Original Article

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Quick Response Code:

Website: www.jacmjournal.org

DOI: 10.4103/jacm.jacm_22_17

Comparative evaluation of sensititre yeast one panel against broth microdilution method for testing *Candida auris* and *Candida haemulonii* susceptibility: A pilot study

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Abstract:

BACKGROUND: Candidal infection due to non-*Candida albicans* is on the rise and implicated to cause significant morbidity and mortality. Although empirical treatment can be provided, the sensitivity pattern varies drastically in different species of *Candida*. The conventional broth microdilution method for antifungal susceptibility is labour intensive. Data on antifungal susceptibility testing (AFST) by colorimetric yeast one sensititre method are available for many *Candida* species but not for *Candida auris* and *Candida haemulonii*.

AIM: The utility of colorimetric yeast one plate was compared to conventional broth microdilution method (CLSI M27-A3 guidelines) for five isolates each of *C. haemulonii* and *C. auris* along with CLSI quality control strain *Candida krusei* ATCC 6258.

MATERIALS AND METHODS: A total of 10 isolates of *C. auris*/*haemulonii*, confirmed by sugar assimilation and DNA sequencing were chosen. AFST was performed by conventional broth microdilution and colorimetric yeast one sensititre plates.

STATISTICAL ANALYSIS: The analysis was performed by Carl Pearson's correlation coefficient.

RESULTS: All isolates showed high MIC (>64 μg/mL) for Fluconazole by both methods. Strains of *C. auris* were found to have high MIC (>64 μg/mL) for Amphotericin B by conventional method. The degree of agreement between the two methods was 100%, 96.83% and 90.03% for Fluconazole, Posaconazole and Itraconazole, respectively, for the 10 isolates. The degree of agreement was 100%, 95.70% and 93.83% and 100%, 97.97% and 86.24% for Fluconazole, Posaconazole and Itraconazole for *C. auris* and *C. haemulonii*, respectively.

CONCLUSION: AFST by colorimetric yeast one plate is less time-consuming, easy to perform and interpret, however further studies with a large number of isolates are required to test Echinocandins and Amphotericin B.

Keywords:

Broth microdilution, *Candida auris*, *Candida haemulonii*, colorimetric method, sensititre yeast one

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Introduction

Candida belongs to normal microbiota of human mucosal tract, gastrointestinal tract and vagina.[1] The expanding population of immunocompromised patients that mandates the use of intravenous catheters, total parenteral nutrition, invasive procedures and the increasing use of broad-spectrum antibiotics, cytotoxic chemotherapies and transplantation are factors that contribute to the increase of

How to cite this article: Ramamurthi R, Tupaki-Sreepurna D, Kindo AJ. Comparative evaluation of sensititre yeast one panel against broth microdilution method for testing *Candida auris* and *Candida haemulonii* susceptibility: A pilot study. J Acad Clin Microbiol 2018;20:19-21.

these infections.[2] Although *Candida albicans* is the most prevalent species involved in invasive fungal infections globally, the incidence of infections due to non-Albicans species is increasing. This change in epidemiology could be associated with severe immune suppression or illness, prematurity, exposure to broad-spectrum antibiotics and older patients.[3] *Candida auris* had been first isolated from ear swab in Korea and Japan in 2009.[4] Since then, *C. auris* has been isolated from blood samples as well.[5] Furthermore, both *C. auris* and *Candida haemulonii* species are found to exhibit Fluconazole resistance.[6] This poses a serious problem in treating the patients infected with them. Hence, it is clearly evident that speciation of *Candida* is necessary as each species has a specific sensitivity pattern. The antifungal susceptibility testing (AFST) for yeast by conventional broth microdilution method as recommended by CLSI M 27-A3 is labour intensive.[7] YeastOne sensititre plate is commercially available to measure antifungal susceptibility. It is easy to perform and interpret. Various studies have been carried out comparing the susceptibility pattern obtained for various common *Candida* species by both the methods.[8-10] However, there are no studies to compare the susceptibility pattern for *C. auris* and *C. haemulonii*. Hence, this study has been undertaken to compare the antifungal susceptibility pattern for *C. auris* and *C. haemulonii* by both conventional broth dilution method and sensititre plate.

Materials and Methods

Study design

The study was designed to compare MICs obtained by theYeastOne plate with those obtained by the CLSI reference M27-A3 broth microdilution method. MIC readings were taken after 24 h of incubation. Colorimetric MICs for each isolate-drug combination were compared to M27-A3 MICs. In addition, AFST was performed for ATCC quality control (QC) strain *Candida krusei* ATCC 6258.

Clinical isolates

A total of 10 isolates from the culture collection of Sri Ramachandra University were included. The isolates were recovered from blood samples. These strains were speciated based on sugar assimilation and gene sequencing. Five *C. auris* and five *C. haemulonii* along with *Candida krusei* ATCC 6258 were tested. All isolates were evaluated by the two procedures.

Antifungal agents

The YeastOne plates containing serial drug dilutions of Caspofungin (Merck Research Laboratories, Rahway, N. J.), posaconazole (Schering-Plough Research Institute, Kenilworth, N. J.), Voriconazole (Pfizer Pharmaceuticals, New York, USA) and the established agents amphotericin B (Bristol-Myers Squibb Pharmaceutical Research Institute) and Fluconazole (Pfizer Pharmaceuticals), were provided by TREK diagnostics, USA. Drug dilutions of Amphotericin B, Caspofungin, and the new triazoles ranged from 16 to 0.008 μg/ml, and fluconazole concentrations ranged from 128to 0.12 μg/ml inYeastOne plates. YeastOne plates, which are individually packed in foil and silica gel desiccant, were stored at ambient temperature until testing. The reference microdilution plates were prepared following the CLSI M27-A3 guidelines.

Inoculum preparation

Stock inoculum suspensions of the yeasts were obtained from 24 h cultures on Sabouraud's dextrose agar (HiMedia Laboratories, India) at 35°C. The turbidity of each yeast suspension was adjusted to 0.5 McFarland standards.

Sensititre YeastOne colorimetric antifungal plate procedure: on the day of the test, the dried YeastOne plates were rehydrated with the working yeast suspension (approximately 1.5×10^3 colony-forming unit/ml) by dispensing 100 μl into each well. The YeastOne plates were sealed and incubated at 35°C in a non-CO_2 incubator and were read after 24 h of incubation. Colorimetric MICs were interpreted as the lowest concentration of antifungal solutions that remained blue or unchanged (indicating no growth). QC isolate was tested in the same manner.

Standard broth microdilution method: performed as per CLSI M 27-A3 guidelines. RPMI 1640 medium with L glutamine, without sodium bicarbonate, adjusted to pH 7.0 using MOPS buffer was used. The inoculum was adjusted to 0.5 McFarland turbidity standard and was diluted 1:500 times in RPMI 1640 medium to obtain the final inoculum suspension. A volume of 100 μL of inoculum was to 100 μL of serial dilution of drug concentrations in dimethyl sulfoxide and incubated at 37°C. The concentrations of the drug were similar to Yeastone sensititre plate.

Data analysis

The degree of agreement between the two methods was calculated using correlation coefficient which was calculated using Carl Pearson's formula.

Results

All the strains of *C. auris* and *C. haemulonii* were found to be resistant to Fluconazole (MIC >64 μg/mL) by both the methods. Strains of *C. auris* were found to have a high MIC (>64 μg/mL) for Amphotericin B by conventional broth microdilution method but not by colorimetric method.

Discussion

The development of reproducible reference method for performing antifungal susceptibility for various *Candida* species has been under development. Determination of endpoint differs for different antifungal agents due to varied mechanism of actions. Fungistatic groups have a trailing phenomenon making interpretation of results even more difficult. Hence, determination of endpoint by colorimetric method using alamar blue as redox indicator has been studied for various *Candida* species. The colorimetric method has been shown to have 85%–99% degree of agreement, (by Carl Pearson's coefficient) least being for Ketoconazole and maximum for Amphotericin B. Among various species of *Candida*, *Candida tropicalis* was found to have a low degree of agreement.

In this study, all isolates showed high MIC (>64 μg/mL) for Fluconazole by both the methods. Strains of *C. auris* were found to have a high MIC (>64 μg/mL) for Amphotericin B by conventional method Degrees of agreement between two methods were 100%, 96.83% and 90.03% for Fluconazole, Posaconazole and Itraconazole, respectively. The degree of the agreement was 100%, 95.70%, 93.83% and 100%, 97.97%, 86.24% for Fluconazole, Posaconazole and Itraconazole for *C. auris* and *C. haemulonii* respectively. Considering ±2 log difference as within the range, the degree of agreement increased by 19% for itraconazole, 16% for Voriconazole and 3% for Posaconazole. Except for Amphotericin B, the MIC by calorimetric method was two dilutions lesser than that obtained by the conventional method. The sensititre YeastOne method investigated in this study appears to be reliable and reproducible against the gold standard except for Amphotericin B for *C. auris*. However, since only five isolates of *C. auris* were tested, further studies are required to conclude on susceptibility to Amphotericin B by colorimetric method.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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