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Original Research Article

Antifungal susceptibility of clinically significant candida species by disk diffusion method

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ABSTRACT

Context: Antifungal susceptibility of candida.**Aims:** To perform antifungal susceptibility testing on candida isolates by disk diffusion method & study its susceptibility pattern.**Settings and Design:** The present study was conducted in the department of Microbiology in a tertiary care hospital in Hyderabad from January 2013 to June 2014, with prior approval of the Institutional Ethics Committee. The present study was designed to perform antifungal susceptibility test on Candida isolates by Disk Diffusion Method and study its susceptibility pattern.**Materials and Methods:** 102 Candida isolates were subjected to Antifungal susceptibility testing by Disk diffusion method using Mueller-Hinton Agar + 2% Glucose and 0.5 µg/mL Methylene Blue Dye (GMB) Mediums per CLSI guidelines.**Statistical analysis used:****Results:** Antifungal susceptibility test shows that *C. albicans* is more susceptible to all the antifungal agents tested. Resistance to azole group of drugs was more pronounced in non-albicans candida spp. Voriconazole seemed to be superior to Fluconazole with a better susceptibility in the Fluconazole resistant strains also.**Conclusions:** Findings of the antifungal susceptibility test suggest that Candida spp., differ in their susceptibility to antifungal agents. Antifungal susceptibility testing of Candida isolates will be helpful in guiding physicians to select the appropriate antifungal drug so that therapeutic failures can be avoided thus decreasing patient morbidity and mortality.© This is an open access article distributed under the terms of the Creative Commons Attribution License (<https://creativecommons.org/licenses/by/4.0/>) which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

1. Introduction

The Candida species are ubiquitous yeasts which are a part of normal flora of the alimentary tract of mammals and the mucocutaneous membranes of humans.¹ It becomes an opportunistic pathogen as a result of one or more underlying pre-disposing factors.² Until recently Candida albicans was the most common yeast isolated from infections. Epidemiologic data from the past decade reveal a paradigm shift in Candida infections with non -albicans Candida species such as *C.glabrata*, *C.tropicalis*, and *C.krusei* as emerging important pathogens.³⁻⁶ This transition has had a significant clinical impact due to decreased susceptibility

of these non -albicans yeasts to antifungal agents.⁵ Candida albicans and non-albicans species are closely related but differ from each other with respect to epidemiology, virulence characteristics, and fungal susceptibility. The Combined effects of increasing number of fungal infections, growing number of patients at risk, the increasing rate of fungal resistance, and the expanded antifungal armamentarium have led to an increased recognition of the need for standardized laboratory testing for antifungal drug susceptibility.⁷ Agar-based susceptibility testing methods have been focus of interest for many researchers, due to their simplicity, reproducibility and lack of requirements for specialized equipment.⁸ Various Indian workers⁹⁻¹¹ used Mueller Hinton Agar supplemented with 2% Glucose and 0.5µg/ml Methylene Blue Dye for Antifungal Susceptibility

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testing by Disk Diffusion Method and reported an increased resistance of non-albicans *Candida* isolates, to Azole group of drugs. The present study was designed to perform antifungal susceptibility test on *Candida* isolates by Disk Diffusion Method and study its susceptibility pattern.

2. Materials and Methods

The present study was conducted in the department of Microbiology in a tertiary care hospital in Hyderabad from January 2013 to June 2014, with prior approval of the Institutional Ethics Committee. A total of 102 *Candida* isolates from different clinical samples were processed for species identification. *Candida albicans* was the major species isolated followed by *C. tropicalis*, *C. parapsilosis*, *C. krusei*, *C. glabrata* and *C. dubliniensis*. All the speciated *Candida* isolates were subjected to Antifungal susceptibility testing by disk diffusion method using Mueller-Hinton Agar + 2% Glucose and 0.5 µg/mL Methylene Blue Dye (GMB) Medium as per CLSI guidelines.¹² Antifungal disc used: Amphotericin – B (100U), Clotrimazole (10µg), Fluconazole (25µg) and Voriconazole (1µg). 0.5 McFarland standards was used to standardize the inoculum density. *C. albicans* ATCC 90028 and *C. parapsilosis* ATCC 22019 were used as Controls. All the culture media, Antifungal disc, and control strains were obtained from Himedia Laboratories, Mumbai.

3. Results

The antifungal susceptibility pattern of *Candida* species is shown in the following table.

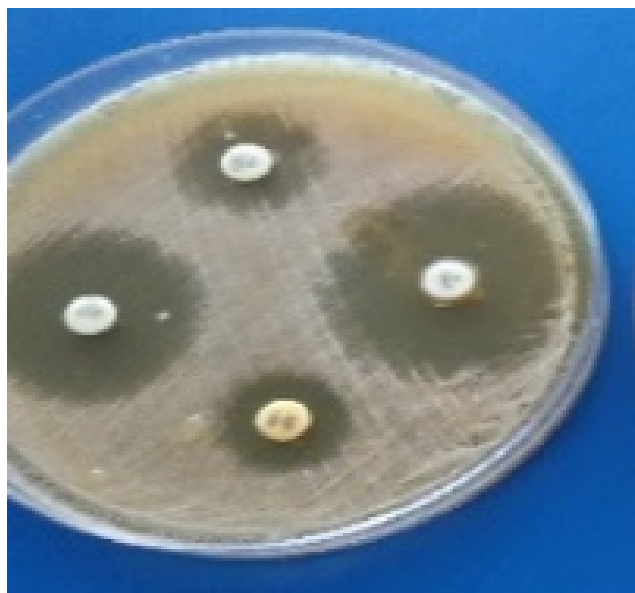


Fig. 1: Antifungal susceptibility pattern of *Candida* isolates on Mueller- Hinton Agar + GMB

4. Discussion

Candida species differ in their susceptibility to antifungal agents. On comparison of susceptibility pattern of *C. albicans* and non-albicans *Candida* species it was observed that *C. albicans* is more susceptible to all the antifungal agents tested. In the present study all the *Candida albicans* showed 100% susceptibility to Amphotericin-B. Similar findings were reported by other studies.^{13,14} It was observed that *C. albicans* showed high fluconazole susceptibility rate (95.83%) in the present study, which is consistent with other reports by Akortha et al.,¹⁵ Amar et al.,¹⁶ & Pahwa et al.,¹⁷ who also observed high Fluconazole susceptibility rates of 95.7%, 92.53% and 99% respectively. 92.53% of *C. albicans* were susceptible to Clotrimazole in a study by Amar et al.,¹⁶ which can be compared with the present study (93.7%). Pahwa et al.,¹⁷ reported 99% of *C. albicans* as susceptible to Voriconazole, which can be compared with 97.91% in present study.

Among non albicans *Candida* spp., susceptibility to Amphotericin-B and Voriconazole varied between 83.33% to 100 %, Clotrimazole between 50% to 87.5% and Fluconazole between 25% to 100%. Non-albicans *Candida* showed higher resistance to all the drugs than *C. albicans*. Maximum resistance was shown by *C. krusei* (100%) and *C. glabrata* (75%) in the present study. Findings from the present study indicate that all the isolates of *C. krusei* were resistant to Fluconazole. Similar findings were shown by Hamza et al.¹⁸ Non- albicans *Candida* has shown an increased resistance to azoles as in other studies.^{19,20} Azole resistance candidiasis appears to be on the rise. The resistance to Fluconazole is of great concern because, it is most commonly used Azole for superficial as well as deep-seated candidiasis. It was observed that out of the total 19 isolates which showed resistance to Fluconazole, 14 isolates were sensitive to Voriconazole. Voriconazole seemed to be superior to Fluconazole with a better susceptibility in the Fluconazole resistant strains also. This may be due to the more effective binding of Voriconazole to Cytochrome P450 isoenzyme of *Candida* species.

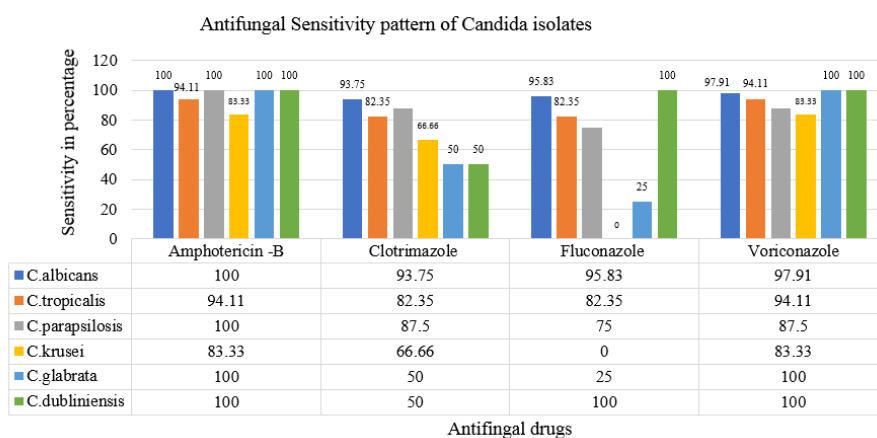
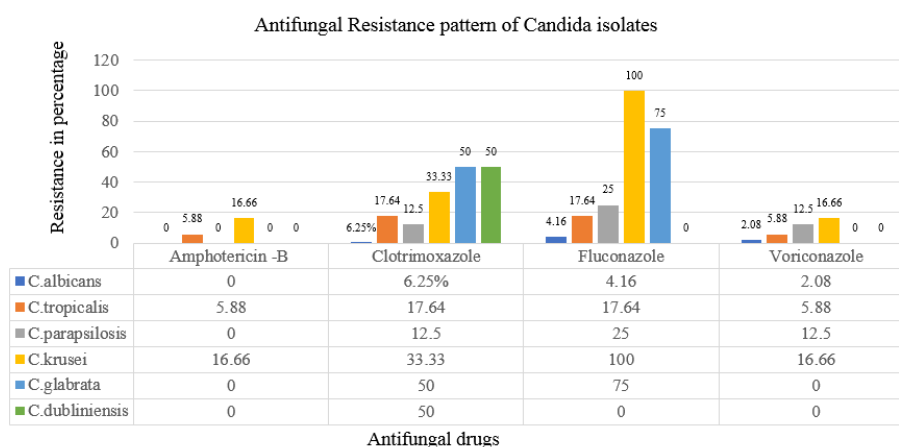
It was observed that infections with non-albicans *Candida* species (NAC) have shown decreased susceptibility to azole group of drugs.

5. Conclusion

Findings of the antifungal susceptibility test suggest that *Candida* spp., differ in their susceptibility to antifungal agents. On comparison of susceptibility pattern of *C. albicans* and non-albicans *Candida* species it was observed that non-albicans *Candida* showed higher resistance to all the drugs than *C. albicans*. Resistance to azole group of drugs was more pronounced in non-albicans *Candida* spp. Antifungal susceptibility testing of *Candida* isolates will be helpful in guiding physicians to select the appropriate

Table 1: Antifungal susceptibility pattern of *Candida* species.

Candida spp.	Amphotericin -B		Clotrimazole		Fluconazole		Voriconazole	
	S	R	S	R	S	R	S	R
<i>C.albicans</i> (n =48)	48(100%)	-	45(93.75%)	3(6.25%)	46(95.83%)	2(4.16%)	47(97.91%)	1(2.08%)
<i>C.tropicalis</i> (n =34)	32(94.11%)	2(5.88%)	28(82.35%)	6(17.64%)	28(82.35%)	6(17.64%)	32(94.11%)	2(5.88%)
<i>C.parapsilosis</i> (n =8)	8(100%)	-	7(87.5%)	1(12.5%)	6(75%)	2(25%)	7(87.5%)	1(12.5%)
<i>C.krusei</i> (n =6)	5(83.33%)	1(16.66%)	4(66.66%)	2(33.33%)	0	6(100%)	5(83.33%)	1(16.66%)
<i>C.glabrata</i> (n =4)	4(100%)	-	2(50%)	2(50%)	1(25%)	3(75%)	4(100%)	-
<i>C.dubliniensis</i> (n =2)	2(100%)	0	1(50%)	1(50%)	2(100%)	0	2(100%)	0

**Fig. 2:** Antifungal sensitivity pattern of *Candida* isolates**Fig. 3:** Antifungal Resistance pattern of *Candida* isolates

antifungal drug so that therapeutic failures can be avoided thus decreasing patient morbidity and mortality.

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7. Conflicts of Interest

All contributing authors declare no conflicts of interest.

8. Source of Funding

None.

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