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Identification and Antifungal Susceptibility of Candida Species Isolated from Various Clinical Samples at a Tertiary Care Hospital

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A B S T R A C T

Candida species especially Non albicans *Candida* are emerging as significant pathogens. Species level identification of *Candida*is essential as certain species are inherently resistant to certain antifungal agents. The present study was done to evaluate the antifungal susceptibility pattern of *Candida* species by Disc diffusion method which is helpful for guiding therapy. All clinical samples sent to the Microbiology laboratory were inoculated on to Blood agar and MacConkey agar. The suspected colonies of *Candida* were identified by Gram stain and inoculated onto CHROM *Candida* differential agar for speciation. Antifungal susceptibility testing was done by disc diffusion method. *C. albicans* (48.7%) was the commonest species isolated followed by *C. tropicalis* (21%), *C. glabrata* (18.4%) and *C. krusei* (11.9%) respectively. All the isolates were sensitive to Amphotericin B. The overall resistance of *Candida* species to Fluconazole and Voriconazole is 21.1% and 11.9% respectively. Rapid identification of *Candida* isolates up to species level with antifungal susceptibility pattern is important for the early management of *Candida* infections.

Introduction

Candida species are the major human fungal pathogens that cause both mucosal and deep tissue infections (Sardi JCO *et al.,* 2013). They are capable of causing infections in both immunocompetent individuals and immunocompromised hosts (DeorukhkarSC *et al.*, 2014). *Candida albicans* is the most common cause of candidiasis accounting for about 60-80% of infections. An increase in prevalence of Nonalbicans *Candida* (NAC) species has been noted during last decade (Kanna BV *et al.,* 2017). The accurate species identification of *Candida* is important for the treatment, as not all species respond to the same treatment because of the problem of anti-fungal resistance (Patel LR *et al.,* 2012).

Amphotericin B has been used for the treatment for invasive Candidiasis, but cost and dose related side effects limit its use. Azole group of drugs are commonly used in treating many forms of *Candida* infections for a long time. However, their prolonged use has led to the development of drug resistance in *C. albicans* and other species (Mondal S *et al.,* 2013). The goal of performing anti-fungal susceptibility testing is to produce actionable data for the treating clinician on the

susceptibility, intermediate (or dose-dependent) susceptibility or resistance phenotype of an organism (SanguinettiM *et al.,* 2018). The disk diffusion method is easy to perform in a clinical laboratory, the materials required are more cost-effective (JabeenK *et al.,* 2016).Thus the present study was undertaken to know the antifungal susceptibility pattern of *C. albicans* and other *Candida* species in our setup.

Materials and Methods

This cross-sectional study was done in the Department of Microbiology of a rural tertiary care hospital. Periodic sampling was done for a period of 12 months from April 2019 to March 2020. The study includes *Candida* isolates from various clinical samples sent routinely to the Microbiology laboratory. Patients who are on any form of antifungal therapy 6 weeks prior to sample collection were excluded from the study.

All the clinical samples were inoculated onto Blood agar and MacConkey agar and incubated aerobically at 37^0C for 24-48 hours. The suspected colonies of *Candida* were identified by Gram stain and subjected to germ tube test and inoculated onto CHROM *Candida* differential agar.

Germ tube test is done to differentiate *C.albicans* and *C.dublinenses* from other *Candida* species. Small inoculums of *Candida* obtained from an isolated colony is suspended in 0.5ml of serum and is incubated at 37°C for not more than 3 hours. A drop of this suspension is placed on a slide, coverslip was placed and examined under microscope for the presence of germ tubes (Forbes BA *et al.,* 2007).

Further speciation of the *Candida* isolates was done by culturing it on CHROM *Candida* differential agar. CHROM agar was prepared as per the manufacturer's instructions and incubated at 30^0c for 24-48 hours. Species identification was done by the morphology and color of the colony (Figure 1). The isolates that remained doubtful in their appearance on CHROM agar was considered as unidentified and excluded from the study

Antifungal susceptibility testing by disc diffusion method

The media and antifungal discs used in the testing were from HiMedia Laboratories, Mumbai. Fungal susceptibility toroutinely used drugs like Amphotericin B (100 units), Fluconazole(25 μg) and Voriconazole (1 μg) was done by the disc diffusion method using Mueller-Hinton agar which is supplemented with 2% glucose and 0.5 µg/ml methylene blue. Incorporation of methylene blue in the medium has been found to improve the *Candida* growth and provide sharp zones of inhibition for the azole group of drugs.

The *Candida* colonies were mixed in 0.85% normal saline (5ml) and turbidity was adjusted to 0.5 McFarland standard. A sterile swab was used to inoculate the plate by making lawn culture and rotating the plate in three directions as used for testing antibacterial agents. Antifungal discs were placed on the inoculated plates and incubated at 37°C for 24–48 hours. The diameter of the zone of inhibition was measured and results were interpreted as per CLSI (Clinical Laboratory Standards Institute) guidelines (Figure 2).

Results and Discussion

In the present study, 152 *Candida* species were isolated from various clinical samples. Most of the isolates were from High vaginal swab (56.6%) followed by Urine (29%), Pus (5.9%), Sputum (3.3%), Blood (2.6%), Endotracheal tube (1.9%) and Cerebrospinal

fluid (0.7%) respectively. *C. albicans* (48.7%) was the common species isolated followed by *C. tropicalis* (21%), *C. glabrata* (18.4%) and *C. krusei* (11.9%) respectively (Table 1).

All *Candida* isolates were subjected to antifungal susceptibility testing by disc diffusion method. All the isolates were sensitive to Amphotericin B. The overall resistance of *Candida* species to Fluconazole and Voriconazole is 21.1% and 11.9% respectively. The results of antifungal susceptibility of individual *Candida* species are shown in Table 2.

Table.1 Distribution of *Candida* species

Sample	*Candida* species				Total
	C. albicans	*C. tropicalis*	*C. glabrata*	*C. krusei*	
High Vaginal swab	42	15	19	10	**86 (56.6%)**
Urine	20	12	07	05	**44 (29%)**
Pus	06	01	01	01	**09 (5.9%)**
Sputum	01	04	00	00	**05 (3.3%)**
Blood	02	00	01	01	**04 (2.6%)**
Endotracheal tube	02	00	00	01	**03 (1.9%)**
Cerebrospinal fluid	01	00	00	00	**01 (0.7%)**
Total	**74 (48.7%)**	**32 (21%)**	**28 (18.4%)**	**18 (11.9%)**	**152(100%)**

Table.2 Antifungal susceptibility of *Candida* species

	Amphotericin B		Fluconazole		Voriconazole	
	S	R	S	R	S	R
C. albicans (n=74)	74 (100%)	00	63 (85.1%)	11 (14.9%)	63 (85.1%)	11 (14.9%)
C. tropicalis (n=32)	32 (100%)	00	30 (93.8%)	02 (6.2%)	31 (96.9%)	01 (3.1%)
C. glabrata (n=28)	28 (100%)	00	27 (96.4%)	01 (3.6%)	28 (100%)	00
C. krusei (n=18)	18 (100%)	00	00	18 (100%)	12 (66.7%)	06 (33.3%)
Total (n=152)	152 (100%)	00	120 (78.9%)	32 (21.1%)	134 (88.1%)	18 (11.9%)

Fig.1 *Candida* species on CHROM agar

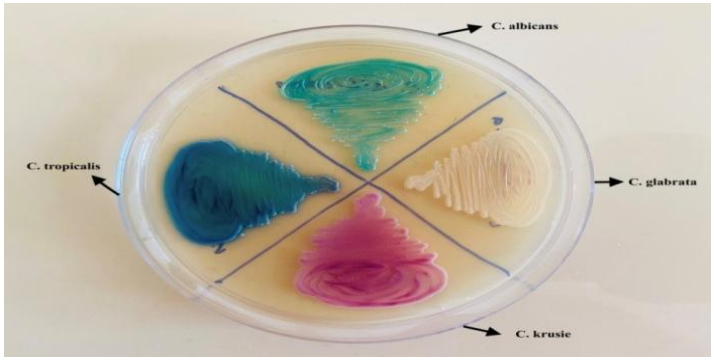
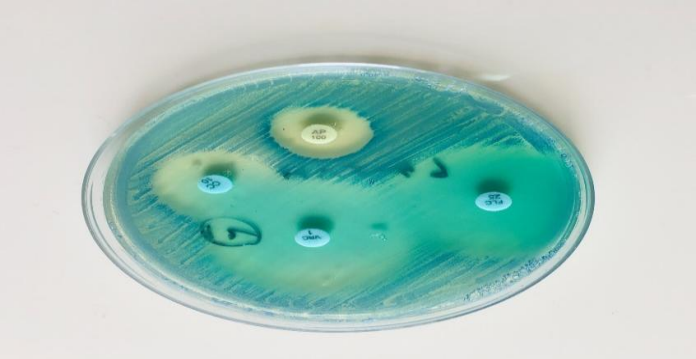


Fig.2 Antifungal susceptibility testing by Disc diffusion method



Candida species is found to be commensal and interruption of host defense is required for them to act as pathogens. As there is an increase in the number of patients who are immunocompromised, aged, receiving antibacterial and aggressive cancer chemotherapy or undergoing invasive surgical procedures and organ transplantation, Candidiasis has emerged as an alarming opportunistic disease (Marak *et al.,* 2018). The extensive use of antimycotic drugs for prolonged therapeutic courses has led to change in the relative prevalence of various species of *Candida* (Jayalakshmi *et al.,* 2014).

The changes in the distribution of *Candida* species have been observed for many years and the rates of Nonalbicans species such as *C. glabrata*, *C. tropicalis* and *C. krusei* have been increasingly reported. *C. albicans* still is the most common agent in many studies (Dagi *et al.,* 2016, Khadka *et al.,* 2017, Lavanya *et al.,* 2019). Our study also shows predominance of *C. albicans* followed by *C. tropicalis*, *C. glabrata* and *C. krusei*.

CHROM agar is a simple, rapid and inexpensive method with good sensitivity and specificity for identification of such species. An additional advantage of HiCromeCandida Differential Agar is ability to detect mixed cultures (Vignesh Kanna *et al.,* 2017).

Because of the increasing incidence of Candida infection along with the emergence of drug resistant phenotypes, it is essential to provide the clinicians with the antifungal susceptibility pattern for better treatment outcomes (Jayachandran *et al.,* 2018). In the present study, all the isolates were sensitive to Amphotericin B which is similar to the results reported by (Marak *et al.,* 2018, Arora *et al.,* 2017 and Jangla *et al.,* 2018). However, Amphotericin B showed a resistance percentage of 1.2% in a study done by Jayachandran *et al*., Khotari *et al.,* has reported a resistance of 8% for Amphotericin B. Candida strains have not shown resistance to Amphotericin B in our study. 21.1% of the strains were resistant to Fluconazole and 11.9% showed resistance to Voriconazole. Our findings are similar to previous other studies (Marak *et al.,* 2018 and Sumana *et al.,* 2017). *C. krusei* exhibits intrinsic resistance to Fluconazole both in-vivo and in-vitro (Orozco *et al.,* 1998). In our study, all the *C.krusei* isolates were resistant to Fluconazole.

The advent of antifungal drug resistance and isolation of Nonalbicans *Candida* with inherent drug resistance can be attributed to the indiscriminate usage of antifungal drugs, immunocompromised states like HIV and cancer chemotherapy requiring prophylactic and empirical antifungal therapy

(Jayachandran *et al.,* 2018). Periodic antifungal resistance surveillance protocols must be formulated for studying the trend of antifungal resistance in a particular area. This will also guide in choosing the empiric/ prophylactic drug before the antifungal resistance pattern is available. Disc diffusion method is easy to perform and results can be interpreted by 24 hours and hence can be used for diagnostic purposes on daily basis (Jayachandran *et al.,* 2018).

In conclusion the infections due to *Candida* species are increasing in the recent few decades. The shift towards the Non albicans *Candida* as a major etiological agent has generated the concern. Several NAC species are inherently resistant to common antifungal agents. Hence, rapid identification of *Candida* isolates up to species level with antifungal susceptibility pattern is important for the early management of *Candida* infections and to prevent treatment failures. Changing trends in the antifungal susceptibility pattern recommends routine antifungal susceptibility testing of *Candida* isolates in clinical microbiology laboratories which helps in the judicious use of antifungal drugs in patients and thus helps in preventing resistance.

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