P363
Mycoflora diversity in stored water from selected households in Nairobi

Olga Mathew1, Bridgit Kimani1, Leah Waithera¹, Shelia Okoth2
1Kenya Medical Research Institute (KEMRI) (K40), Nairobi, Kenya
2University of Nairobi, Nairobi, Kenya

Poster session 5, September 23, 2022, 12:30 PM - 1:30 PM

Introduction: Water is a very vital natural resource for all life on earth as it is used widely. Water plays an important role in the world economy; the uses can be categorized into commercial use where it is used in hotels, restaurants, offices, and other commercial activities. Fungi are ubiquitous in nature; they produce spores that are small-sized, able to stay airborne for a long duration, and transported over long distances during air dispersal. These are major sources of allergies and airway irritants that are detrimental to health. Biofilms in water distribution systems and storage containers provide favorable habitat for microorganisms as they accumulate better in solid-liquid interfaces that enable them to be embedded in the gelatinous matrix of extracellular polymers excreted by the microorganisms leading to persistence of microbes from environmental stresses. Fungi hydrophobicity and adaptability have enabled them to assemble and colonize different surfaces in domestic settings. The potential health effects caused by fungi in treated water are not well highlighted and thus the need to carry out this study to investigate the mycoflora isolated from stored water.

Methods: This was a cross-sectional study conducted from 2019-2021, whereby 120 water samples were collected from stored containers in households in Nairobi county Kenya. The fungal strains were plated onto Sabouraud’s dextrose agar (SDA), Potato Dextrose Agar, and Crude soda Agar media with chloramphenicol (0.05 mg/ml) (Oxoid, U.K.). The plates were incubated for 7 days at 25°C. Fungal identification was done by direct microscopy and morphological features.

Results: A total of 102 fungal species were isolated from water samples. Yeasts 68 (67.5%), Rhodoturula 34 (33.6%) accounted for the unicellular fungi. Among filamentous mycoflora, the most common isolated fungi were Aspergillus species 13(12.6%), followed by Aspergillus spp 4 (3.9%) with Mucor and Rhizopus species accounting for 1 (0.9%) respectively. Yeasts species were the most common species isolated from water species and Aspergillus species were more frequently isolated from filamentous fungi.

Conclusion: Yeasts species were the most common species isolated from water species and the roles they play in biofilm needs to be further investigated.

P354
Quantitative monitoring of Aspergillus fumigatus mycelial growth by optical density

Ken Miyazawa1, Takashii Umeyama1, Yasutaka Hoshino1, Shogo Takatsuka1, Yasunori Murase2, Kenitsu Abe2, Yoshihugu Miyazaki3
1National Institute of Infectious Diseases, Tokyo, Japan
2Tokoku University, Sendai, Japan

Poster session 5, September 23, 2022, 12:30 PM - 1:30 PM

Objective: Filamentous fungi generally form hyphal pellets in liquid culture. This property prompts filamentous fungi from applying the growth monitoring methods used for unicellular organisms such as yeast and bacteria. We have analyzed the biological functions of cell wall polysaccharide α-1,3-glucan and extracellular polysaccharide galactomannan-glucan (GAG) in Aspergillus species, and revealed that both α-1,3-glucan and GAG contribute to the pellet formation. Here we constructed the
double disruption mutant of α-1,3-glucan and GAG biosynthesis-related genes (Δags1Δgtb3) in Aspergillus fumigatus AF53 strain, and used the mutant for quantitative monitoring of the mycelial growth by optical density.

Methods: To measure the optical density of conidia and mycelia in shake-flask culture, conidia (final concentration, 1.0 × 10^7/ml) of AF53 or Δags1Δgtb3 strains were inoculated into 50 ml of Aspergillus minimal medium (AMM) medium in a 200-ml Erlenmeyer flask and rotated at 140 rpm at 37°C. At each sampling point, the cultures (2 ml) were withdrawn, and 100 μl of the culture was mixed with 100 μl of 4% paraformaldehyde solution in a 96-well plate. OD₆₀₀ was measured in a microplate reader. To evaluate the susceptibility of the Δags1Δgtb3 strain to antifungal agents, conidia (final concentration, 1.0 × 10^7/ml) were inoculated into 500 μl of RPMI medium containing an antifungal agent (voriconazole, itraconazole, amphotericin B, flucytosine, or micafungin) in a 48-well plate and rotated at 300 rpm using a microplate mixer at 37°C for 15 h, and OD₆₀₀ was measured in triplicate.

Results: In AMM flask cultures, colonies became visible in AF53 from 9 h, and their size increased with time, whereas the Δags1Δgtb3 hyphae were constantly dispersed (Fig. 1a). The OD₆₀₀ measurements of AF53 suggested no correlation between apparent AF53 growth and OD₆₀₀ (Fig. 3b). In the Δags1Δgtb3 strain, the first and third quartiles were 0.633 and 0.995 at 15 h (Fig. 1b), suggesting that the measurement of OD₆₀₀ is suitable for monitoring the growth of the mutant. As an application of the monitoring method, the Δags1Δgtb3 strain was grown in an RPMI medium containing the indicated antifungal agent for 15 h in a 48-well plate (Fig. 2). Growth was repressed in the presence of antifungal agents tested, except for micafungin (Fig. 2). Growth was completely inhibited at 2 μg/ml of voriconazole, 1 μg/ml of itraconazole, 0.5 μg/ml of amphotericin B, and 2.5 μg/ml of flucytosine (Fig. 2), which was in agreement with MICs determined by CLSI M38-A2.

Conclusion: We established a convenient strategy to monitor A. fumigatus hyphal growth. Our method can be directly applied to screening for novel antifungals against Aspergillus species.