Evaluation of purity, viability, stability and biochemical characteristics of clinical fungal isolates preserved by three different methods

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Abstract

Introduction: In era of organ transplants and immunocompromised patients, systemic mycosis has emerged as a major cause of morbidity and mortality among these patients. Research on these clinical fungal isolates is the key to the development of antifungal diagnostics and therapeutic agents. Mycology laboratories has to preserve these pathogenic fungal strains for various experiments. These laboratories requires a preservation method that ensures purity, viability, stability and biochemical characteristics of the fungal isolates during the storage period.

Objectives: To determining the purity, viability, stability and biochemical characteristics of clinical fungal isolates subjected to three different, but simple and inexpensive preservation methods.

Materials and Methods: A total of 50 clinical isolates of yeast and mold were subjected to different preservation **Methods:** All the isolates were evaluated for purity, viability, stability and biochemical characteristics before storage and after 6 months and one year. Stability testing was based on antifungal susceptibility testing as recommended by CLSI.

Results: All the three preservation methods, ensured survival of all the fungal strains for one year duration. Periodic subculture method on agar slope demonstrated mild colony morphological changes in 2 molds and high contamination rate. Water culture technique and oil overlay technique, demonstrated protection of purity, viability, stability and biochemical characteristics of all the fungal isolates up to one year of storage.

Conclusion: Comparison of different fungal storage methods suggested that both water technique and oil overlay technique can be utilized as a simple, effective and inexpensive methods for long term preservation of clinical fungal isolates.

Keywords: Preservation, Storage, Yeast, Molds, CLSI.

Introduction

Fungal infections have become a leading cause of morbidity and mortality in patients with immunocompromised state. [1] Mortality remains high in these patients, despite administration of potent antifungal drugs.[1] Researchers and laboratory studies require a constant supply of fungal isolates.[2] Mycology laboratories are responsible for the diagnosis of fungal infections, as well as conservation of standard strains. These laboratories have to ensure long term preservation of fungal isolates. The preservation method used should be such that the purity, viability, stability and biochemical characteristics of the fungal isolates should be maintained during the preservation period.[3]

Many reviews, highlighting benefit and limitations of these different storage methods are available.[4] The present study was aimed at determining purity, viability, stability and biochemical characteristics of clinical fungal isolates subjected to three different, but feasible preservation methods.

Materials and Methods

This study included, 50 clinical fungal isolates obtained from patients with diverse mycoses. The study sample included both pathogenic yeast (n=30) and mold (n=20) isolates. The list of these fungus isolates used in the study is provided in Table 1.

1. Preservation methods

Periodic subculture on agar slopes: Recommended standard method to subculture fungal isolates on Sabouraud dextrose agar (SDA) was followed.[5] SDA (Himedia) slant in screw cap glass test tubes used and incubated at a room temperature (25–28 ± 1 ^{0}C). Following good cultures growth, these tubes were are stored in a refrigerator at 4-6^{o}C.

Water Culture Technique: In this techniques the clinical fungal isolates, were inoculated into slants of Potato dextrose agar (PDA, Himedia) in screw-cap glass test tubes. These tubes were incubated at 25 °C for 24-48 hrs for yeast isolates and upto 2 weeks for molds isolates. Colonies with adequate sporulation, were processed for long term preservation. Sterile distilled water (2ml) was added onto each culture and scraping of the aerial growth was done (Fig.1). The milky suspension of fungus isolates were removed with the help of the pipette and the suspension was transferred to a screw-cap glass test tubes and stored at 25 °C on laboratory shelves.[6]

Oil Overlay Technique[7]: Fungal isolates were inoculated and grown on PDA screw-cap glass test tube slants and incubated at 25 ° C for 2 weeks. Fungal cultures were covered with sterilized laboratory grade mineral oil, to a depth of 0.5 cm above the top of the slant. The tubes were kept upright on the shelves and stored at room temperature. (Fig. 2)

Morphology and biochemistry properties testing: Morphology and biochemistry properties of the fungal strains were tested by standard procedures.[7,8]

Purity and Viability testing of fungal isolates: The purity and viability of the fungal strains were regularly tested, after 1 month, 6 months and one year of storage. Viability of fungus isolates was determined by transferring small suspension onto SDA and incubation at 25 °C for three weeks. Cultures with no growth after 3 weeks of incubation were retested with repeating subculture. Isolates with no growth after the second subculture, were considered as non-viable.

Stability testing of fungal isolates: The stability of all yeasts and molds isolates werevalidated by antifungal susceptibilities testing by the microbroth dilution method as described by Clinical and Laboratory Standards Institute (CLSI).[9,10] Antifungal powder used were Amphotericin B, fluconazole, and voriconazole (Fig. 3). Schedule for antifungal susceptibilities testing were before storage and after 1 years of preservation.

Results

All 50 fungal strains tested had survived for one year in all the three preservation methods. Morphology testing showed, mild deterioration in the colony morphology of 2 molds. Both these molds isolates were preserved by the periodic subculture method on agar slope. Biochemical characteristics of all the isolates remained unchanged, despite preserving them for one year.

Purity testing revealed contamination in 2 fungus strains preserved by periodic subculture on agar slope. No contamination was noted in water culture technique and oil overlay technique.

All fungus isolates of both yeast and molds, maintained stability after 6 months and one year of storage. MICs all the isolates after storage duration was found to be either the same or within three dilutions of, the MICs before storage. This is as per stability criterion recommended by CLSI.[9,10]

Table 1: Fungal species and number of isolates included in study

Species	Number of isolates
Yeast (n=30)	
Candida albicans	10
Candida tropicalis	6
Candida parapsilosis	6
Candida krusei	4
Candida guilliermondii	4
Molds (n=20)	
Aspergillus flavus	5
Aspergillus fumigatus	5
Aspergillus niger	3
Fusarium incarnatum	2
Rhizopus arrhizus	1
Alternaria spp.	1
Cladophialophora bantiana	1
Exophiala spinifera	1
Scedosporium apiospermum	1

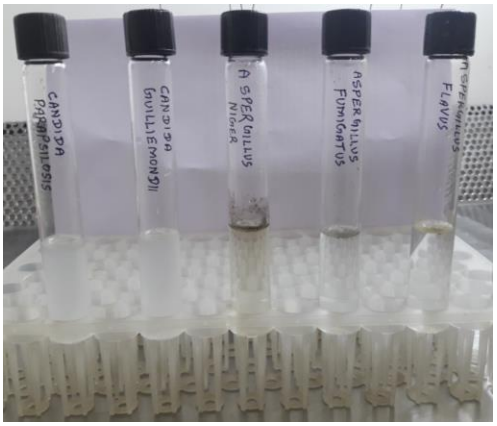

Fig. 1: Water Culture Technique for Preservation

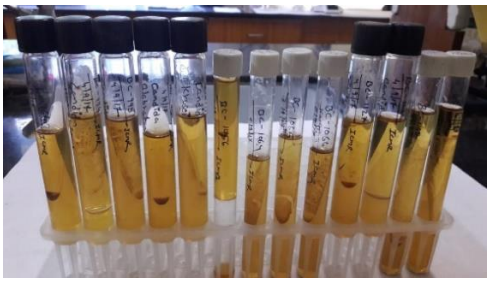

Fig. 2: Oil Overlay Technique for Preservation

Fig. 3: Stability Testing by Antifungal Susceptibility Testing -Broth Microdilution Method

Discussion

The preservation of fungal isolates with unaltered physical and biochemical state is of critical importance in medical mycology laboratory. This study demonstrated, that all three preservation techniques namely periodic subculture on agar slope, water technique and oil overlay technique maintains 100% viability of pathogenic fungus isolates for duration of one year. Similar findings, with gradual decrease in viability after one year has been reported by some researchers.[6] While distilled water and mineral oil overlaytechnique of the preserved cultures had revealed

the least contamination rate compare to periodic subculture on agar slope. This finding is in concordance with other studies utilizing same preservation methods.[11,12]

Pathogenic fungal isolates preserved on agar slope by periodic subculture methods adapts to the new milieu and undergomutational changes. This may be reflected as changes in morphological and biochemical characteristics of the isolates.[2,13] Our study have shown similar morphological change in two mold isolates. Oil overlay technique also utilizes agar but due to low oxygen tension growth and metabolic activities are reduced. As fungal isolates continues to grow slowly due to presence of agar in oil overlay technique, they undergo morphological and biochemical changes in long term preservation.[12,14] Water overlay method, fungal spores are suspended in sterile water and these isolates are subjected to minimal morphological changes. However due to lack of nutrient very long term preservation in water method may not be viable for some species of fungus.[15]

Conclusions

Our study demonstrates that water technique and oil overlay technique are simple, effective and inexpensive methods for the long-term preservation of mostclinical fungal isolates.

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