P081

Azole-resistant *Aspergillus fumigatus* among NIH hospitalized patients with underlying primary immunodeficiencies

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

Objectives: *Aspergillus fumigatus* causes a variety of diseases in humans. The drugs recommended for treatment of *Aspergillus* diseases are the mold-active azole antifungals. However, a wide range of mutations in *A. fumigatus* confers azole resistance, which commonly involves modifications in the cyp51Agene, the target for azole antifungal drugs.

Methods: We investigated 255 clinical *A. fumigatus* isolates obtained from patients hospitalized at National Institutes of Health Clinical Center, Bethesda, Maryland, USA. The species-level identification of each isolate was evaluated by colony morphology, microscopic characteristics, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF), and PCR-sequencing of the β-tubulin gene. We also studied sequence-based analysis of the Cyp51A gene for the azole-resistant isolates.

The azole antifungal susceptibility profile of each isolate was initially evaluated using 4-well triazole screen plates (Microbiology Associates LLC, Rockville, MD, USA) containing itraconazole (4 μg/ml), voriconazole (2 μg/ml), posaconazole (0.5 μg/ml), and growth control. The full array of antifungal susceptibility was confirmed using microbroth dilution method according to Clinical and Laboratory Standards Institute CLSI M38-A3 guidelines.

Results: Of 255 *A. fumigatus* isolates, 12 grew on the wells containing azoles, indicating an azole-resistant phenotype. The results were read and recorded after 24 and 48 h of incubation at 35-37°C. Majority of our isolates had visible growth at 24 h. Sequence analysis of the CYP51A gene indicated the presence of M220K mutation in all 12 isolates and no mutations in the other isolates.

The fact that the azole resistance was found in *A. fumigatus* isolated from patients with previous azole exposure, underscores the possibility that prevalence of azole-resistance might be underestimated in various patient populations because *in-vitro* susceptibility testing of *A. fumigatus* is not routinely performed.

Conclusion: In conclusion, prevalence of azole resistance in clinical *A. fumigatus* isolates obtained from NIH patients underlying primary immunodeficiencies was 4.7%; all the resistant isolates exhibited azole-resistance mutation in Cyp51A gene.

Our finding adds to the growing list of regions where acquired resistance in *A. fumigatus* of is documented. Our results also indicate that 4-well triazole screen plates are a reliable tool for azole-resistance screening and the selection of isolates that require a full panel of antifungal susceptibility testing.

P082

Synergistic activity, anti-adherence and anti-fungal abilities of fluconazole and voriconazole combined with thymol and carvacrol against *C andida* species

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

Objectives: The current study aimed to assess the anti-adherence and antifungal activities of thymol and carvacrol against *Candida albicans*, *C. glabrata*, and *C. krusei* isolates obtained from patients with oral candidiasis concerning growth inhibition and fungal death as compared to the synthetic antifungals such as fluconazole and voriconazole.

Methods: The susceptibility assay for the test compounds was performed using the disk diffusion method against all *Candida* isolates. Also, anti-adherence activity was examined using a rapid and highly reproducible 96 well microtiter-based method.

Results: Both natural phenols and antifungal drugs revealed various efficacies against studied *Candida* species. The susceptibility to fluconazole and voriconazole were 100% for *C. albicans*, 50% and 90% for *C. glabrata*, and 0% and 100% for *C. krusei* isolates, respectively. The mean diameter of the inhibition zone was greater for thymol than carvacrol in *C. albicans* (19.89-0.80 mm vs 17.05-0.61 mm), *C. glabrata* (18.87-0.71 mm vs 15.77-0.57 mm), and *C. krusei* (15.11-0.91 mm vs 13.91-1.04 mm) isolates tested.

Thymol showed more effective inhibition on adherence of all *Candida* species than other treatments. The mean relative adherence ratios for *C. albicans*, *C. glabrata*, and *C. krusei* were 0.50, 0.60, and 0.64, respectively.

Conclusions: This study demonstrated significant inhibitory properties of thymol and carvacrol on the adherence and growth of azole susceptible- and -resistant *Candida* isolates. Also, thymol was more effective for preventing the adherence of yeast cells to polystyrene in comparison to carvacrol.

P083

Susceptibility pattern of fungal isolated from patients with otomycosis

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

Objectives: Antifungal resistance is posing several new concerns to clinicians. Increased rates of azole and echinocandin resistance in various non-*albicans Candida* species and azole resistance in *A. fumigatus* may arise due to clinical or environmental exposure to these drugs. The study evaluated the antifungal susceptibility for clinical fungal isolates causing otomycosis.

Methods: A total of 89 *Aspergillus* isolates containing *A. niger* (58 isolates), *A. flavus* (19 isolates), *A. fumigatus* (12 isolates), and 25 *Candida* isolates containing *C. parapsilosis* (14 isolates), *C. orthopsilosis* (6 isolates), and *C. albicans* (5 isolates) collected from individuals with confirmed otomycosis during October 2020-November 2021 were tested for antifungal susceptibility testing (AFST). AFST of ketoconazole, voriconazole, tioconazole, amphotericin B, miconazole, fluconazole, nystatin, and itraconazole was conducted using the broth microdilution method based on CLSI (M38-A2, M27-A3) protocols. Conidia of molds and colonies of yeasts were harvested from fungal cultures on SDA incubated at 35°C; the turbidity of the suspension was then adjusted to OD630 nm = 80%-82%T for molds and 75%-77%T for yeasts.

Results: Mainly, all antifungals examined were effective against most *Aspergillus* isolates, aside from tioconazole (GM = 5.54767 μg/ml) and nystatin (GM = 2.10151 μg/ml). Terbinafine (GM = 1.69824 μg/ml) had minimal *in vitro* effects (Table 1). Nystatin (GM = 2.94853 μg/ml) and itraconazole (GM = 1.08673 μg/ml) showed higher GM MICs against all *Candida* species isolates. Conversely, amphotericin B (GM = 0.07129 μg/ml) in *Aspergillus*, ketoconazole (GM = 0.02570), and voriconazole (GM = 0.03686 μg/ml) in *Candida* showed the highest antifungal activity (Table 2). Regarding the CLSI-M59 document for ECV, one *A. niger* (MIC 8 μg/ml), *A. flavus* (MIC 2 μg/ml), and *A. fumigatus* (MIC 2 μg/ml) isolates were non-wild type against itraconazole. A total of 3 *A. niger* non-wild type isolates with MIC 4 μg/ml against voriconazole were inspected (Table 1). Three *C. albicans* isolates with high itraconazole MICs (two 8 μg/ml and one 16 μg/ml) were observed (Table 2). Even though the MIC50 of *Aspergillus* niger for tolnaftate was 0.37 μg/ml, 9 isolates with high MICs (16 μg/ml) were found.

Conclusion: The foremost commonest yeast isolates in this study, *C. parapsilosis*, exhibit significant sensitivity to various antifungals, including ketoconazole, voriconazole, tioconazole, amphotericin B, miconazole, fluconazole, and itraconazole. However, contrary to other studies, nystatin had high MICs and is not recommended as an effective drug. Since the pattern of antifungal susceptibility is varied among the cryptic species of *Aspergillus* sections, we recommend that physicians request a drug susceptibility testing before antibiotic therapy to prevent the development of resistance.