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IP International Journal of Medical Microbiology and Tropical Diseases

Journal homepage: https://www.ijmmtd.org/

Original Research Article

Recent trends in the Susceptibility pattern of *Candida* to Fluconazole and Amphotericin B at a tertiary care center in South India

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ARTICLE INFO

Article history:
Received 21-03-2024
Accepted 01-04-2024
Available online 17-04-2024

Keywords:
Vitek 2
Candida
Disc diffusion
Microbroth dilution
AST

ABSTRACT

Aims and Objectives: To determine the fluconazole and Amphotericin B susceptibility pattern among *candida* isolates by disk diffusion, Vitek-2 method, and micro-broth dilution methods (MBD).

Introduction: Fungal infections are now becoming more prevalent than bacterial in causing opportunistic and nosocomial infections. *Candida species* are among the most common invasive fungi that are seen in most patients with predisposing co-morbidities such as diabetes and hypertension. Therefore; identification of the species and antifungal susceptibility testing is essential for proper patient management as various species respond differently to antifungals and for the prevention of emergence of drug resistance.

Materials and Methods: *Candida species* isolated from different specimens were included in the study, speciation of the isolates was achieved by Vitek 2 automated machine and also with corn meal agar inoculation (CMA). The comparison of antifungal susceptibility testing was done by three different methods which included Vitek 2 systems, disk diffusion, and Micro-broth dilution methods. The antifungal susceptibility was tested for fluconazole and Amphotericin B.

Results: A total of 50 *Candida* isolates were randomly selected and speciated. Species-wise distribution showed *C. tropicalis* to be the most common one accounting for 34 (69%) followed by *C. albicans* 12(24%) and 4 (12%) of them were *C. parapsilosis*. The overall resistance among these isolates was as follows: by micro –broth dilution fluconazole resistance to *Candida species* was 6% and to Amphotericin B was 8%. By disc diffusion, fluconazole resistance was 10%, Amphotericin B was 14%. With the Vitek 2 system, fluconazole and Amphotericin B showed resistance of 10% and 6% respectively.

Conclusion: Speciation and Antifungal susceptibility testing of *Candida i*solates are of great significance regardless of the specimen isolated from, which helps in the management and knowing the epidemiology of the susceptibility pattern of the *Candida species*. For this to become a reality a reliable, easy, and sensitive method of antifungal susceptibility needs to be used in every Microbiology laboratory as a routine practice.

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1. Introduction

Candidaspecies are the most common fungal pathogens of humankind, causing diseases starting from superficial mucosal infections to candidemia, systemic infections that are a threat to life. Non-*Candidaalbicans species* are now emerging as major pathogens and dominating *C. albicans* in causing human infections.[1] Furthermore, the pathogenesis and prognosis of *Candida* infections are greatly influenced by the host's immune status and also depends on the disease presentations. *Candidaspecies* have now become the most common cause of opportunistic and nosocomial infection

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https://doi.org/10.18231/j.ijmmtd.2024.011
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with increased drug resistance.[2,3] In addition, non-*albicans Candida species* have now become the cause of most of the infections of candidiasis therefore, identification up to the species level is very significant among *Candida* infection.[4]

With the increase in incidence of drug resistance to fluconazole by *Candida species*, it is very important to direct the treatment of *Candida* infections based on antifungal susceptibility pattern. Antifungal susceptibility testing for *Candida* was implemented by the two institutions - Clinical and Laboratory Standards Institute (CLSI) and the European Committee for Antimicrobial Susceptibility Testing (EUCAST). The reference methods include micro & macro broth dilution methods and disk diffusion methods for both yeasts and molds Most of the Clinical microbiology laboratories rely on these methods of antifungal susceptibility testing methods to select the agent of choice for guiding treatment for fungal infections, and to know the local and the global epidemiology of antifungal resistance. The proposed broth macro-dilution method is complex and impractical for use in routine laboratory settings. These reference techniques are time-consuming and more adapted for reference laboratories and large epidemiological surveillance studies. Micro-broth dilution method being the gold standard technique, Disk diffusion susceptibility testing is mostly used for antifungal susceptibility and is also a better alternative for antifungal susceptibility in routine clinical microbiology laboratories. Therefore; the current study was aimed at evaluating the different methods for antifungal susceptibility testing among clinical isolates of *Candida*.[5]

2. Materials and Methods

This study was conducted in the Microbiology department for a period of one year from January 2020 to December 2020 after getting approval from the institutional ethical committee (JSS/MC/PG/6929/2019-20) and informed consent was taken from patients. A total of 50 *Candida* isolates were randomly selected and subjected to the Vitek-2 Compact system for species identification and antifungal susceptibility testing. Speciation of *Candida* isolates was again confirmed by studying the morphology on corn meal agar (CMA). The same isolates were also tested for antifungal susceptibility by microbroth dilution and disk diffusion methods against two antifungals: fluconazole 25μg, and Amphotericin B 100 UI.

The disk diffusion method was performed on Muller-Hinton agar supplemented with 2% glucose and 0.5 methylene blue dye as per CLSI guidelines using manually prepared fluconazole 25μg discs and Amphotericin 100UI discs commercially procured from Himedia. MBD was performed using microtiter plates and DMSO was used for dissolving amphotericin B, RPMI as media as per CLSI guidelines, density was read using spectrophotometer. ATCC 90028 *C. albicans* was used as a quality control strain. The antifungal susceptibility of the isolates was interpreted as per CLSI guidelines.

3. Results

Among 50 Candida infected patients, males were dominating with 70% (35/50) compared to females at 30% (15/50). In the study, according to age-wise distribution of the patients, we found that the patient age group between 51-60 years were infected more with *Candida* (28%, 14/50) followed by the 61-70 age group (26%, 13/50). Age groups of 41-50 years and 71-80 years were the next common group to be involved with 12%.

The Clinical diagnosis among these 50 patients included diabetes, which accounted for 42% (21/50). Pneumonia was the second most common diagnosis encountered in these 50 patients accounting for 32 % (16/50), of which 8 % (4/50) had pneumonia due to COVID-19. Hypertension was the diagnosis in 15/50 (30%). In addition to this, we had cases of urinary tract infections (UTIs) 26% (13/50), nephritis14% (7/50), acute kidney injury patients 10% (5/50) and patients with urinary obstruction in 4% (2/50). In total, kidney-related infections accounted for about 27/50 (54%) of all the patients. Other diagnoses among these patients were hypothyroidism, hyperthyroidism, and ischemic heart disease as seen in Table 1.

Table 1: linical diagnosis among the patients having *Candida* infection

Clinical predisposing factors	Frequency	Percentage (%)
Type 2 Diabetes mellitus	21	42
Hypertension	15	30
Pneumonia	16	32
Urinary tract infection	13	26
Hypothyroidism	3	6
Hyperthyroidism	1	2
Ischemic heart disease	5	10
Urinary obstruction	2	4
Nephritis	7	14
Acute kidney infection	5	10

The various specimens were sent from the above patients for culture, which altogether yielded 50 *candida* isolates. The maximum isolates were obtained from a urine sample yielding 39/50 (78%). endotracheal aspirates grew 6/50 (12%) isolates, ear swabs grew 2/50 (4%) isolates, one isolate in pus (from a diabetic foot patient), blood and skin scrapings respectively.

These 50 *Candida* isolates selected were subjected to Vitek- 2 Compact and corn meal agar for identification. Of them, 34/50 (68%) were identified to be *C. tropicalis*, 12/50 (24%) as *C. albicans*, and 4/50 (08%) were found to be *C. parapsilosis* by Vitek which were confirmed by corn meal agar inoculation as depicted in theTable 2.

Table 2: Species-wise distribution of *Candida* isolates

Isolates	Number of isolates (n=50)	Percentages
C. albicans	12	24%
C. tropicalis	34	68%
C. parapsilosis	04	08%

All *Candida* isolates were subjected to antifungal susceptibility testing with the two antifungals, fluconazole and amphotericin B by three methods Vitek 2 system, disk diffusion, and microbroth dilution. (Table 2)

By disk diffusion method, 10% (5/50) of *Candida* isolates were resistant to fluconazole, 14% to Amphotericin B. Species wise resistance showed 1 *C. albicans* to be resistant to fluconazole 1/12 (8%), 3 *C. albicans* (3/12 (25%) were resistant to amphotericin B. Among *C. tropicalis*, 4/34 (11.8%) were resistant to fluconazole, and 2/34 (5.9%) demonstrated resistance to amphotericin B in this method. In *C. parapsilosis*, there was no resistance showed by this species against fluconazole, however; 2/4 (50%) isolates shown resistance to amphotericin B. (Figure 1)

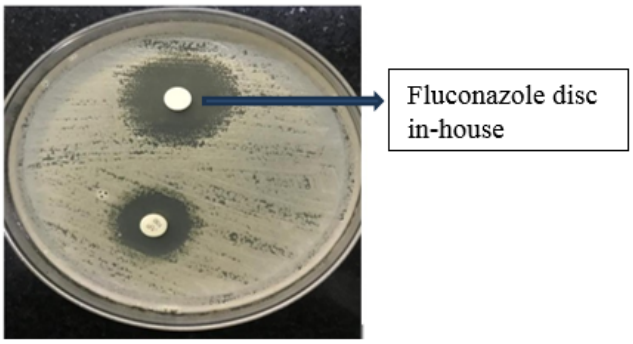

Figure 1: Disk diffusion plate showing susceptibility to fluconazole and amphotericin B

In the micro-broth dilution method, *C. albicans* showed no resistance against fluconazole as shown in (Figure 2) but one isolate displayed susceptible dose-dependent to fluconazole and 6/12 (50%) resistant to amphotericin B. 3/34 (8.8%) of *C. tropicalis* were resistance to fluconazole, 4/34 (11.8%) to amphotericin B. 3 isolates exhibited SDD against fluconazole 3/34(8.8%) for this species. For *C. parapsilosis* there was no resistance recorded for fluconazole and amphotericin B.

MIC detection by VITEK 2 system – By this method, 1 *C. albicans* was resistant to fluconazole and amphotericin B (8.3%). *C. tropicalis* showed a resistance of 4/34 (11.8%) against fluconazole and 2/34 (5.9%) against Amphotericin B. There was no resistance reported for *C. parapsilosis* against fluconazole and Amphotericin B by this method.

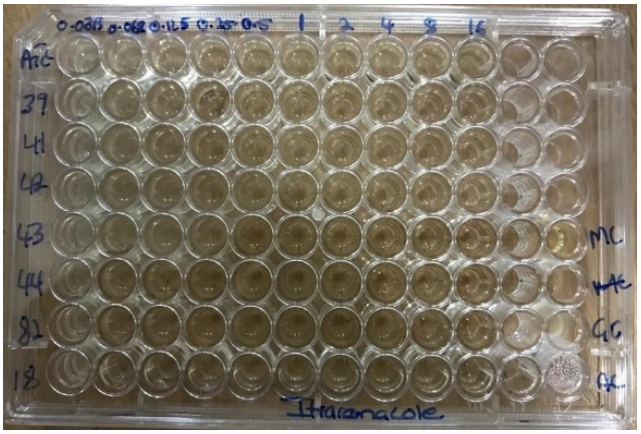

Figure 2: Micro broth dilution method for fluconazole

The results of different methods for susceptibility against is shown in Figure 3. Although the microbroth dilution method is a standard reference, it is tedious and time-consuming, and cannot be performed daily, whereas, Vitek -2 is a convenient MIC-based automated method that can be used for the determination of the susceptibility pattern where the facility is available.

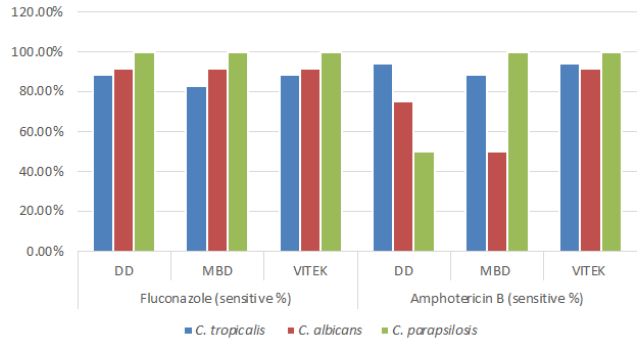

Figure 3: Comparative table to show susceptibility results of all the 3 methods to fluconazole and Amphotericin B (DD- Disk diffusion, MBD- Micro broth dilution)

4. Discussion

Candida **,** an opportunistic pathogen can lead to mucosal candidiasis - which includes oral thrush, oropharyngeal candidiasis, pseudo-membranous candidiasis, vulvovaginal candidiasis, cutaneous candidiasis such as candidial folliculitis and systemic candidiasis. Optimum and repeated sample collection are a must to prove their role as pathogens at many sites.[1,6]

Due to increased incidences of candidiasis and the repeated uses of antifungal agents, there is an increased need for effective and constant antifungal susceptibility testing methods in all clinical microbiology laboratories, which will help in monitoring the susceptibility of fungus to specific

anti-fungal drugs and help in choosing the right antifungal drug for optimum treatment of specific fungal disease.[7]

A total of 50 *Candida* isolates that were identified by the Vitek 2 method were part of this study. Among the total patients, 70% were males and 30% females, so this was probably due to catheterization which was more seen in male patients. This is similar to the study conducted by Shukla R et al in which 67% of males had *Candida* infection and 33% of females had candidiasis.[1] The age group with the most *Candida* infections were the patients >50 years age group which is similar to the study by Teeba Hashim M et al.[8]

The most common predisposing factor in many of the patients was Diabetes mellitus accounting for 38% followed by hypertension which in most cases was associated with diabetic mellitus; this is in agreement with the study conducted by Shukla et al and Teeba Hashim M et al.[1,8] Hypertension was a comorbid condition in 30% of the cases, this is correlating to the study by Shukla R et al who also found hypertension as a predisposing factor for *Candida* infection.[1] About 24% and 10% of the patients had broncho-pneumonia and ischemic heart diseases (IHD) respectively. Among those patients with bronchopneumonia, 8% of patients had COVID-19 and the rest had bacterial infection. The study by Gen Song et al revealed that 4% of the total patients had *Candida* infection as a co-infection due to COVID-19.[9]

In this study, it was also observed that 6% of the patients had hypothyroidism and 2% of them were diagnosed to have hyperthyroidism. Macura et al observed more frequent and severe onychomycoses with *C. albicans* infection in patients with hypothyroidism and hyperthyroidism.[10]

In the current study, *Candida species* isolated were *C. tropicalis* 34 (68%) *C. albicans* 12(24%) and *C. parapsilosis* 4 (08%) from various specimens, this has the same pattern as the studies conducted elsewhere.[11,12] The resistance pattern for the *Candida species* shows that *C. tropicalis* isolates showed 12% resistance to fluconazole and 6% to amphotericin B. The study by Urvashi et al showed that there was 59% and 9.4% resistance for *C. tropicalis* against fluconazole and amphotericin B which is slightly higher than our study.[13]

C. tropicalis showed 88.2% susceptibility for fluconazole by disk diffusion method, 82.4% by microbroth dilution (MBD), and 88.2% by VITEK-2. In comparison with the study by El-Ganiny AM, et al (87.9%) (7) also documented similar results to fluconazole by disk diffusion method. *C. tropicalis* showed 88.2% susceptibility against amphotericin B by microbroth dilution. In contrast, the study by Gamze Alçi et al 97.7% (6) susceptibility for amphotericin B.

C. albicans displayed 8.3% resistance to fluconazole, and 25% resistance to amphotericin B. However; the study conducted by El-Ganiny AM et al showed a resistance rate of 10% against fluconazole and found susceptibility of 90% against fluconazole among *C. albicans*. (7) *C. albicans* showed a sensitivity of 75% by disc diffusion, 50% by microbroth dilution, and 91.7% by VITEK 2 method to Amphotericin B. However, the study conducted by Gamze Alci et al shows a sensitivity of 95% for *C. albicans* by micro broth dilution method. (6) This indicates that a standard MIC-based method should be used for testing results of which have to be correlated with clinical scenarios.

C. parapsilosis displayed no resistance to fluconazole, however there was 50% resistance to Amphotericin B by disk diffusion. There was no resistant pattern for *C. parapsilosis* and this goes hand in hand with the other study conducted by Mirshekar et al.[3]

5. Conclusion

Our study has shown that the non-*albicansCandida species* are on the rise and due to the frequent use of many of the antifungal drugs, resistance to these drugs is also emerging. This is giving us clear reasons to always speciate and test every species for its antifungal susceptibility pattern for better patient management. In addition to fluconazole, amphotericin resistance is also on the rise. C. *albicans* showed increased fluconazole resistance compared to *C. tropicalis* and *C. parapsilosis*. Susceptibility results were almost similar by all three methods to fluconazole while *C. albicans* showed varied susceptibility results to amphotericin B by different methods necessitating the use of standard MIC-based resistance detection test and clinical correlation with patient condition.

6. Source of Funding

None.

7. Conflict of Interest

None.

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Cite this article: Sem JN, Morubagal RR, Shivappa SG, Gowda RS, Mahale RP, Urs TA, Chitharagi VB. Recent trends in the Susceptibility pattern of *Candida* to Fluconazole and Amphotericin B at a tertiary care center in South India. *IP Int J Med Microbiol Trop Dis* 2024;10(1):62-66.