Background. Biofilms of Trichosporon asahii are known to resist the effects of antifungal drugs, but the study of their susceptibility to various abiotic stresses remains sparse. This study was thus undertaken to compare the level of resistance of T. asahii biofilm and planktonic cells to various stress factors.

Methods. In this study, one T. asahii clinical isolate identified by amplifying ITS sequencing and one reference strain (NCCP940033) were used. Biofilm and planktonic cells of T. asahii were exposed to increasing concentrations of NaCl (0.5–6 M) and d-sorbitol (3–13 M) for inducing osmotic stress; H₂O₂ (5–50 mM), menadione sodium bisulfate (0.048–100 mM) and ox bile (1–12%) for oxidative stress; P₂O₅ 1 to 13 for P₂O₅ stress; Congo red (600–10,000 µg/mL) for cell wall stress; CaSO₄·5H₂O (Y/ZnSO₄/FeSO₄, 1.25–2.56 mM) and MgSO₄ (250–3,000 mM) for metal stress. The biomass and metabolic activity of biofilms were quantitatively determined by crystal violet method and XTT reduction assay, respectively. Further, spot assay of serially diluted planktonic cells was performed on agar plates containing stress and non-stress control to determine relative percentage growth of strains.

Results. Biofilm cells of both the strains exhibited significantly higher (ANOVA) stress resistance than planktonic cells and on an average showed at least 100 times more resistant to stresses than planktonic cells [Minimum Biofilm Eradication Concentration (MBEC) vs. Minimum Inhibitory Concentration (MIC); H₂O₂ >50 mM vs. 10 mM, Ox bile >12% vs. 2%, Menadione >100 mM vs. 0.39 mM, Zn/Fe/Cu >2,560 mM vs. 10 mM, Mg >3,000 mM vs. 1,000 mM, NaCl >6 M vs. 1.5 M, d-sorbitol >13 M vs. 5 M and Congo red >10,000 µg/mL vs. 800 µg/mL. Besides optimal P₂O₅ 5–10, extreme acidic and alkaline pH 1–13 led to complete inhibition of viable planktonic cells. Higher biomass reduction (77.2%) and highest viability inhibition (69%) of biofilm were observed at pH 3 and 13, respectively. Menadione reduced 86.9% biomass and 89.3% viability which accounted the highest biofilm inhibition.

Conclusion. This is the first report on comparing the susceptibility of planktonic and biofilm T. asahii cells to various stress factors. The increased resistance of T. asahii biofilm may serve as an advantage against the host adversary.

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265. Clinical Epidemiology of Invasive Fungal Infection with Aspergillus and Mucor Species in a Tertiary Care Hospital
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Background. Patients undergoing hematopoietic stem cell transplantation and patients with hematologic malignancies are at increased risk for acquiring invasive fungal infection (IFI) due to immune system impairment from chemotherapy. Affected patients require prolonged antifungal therapy with the risk of associated toxicity and extended hospitalization due to delay of accurate diagnosis. There is a lack of effective serologic biomarkers and hesitancy to proceed with tissue diagnosis due to thrombocytopenia or other associated risks. Mortality in oncology patients with invasive mycoses is high, with pediatric mortality rates of 30–40% at 12 weeks following diagnosis.

Methods. All patients that were admitted to Lurie Children’s Hospital between January 2014 and December 2018 and received voriconazole, amphotericin, posaconazole and isavuconazole were identified. The following data were retrospectively collected: CT chest and sinus, (1,3)-β-D-Glucan and Aspergillus galactomannan, ANC and ALC at diagnosis, blood next-generation sequencing, tissue 18s rRNA, fungal culture, duration of neutropenia and lymphopenia, site of infection, time underlining diagnosis and development of IFI, surgical intervention and associated mortality.

Results. A total of 94 unique patients that received voriconazole were identified. There were 6 proven cases of invasive Aspergillus infection the past 5 years, 50% of male, mean age 14 years. Only 25% of patients had positive serum Aspergillus galactomannan and 37.5% had positive β-D-Glucan. Seven cases were due to Aspergillus fumigatus and one case was due to Aspergillus flavus. There were 9 patients with mucormycosis and all but one were culture positive. Three patients with Mucor had mortality of 22.2%. All patients that were admitted to Lurie Children’s Hospital between January 2014 and December 2018 and received voriconazole, amphotericin, posaconazole and isavuconazole were identified.

Conclusion. The majority of pediatric patients with invasive aspergillosis did not have characteristic chest CT imaging findings and serum Aspergillus galactomannan was usually negative. The was no associated mortality in invasive Aspergillus cases, whereas the mortality rate of invasive mucormycosis was 22.2%. Although we have a small sample size, this is significantly lower compared with published literature.

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266. Clinical Profile and Treatment Outcomes of Candida auris Isolates from a Tertiary Care Hospital in South India
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Background. Candida auris is an emerging multidrug-resistant fungus that is rapidly spreading worldwide. In publications from India, it has already accounted for >5% of candidemia in a national survey of ICUs and as much as 30% of candidemia at various clinical syndromes in our case series, the treatment regimens we opted and their clinical outcomes.

Methods. The present study was a retrospective observational analysis of candida auris isolates obtained from patients admitted in a Aster Medcity, Kochi. Duration of study was 4 months (September 2018 to December 2018). Laboratory confirmation of the candida auris isolates was done as per CDC recommendations for Vitek2. Vitek2 was used for obtaining the antifungal sensitivity pattern for these isolates.

Results. We had 18 cases of Candida auris identified. The most common syndrome was surgical site infections, 9 out of 18 (50%), followed by Catheter-Associated Urinary Tract Infection (CAUTI 5/18; 28%). We had 3 patients with Central Line-Associated Blood Stream Infections (CLABSI) and one patient who had secondary peritonitis. The overall mortality was 28% (5/18)—mostly contributed by the CLABSI. All patients that were admitted to Lurie Children’s Hospital between January 2014 and December 2018 and received voriconazole, amphotericin, posaconazole and isavuconazole were identified.

Conclusion. Candida auris is an emerging nosocomial pathogen in India with serious outbreak potential. The anti-fungal susceptibility is indicative of a multidrug-resistant pattern—with favorable MIC to Echinocandin and Voriconazole. Complicated bloodstream infections had high mortality inspite of early Echinocandin use; as they had either mild UTI (fever spikes resolved with catheter removal) or superficial SSI which could be treated with topical wound management.

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267. Fungal Culture Diagnostic Stewardship: An Avenue for Antimicrobial Stewardship in the Immunocompromised Host
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Background. Bronchoalveolar lavage (BAL) is a widely used procedure in the diagnosis of pneumonia in critically ill and immunocompromised hosts. Fungal smears and cultures are commonly performed on these samples. We evaluated the yield of various fungi, including but not limited to Candida species, Aspergillus species, and Penicillium species, isolated from BAL samples at our institution to determine the impact of this test and its potential on decision making.

Methods. We identified adult immunocompromised patients who underwent “Bronchoscopy with Immunocompromised Host Protocol (ICH),” which consists of an exhaustive list of diagnostic tests for various pathogenic organisms, over a one year period from January 1, 2017 to December 31, 2017. We reviewed positive fungal cultures led to a change in management and if this was appropriate.

Results. 582 patients underwent bronchoscopy with ICH protocol. There were 285/582 (48.9%) positive fungal cultures of which 177 (62%) grew Candida species. The most common species was Candida albicans (142/177, 80%), 58(18%) were Aspergillus species of which Aspergillus fumigatus was the most common (26/53). 16/285 (5.6%) patients underwent intervention based on the results, 14(87.5%) of which were appropriate interventions based on proven/probable invasive fungal infections criteria as were rest of the 6/16 patients with other fungal organisms (Table 4). Patients with Aspergillus species in BAL cultures are 8 times more likely to have an intervention (OR: 8.7, P = 0.001) while patients with Candida species in BAL cultures are not likely to be intervened upon (OR: 0.26, P = 0.0098) (Table 3).

Conclusion. Although Candida species is commonly isolated in BAL cultures its clinical significance is minimal in the absence of disseminated disease even in immunocompromised hosts. Evaluating the way that Candida cultures are communicated for respiratory samples, along with diagnostic stewardship may be a route for antimicrobial stewardship. Consulting ID service early on is essential in assessing the significance of fungal culture data thereby resulting in appropriate changes in management.

Table 2: Groups with and without intervention based on the type of fungal organisms isolated in BAL cultures. 5 patients with Candida species who had an intervention were treated for other fungal organisms that grew with it rather than for Candida per se.

Table 4: Fungi with intervention based on culture positivity.

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268. Fungal NGS: Identification of Etiological Agents of Invasive Fungal Infection by High-throughput Sequencing
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Background. Invasive fungal infections (IFI) cause severe symptoms that affect immunocompromised and transplant patient populations. Antifungal therapies vary depending on the pathogenic species, and delays in diagnosis can lead to graft loss and an increase in morbidity and mortality. Therefore, rapid identification of fungi causing IFI is critical for informing antifungal therapy. Such actionable genus/species information can be obtained quickly via Next-generation Sequencing (NGS). In this study, an NGS assay was developed to identify fungal species responsible for IFI, allowing for selection of effective antifungal therapies.

Methods. Internal transcribed spacer (ITS) regions 1 and 2 were used for fungal identification. Primers were taken from published research and/or designed/modifed by assessment in fungal sequence alignments. A DNA sequence database was compiled and a reference-assisted assembly approach utilizing % sequence ID and % coverage was developed for species identification. End-point PCR was conducted on DNA extracted from 19 pathogenic fungal species, and mixed communities (MC) for preliminary sensitivity and inclusivity. Sensitivity was assessed using dilutions of template DNA into the PCR reaction. One hundred individual species were correctly identified with this limited data in both single and MC samples. The lower limit of detection was assessed at 5,000 genomic equivalents/mL of eluate. In MC analyses, combinations of 3, 4, 6, and 10 fungal species resolved 100% of the genera present, but failed to resolve species appropriately with only 2 loci evaluated. Unexpectedly, 3 tested Aspergillus spp. were correctly identified with this limited data in both single and MC samples. The lower limit of detection was assessed at 5,000 genomic equivalents/mL of eluate.

Conclusion. The inclusivity and sensitivity demonstrated here of an NGS approach for identification of etiological agents of IFI support this assay's potential utility as an aid in the treatment of IFI in at-risk patient groups. This assay allows for rapid identification (<4 days) of fungal species to aid clinicians in improving case outcomes.

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