P372

Deciphering transcript isoform switching and utilization in mature biofilm of *Candida glabrata* through a global transcriptomic approach

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Poster session 3, September 23, 2022, 12:30 PM - 1:30 PM

Objective: Eukaryotic cellular systems utilize alternative transcript isoforms generated via an isoform switching mechanism to thwart various stress conditions. To the best of our knowledge, transcript isoform switching and differential transcript isoform usage (DTU) in *Candida glabrata* have not been studied. Therefore, the present study was designed to delineate differential transcript isoform expression (DTE) and transcript isoform switching followed by DTU and the functional impact of transcripts in a mature biofilm of *C. glabrata* (clinical isolate; NCCPF 100037) through an RNA sequencing approach.

Results: DTE analysis generated 7837 transcript isoforms from the *C. glabrata* genome (5293 genes in total), and DTU investigations revealed that transcript isoforms of 292 genes were specifically utilized in the formation of mature biofilms via the isoform switching process. Gene Ontology/pathway analysis and protein-protein interactions further substantiated the functional attributes of switched transcript isoforms selectively used by *C. glabrata* in biofilm formation and survival.

Conclusion: The present study elucidates that specific transcript isoforms utilized in mature biofilms of *C. glabrata* could be used as potential targets for the development of novel antifungal therapeutics.

P373

Does biofilm forming capacity of *Candida tropicalis* vary with echinocandin susceptibility?

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Objectives: *Candida tropicalis* is a common cause of nosocomial candidemia and candiduria. The role of biofilm formation in virulence and antimicrobial resistance in *C. tropicalis* remains under-investigated. We aimed to evaluate the biofilm-forming capacity of *C. tropicalis* isolates exhibiting resistance, borderline resistance, and sensitivity to echinocandins.

Methods: The echinocandin resistant, borderline resistant, and susceptible isolates of *C. tropicalis* were collected based on their minimum inhibitory concentration (MIC) values according to Clinical and Laboratory Standard Institute (CLSI) broth microdilution guidelines. The isolates were subjected to FKS1 gene sequencing. To estimate biofilm production, echinocandin resistant ($n = 2$), borderline resistant ($n = 5$), and susceptible isolates ($n = 3$) were seeded at the cell concentration of 1×10^6 cells/mL in RPMI-1640 with 0.165 M MOPS in polystyrene 96-well microtiter plates and incubated for 24 and 48 h at 37°C. The biofilm was quantified by crystal violet/safranin-based spectrophotometric method and XTT (2,3-Bis-(2-Methoxy-4-Nitro-5-Sulfophenyl)-2H-Tetrazolium-5-Carboxanilide) reduction assay. Statistical analysis was performed using one-way ANOVA with Bonferroni's *post-hoc* test for multiple comparisons among the groups.

Results: FKS1 sequencing analysis revealed S654P mutation in HS1 in both resistant isolates while isolates exhibiting borderline MIC to echinocandins carried wild-type FKS1 gene. Biofilm formation in borderline echinocandin-resistant *C. tropicalis* isolates was significantly ($P < .005$) higher compared with resistant and susceptible isolates. However, no significant difference in biofilm formation was noted among resistant and susceptible isolates.

Conclusion: This study suggests differential biofilm-formation capacity among *C. tropicalis* isolates with reduced susceptibility to echinocandins. However, this warrants further studies before any definitive inference can be made.

P374

Fungal coinfection in patients with SARS-CoV-2: Atertiary care hospital study

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Background: There is a high risk of developing fungal co-infections in patients infected with SARS-CoV-2, few studies have been conducted on the incidence and risk of these secondary infections. The incidence of *Candida* infection due to different *Candida* species is increasing in COVID patients.

Methods: This retrospective study comprised a total of 23 patients with upper respiratory infection investigated in the Department of Microbiology of University College of Medical Sciences (UCMS) and Guru Teg Bahadur Hospital (GTBH), Delhi from April 2021 to June 2021. Identification was done using DNA sequencing.

Results: Of the 23 cases, identification by DNA sequencing, *C. albicans* was present in 34.78%, *C. tropicalis* in 30.43%, *Pichia kudriavzevii* in 26.08%, *C. parapsilosis* in 4.3%%, and *C. auris* in 4.3%.

Conclusion: *C. albicans* is the leading pathogen in our patients, along with the rise in the incidence of *P. kudriavzevii* which is usually an environmental contaminant. Regular surveillance and infection control practices are future ventures to reduce the burden of infection.

P377

Study of the prevalence of candidemia and its antifungal susceptibility pattern in a tertiary care hospital

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Objectives: Study of the prevalence of candidemia and its resistance pattern in a tertiary care hospital.
Methods:

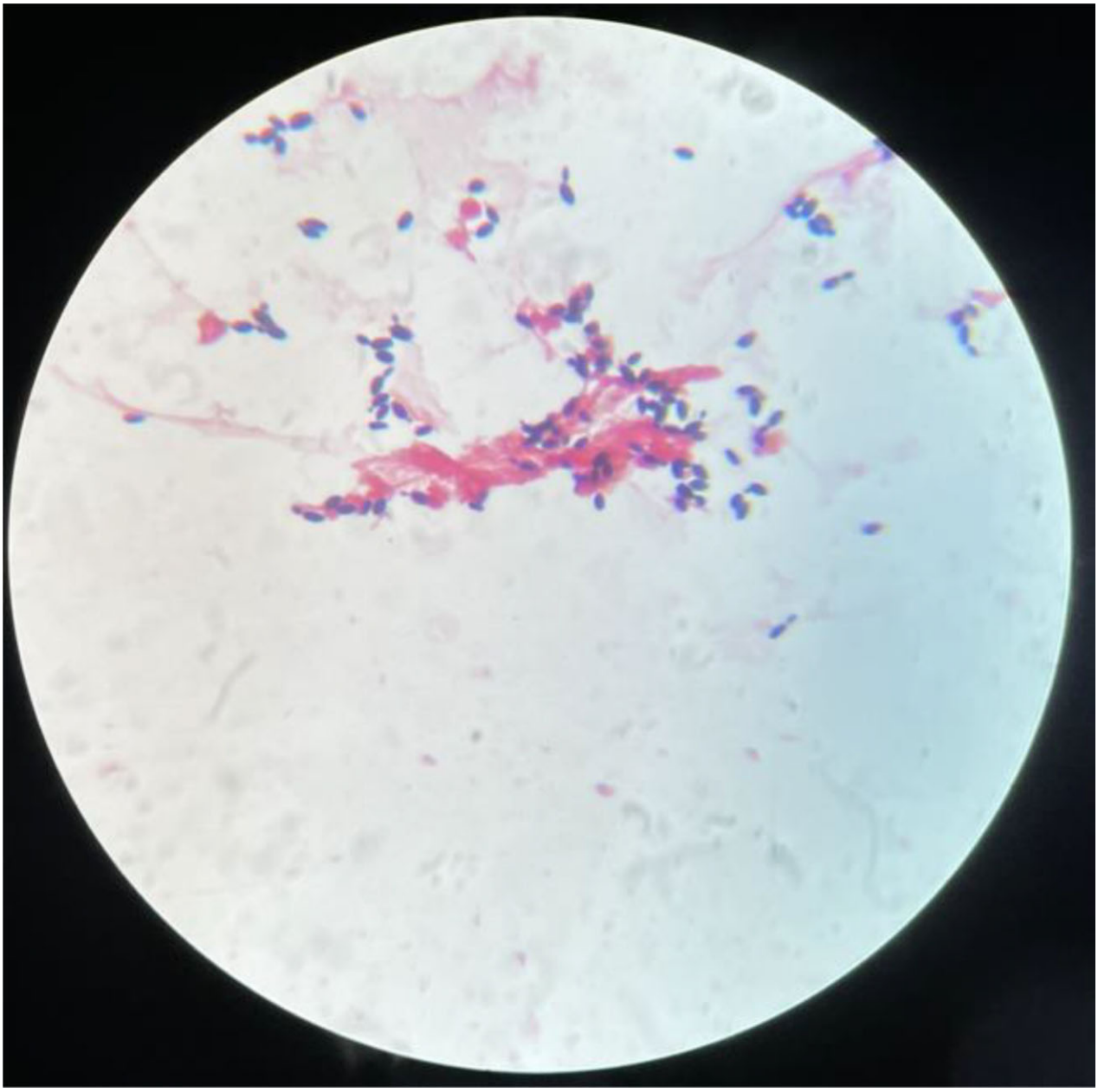
- Retrospective observational study
- Study period—January 1, 2020-December 31, 2021
- Blood culture bottles (aerobic and anaerobic) were received in the laboratory

1) Incubated in BacT Alert 3D (Biomerieux) for 5 days
2) After flagging positive, a direct gram stain was prepared from the culture bottle and informed to the clinician. Culture plating was done on Blood agar and Mac Conkey Agar which were incubated for 18-24 h at 37°C.
3) After 24 h, growth was obtained on the culture plate and identification was done by using MALDI-TOF MS (Biomerieux) and Susceptibility Testing was done using VITEK 2 Compact (Biomerieux).
4) Antifungal sensitivity testing was done using VITEK 2 Compact for fluconazole, voriconazole, caspofungin, micafungin, amphotericin B, and flucytosine.

Results:

- During the study period, 18 235 blood culture bottles were received, and 1652 blood culture bottles were flagged positive. The most common organism causing bloodstream infection belonged to *Burkholderia* spp.
- *Candida* sp (4%) was found to be the sixth most common organism causing bloodstream infection.
- Among *Candida* spp most common isolate was *C. tropicalis* 42% followed by *C. parapsilopsis* 21%, *C. albicans* 21%, and *C. auris* 12%, and the least isolated species was *C. glabrata* 4%.
- Maximum number of *Candida* spp were isolated from intensive care units.
- Susceptibility testing was given by VITEK 2 compact for all *Candida* spp except for *Candida auris*.
- The isolated *Candida* spp showed the least susceptibility to fluconazole (resistant rate R 30.6%) as compared to micafungin, caspofungin, flucytosine, and amphotericin B (R 13.9%, 13.9%, 22.2%, and 5.6%,) respectively.
- *Candida glabrata* showed less sensitivity toward caspofungin as compared to other antifungals.

Conclusion: *Candida* spp. are a part of the normal flora of healthy hosts but are also found to be a major cause of invasive fungal infections which is now found to be one of the leading causes of mortality in hospitalized patients. The emergence of unusual and relatively unknown *Candida* species as nosocomial pathogens with increasing treatment failure, emphasizes the need to isolate and identify all species and to start early definitive treatment according to the susceptibility pattern reported to decrease mortality and morbidity rates.



P378

Insecticidal potential of isolated fungal species in targeting *Drosophila melanogaster* and *Zaprionus indianus*

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Objectives: Fruit flies are polyphagous insects that attack a variety of commercially significant plants, which resulted in the build-up of insecticide resistance making the research focus shift toward alternative pest management tools in order to reduce risks to humans, environment, and non-target organisms.

Methods: Fungal species were isolated and molecularly characterized from Drosophila culture medium. Virulence assay was conducted against third instar larvae and adults of *Drosophila melanogaster* and *Zaprionus indianus*. Percent adult emergence and larval mortality were calculated.

Results: Three species: *Meyerozyma caribbica*, *Pichia kudriavzevii*, and *Aspergillus flavus* were identified by ITS region sequencing. *A. flavus* was the most virulent against larvae and adults of *D. melanogaster* and *Z. indianus* followed by *P. kudriavzevii* and *M. caribbica* (44%-100% mortality). Lethal time to 90% mortality (LT90) ranged from 4.5 to 7 days (*P. kudriavzevii*) and 3.2 to 4.5 days (*A. flavus*).

Conclusion: These preliminary findings suggest that the isolated fungal species can be deployed in targeting the developmental life stages of Drosophila species and hence, controlling invasive insect pests in an eco-friendly way. The use of these biological control agents could further minimize the use of harmful insecticides which has substantial global health benefits.