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

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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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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.

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Susceptibility pattern of fungal isolated from patients with otomycosis

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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.

Table 2. Antifungal susceptibility pattern of *Candida* strains isolated from otomycosis patients

Species		FLC	KTC	MCZ	NYT	TCZ	ITC	AMB
C. parapsilosis (n=14)	Range (µg/mL)	0.25-2	0.016-0.063	0.016-2	2-4	0.016-0.5	0.5-1	0.032-0.5
	GM (µg/mL)	0.64044	0.0269	0.49994	2.82842	0.05383	0.78071	0.13798
C. orthopsilosis (n=6)	Range (µg/mL)	0.25-2	0.016-0.032	0.5-2	2-4	0.016-0.5	0.5-2	0.032-0.25
	GM (µg/mL)	0.62996	0.02476	0.89989	2.82842	0.12493	1	0.01113
C. albicans (n=5)	Range (µg/mL)	0.5-1	0.016-0.063	0.063-0.125	2-8	0.016-0.063	0.5-8	0.063-0.5
	GM (µg/mL)	0.37892	0.02365	0.07179	3.48220	0.03121	0.16685	0.07568
All *Candida* species (n=25)	Range (µg/mL)	0.25-2	0.016-0.063	0.016-2	2-8	0.016-0.5	0.5-16	0.032-0.5
	GM (µg/mL)	0.57434	0.02570	0.38955	2.94853	0.05907	1.08673	0.14356

Note: Antifungal agents*: FLC: fluconazole, KTC: ketoconazole, MCZ: miconazole, NYT: nystatin, TCZ: tioconazole, ITC: itraconazole, AMB: amphotericin B, VRC: voriconazole, GM: geometric mean

P084

***Trichosporon* osis awakening: molecular and antifungal susceptibility study on Trichosporonosis in North India and how antifungal stewardship can contribute in control**

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Background: *Trichosporon* spp are yeast-like organisms belonging to class Basidiomycetes which are usually commensals. It can cause diseases ranging from superficial infection, commonly known as White Piedra, to disseminated infections (Trichosporonosis). Cases of invasive Trichosporonosis have increased worldwide. It has now become the second most common cause of fungemia after *Candida* spp, especially in immunocompromised individuals.

Methods: We conducted a hospital-based prospective chart review of six patients with nosocomial infections caused by *Trichosporon* spp. Demographic data, clinical history, comorbidities, and outcomes after treatment were collected. Samples were processed using conventional media, biochemicals, and confirmed using automated system, MALDI-TOF and VITEK-2

Results: A total of 8 patients developed UTIs and 4 developed disseminated bloodstream infections. All patients had associated co-morbidities. All patients had history of treatment with antimicrobials which were ineffective. In all, 8 patients were identified to be infected with *T. asahii* and 4 with *T. mucoides*. All patients showed improvement with azoles. We also encountered a case of breakthrough Trichosporonosis.

Conclusion: Invasive Trichosporonosis developed in patients with associated risk factors. *Trichosporon* spp presents diagnostic and therapeutic challenges. It is usually confused with disseminated candidiasis leading to incorrect treatment and increased risk of breakthrough Trichosporonosis. Prolonged undiagnosed infection can lead to disseminated infection. New triazoles have shown to be effective against *Trichosporon* spp.

P085

Prevalence and antifungal susceptibility of *Candida* spp. from the sputum sample of patients in a tertiary care hospital in Sikkim

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Objectives: The role of *Candida* in sputum culture is unclear and is generally not treated when present in sputum samples. The objective of this study is to describe the clinical findings of patients with *Candida* spp. in sputum and their antifungal susceptibility pattern to know the local epidemiology of antifungal resistance.

Methods: Patients with respiratory symptoms attending the tertiary care hospital during the study period of 6 months from June 2021 to December 2021. A total of 23 sputum samples were processed in the microbiology laboratory. Samples were processed following conventional mycological procedures including direct microscopy (visualization of budding yeast cell on grams stain), growth on sabouraud dextrose agar, Germ tube test, and CHROMagar. The isolates were identified by rapid identification (ID) of yeast and yeast-like organisms in the BD PhoenixTM Automated Microbiology System.

Antifungal Susceptibility Testing (AFST) was carried out by disk diffusion susceptibility testing, Zone interpretation criteria as per M44/A2 protocol of CLSI were used. Antifungal used were amphotericin B (20 mcg), itraconazole (10 mcg), fluconazole (25 mcg), and voriconazole (1 mcg). Results of tests done on 23 isolates were collated and analyzed retrospectively.

Clinical profile of the patients was taken retrospectively from record section and analyzed.

Results: Of the 23 patients, most common presentation was fever followed by cough and dyspnea. A total of 34% patients were receiving some form of steroid (injectable or inhalational). Only 2 patients were COVID positive by RT-PCR and 7(30%) patients had some radiological findings like consolidation, emphysematous changes, etc. Immunodeficiency condition was seen in 4 (17%) patients like tuberculosis and diabetes mellitus.

Of the 23 samples, *C. albicans* showed prevalence of 91% as compared with *C. tropicalis* (5%) and *C. glabrata* (4.8%). AFST showed *Candida* spp. was found to be mostly sensitive to voriconazole and fluconazole. Resistance to amphotericin B was seen in most *Candida* spp. Itraconazole was not susceptible to even one isolate only 4 samples were intermediate (Fig. 1).

Conclusions: Infections with *Candida* spp. are usually of low virulence and are associated with a few well-defined risk factors as immunocompromised state, malignancy, and steroid therapy. Understanding these risk factors, identifying the species with changing trends in antifungal resistance, instituting infection control practices to reduce morbidity and mortality in critical care areas can improve outcomes.

Surveillance of the rates of *Candida* infection in critical areas, reporting of outbreaks and continuous monitoring of antifungal susceptibility patterns will help in choosing the best therapeutic management of complicated cases. Comparison of trends in infection rates amongst hospitals between various Indian cities and their resistance patterns can reveal vital information regarding the breakdown of infection control measures. Most *Candida* infections are of low virulence and only become significant in the vulnerable critical care areas. With the rise in prevalence of inherently azole-resistant species and rising use of echinocandins in ICUs, identifying risk factors and controlling the infection early can improve patient outcomes.

Figure 1. Anti-Fungal susceptibility test (AFST) pattern seen in *Candida spp.*