well understood and so far only two reports of azole resistance in *A.flavus* suggesting *Cyp51C* to be the target gene for mutations are on record. Herein we studied the prevalence of azole resistance in clinical *A.flavus* isolates originating from clinical specimens of patients in a tertiary Chest referral hospital, Delhi, India and analyzed the resistance mechanism involved.

Material/methods: A total of 120 *A.flavus* isolates originating from patients of suspected bronchopulmonary aspergillosis and rhinosinusitis were screened for antifungal susceptibility testing using CLSI broth microdillution method (CLSI M38-A2). The isolates with VRC MIC values higher than epidemiological cut off values (ECVs) along with wild type isolates were subjected to molecular identification by sequencing of *β-tubulin* and *calmodulin* genes. The *Cyp51C* gene of the VRC non-wild type isolates was sequenced for mutation analysis and compared with the reference *A.flavus* strain(NRRL3357). The homology modeling of *CYP51C* gene amino acids sequence was performed using MODELLER-9.15. Four substitutions were introduced in the model to elucidate their structural effects by Molecular Dynamics simulations using GROMACS-5.0.

Results: Three (2.5%) isolates had VRC MIC above ECV (>1µg/ml). Of the three isolates, two had MICs 2µg/ml of VRC but low MICs of itraconazole and posaconazole. However, a solitary isolate revealed cross-resistance to itraconazole (>16µg/ml), VRC (>16µg/ml) and posaconazole (>8µg/ml). A comparison of sequences of *cyp51C* homologs of non-WT (n=3) and WT (n=2) with the reference

strain (NRRL3357) showed the presence of three missense mutations, namely, M54T, S240A and D254N. In a solitary non-WT isolate, in addition to the above-mentioned mutations, four novel substitutions, namely, S196F, A324P, N423D and V465M were observed. The three isolates originated from individual patients of COPD and nasal polyposis. Of the three patients, two were on VRC therapy for 2 weeks and no information on antifungal therapy was available for a solitary patient. The computational results indicate differences in charge distribution at substitution positions and backbone movements.

Conclusions: The prevalence of azole resistance in *A. flavus* isolates is alarming. This study identified the presence of four novel substitutions in VRC resistant *A. flavus* isolate. The azole resistance in *A. flavus* remains unexplored and many other mechanisms may have been responsible for the elevated MICs in other two non-WT isolates observed in this study.