Isolation, Speciation And Antifungal Drug Susceptibility Of Candida Species Isolated From Various Clinical Specimens In A Tertiary Care Hospital

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ABSTRACT:

Background-

Over the last 20 years, the rates of fungal infection have increased and Candida has emerged as a major cause of human disease. Candida species cause various clinical infections ranging from mucocutaneous infections to life threatening blood stream infections. Emergence of Candida species resistance is on the rise especially to triazoles and amphotericin B has led to use echinocandins, mostly caspofungin in the management of invasive candidiasis. The aim of the study was to identify the spectrum of Candida species and to determine the susceptibility pattern to antifungal agents.

Methods-

A total of 150 Candida isolates were isolated over a period of 2 years. Growth of each isolate was evaluated for colony appearance, Gram stain, Germ tube test, morphological appearance by Dalmau technique using cornmeal agar, sugar utilization test, Culture on CHROMagar for species identification was done for species identification. Antifungal susceptibility was performed as recommended by Clinical and Laboratory Standards Institute (CLSI) M44-A2 document.

Results-

Out of 150 Candida isolates, C.albicans was the most common species. 40% isolates obtained were from age group >60 years. Majority of Candida isolates, 52(34.67%) were from patients who had immunosuppression & chronic drug therapy as predisposing factor. Among the non- albicans Candida species C. tropicalis (27.33%) was predominant isolate followed by C. glabrata (16%). Regarding antifungal susceptibility pattern, Candida species were more resistant to fluconazole (20.67%) followed by ketoconazole (8.66%) and voriconazole (6.67%).

Conclusion-

Due to the variable clinical presentations of Candida infections, it becomes very important to identify these pathogens from all the routine culture specimens received irrespective of clinical diagnosis. Candida spp. differ in their antifungal susceptibility and virulence factors. Thus, routine identification of Candida up to species level along with antifungal susceptibility becomes very essential in diagnostic microbiology laboratory.

Keywords: Antifungal susceptibility testing, Susceptible Dose Dependent, Candida

INTRODUCTION

Fungal infections in immunocompromised individuals are a major cause of morbidity and mortality and *Candida* are among the most common pathogens in these patients.

Candida is an asexual, diploid, dimorphic fungus. *Candida* species belong to normal microbiota of an individual's mucosal oral cavity, gastrointestinal tract and vagina[1] and are responsible for various clinical manifestations from mucocutaneous overgrowth to bloodstream infections.[2] There has been an increase in number of patients who are immunocompromised, aged, receiving prolonged antibacterial and aggressive cancer chemotherapy or undergoing invasive surgical procedures and organ transplantation; therefore, candidiasis has emerged as an alarming opportunistic disease.[3] More than 90% of invasive infections are caused by *C. albicans, C. glabrata, C. parapsilosis, C. tropicalis and C. krusei*.[4]

The emergence of non-albicans Candida species has however been well recognized during the past decade.[5,6] *Candida* species have been shown to cause a similar spectrum of disease ranging from oral thrush to invasive disease, yet differences in disease severity and susceptibility to different antifungal agents have been reported.[7]

The potential clinical importance of species-level identification has been recognized as *Candida* species differ in the expression of putative virulence factors and antifungal susceptibility.[8,9]

Thus isolation, identification, characterization and susceptibility testing of *Candida* species in clinical specimens have become increasingly important for management of fungal infections. The present study was designed to identify the spectrum of *Candida* species in clinical infections and to identify their susceptibility pattern to available antifungal agents.

MATERIAL AND METHODS

A hospital based cross sectional study was conducted for two years (November 2018 to October 2020) by Department of Microbiology, in a tertiary care hospital.

A total of 150 *Candida* isolates from various clinical samples were included in the study while *Candida* isolates from stool and sputum samples were excluded. The primary diagnosis of specimens was performed by wet mount and Gram stain. All suspected samples were inoculated on Sabouraud dextrose agar (SDA) slope supplemented with chloramphenicol and aerobically incubated at 37 °C for 24-48hrs. Any visible growth on SDA slope was processed for further identification. From isolated colony,

macroscopic examination, Gram stain, Germ tube test, morphological appearance by Dalmau technique using cornmeal agar, sugar utilization test was performed. Culture on CHROMagar was performed for species identification.

Antifungal susceptibility testing was performed by Disk diffusion method for Flucanozole (25 mcg) and Voriconazole (1 mcg) using CLSI M44A210 and for Ketoconazole (10 mcg) as per Salehei et al (2012)[11] and Infectious Diseases Society of America (2016),[12] since CLSI has no guidelines for antifungal susceptibility of this drug.

The inoculum was prepared by suspending five colonies of growth in 5 ml of sterile saline and compared the turbidity to 0.5 McFarland Standard. A cotton swab was dipped into the inoculum suspension and evenly streaked onto Mueller–Hinton agar supplemented with 2% glucose and 5 µg/ml methylene blue.[13] *C. albicans* ATCC 90028, C. tropicalis ATCC 750 were used as controls. Antifungal discs containing fluconazole (25 µg), ketoconazole (10 µg), and voriconazole (1µg) were placed on the inoculated media. Zone of inhibition around the disc was measured after incubating the media at 37 °C for 24 h.

Minimum inhibitory concentration (MIC) for fluconazole was performed by Etest for strains resistant to fluconazole by disk diffusion. [14- 16]

RESULTS

A total of 150 *Candida* isolates were isolated from urine (44%), High vaginal swab (26%), Oral swab (10.66%), Pus (9.33%), Blood (5.33%) & other samples (4.66%). Gender wise distribution showed that 56.67% *Candida* isolates were from males and 43.33% from females. The maximum isolates (40%) obtained were from age group >60 years, followed by 20.67% from age group 21-30 years, 18.67% from 31-40 years age group. (Table 1)

Table 1: Distribution of study patients according to age

R NO	AGE IN YEARS	CANDIDA ISOLATES	PERCENTAGE
1	0-10	11	7.33%
2	11-20	3	2.00%
3	21-30	31	20.67%
4	31-40	28	18.67%
5	41-50	11	7.33%
6	51-60	6	4.00%
7	>60	60	40.00%
	Total	150	100

Majority of the isolates 52(34.67%) were from patients who had immunosuppression & chronic drug therapy as predisposing factor. The second common predisposing factor was diabetes mellitus 38(25.33%), followed by pregnancy 22(14.66%), pre-term and LBW babies 18(12.00%) and undetermined factors 20(13.34%). (Fig. 1)

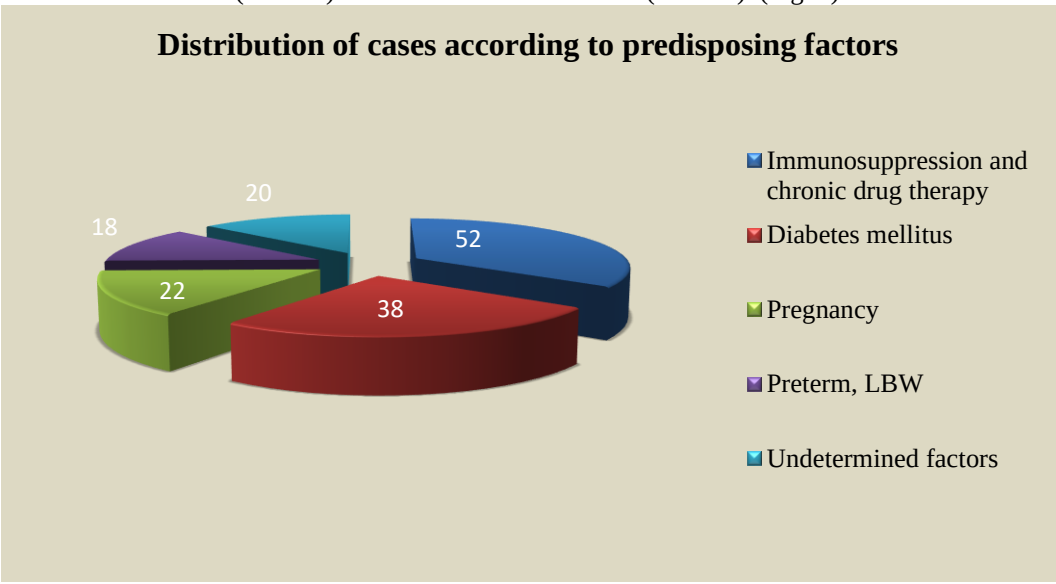

Figure 1: Distribution of cases according to predisposing factors

Based on Morphological and CHROMagar five types of *Candida* species were differentiated. *C.albicans* (46%) was the most frequently encountered species & maximum number of isolates were from urine & High Vaginal Swab (37% and 28.98 % respectively).

Amongst the NAC spp., *C. tropicalis* (27.33%) was the commonest isolate followed by *C.glabrata* (16%), *C.parapsilosis* (6.66%), *C.krusei* (4%). The different species of *Candida* reported from various specimens in our study is shown in Table 2.

Table 2: Distribution of frequency of *Candida* species in various clinical samples

CLINICAL SPECIMEN	ISOLATES					
	C.ALBICANS	C. NON-ALBICANS	C.TROPICALIS	C.GLABRATA	C.PARAPSILOSIS	C.KRUSEI
URINE (66)	26 (37.68%)	40 (49.38%)	22 (53.65%)	11 (45.83%)	5 (50%)	2 (33.33%)
HIGH VAGINAL SWAB (39)	20 (28.98%)	19 (23.45%)	12 (29.26%)	6 (25%)	-	1 (16.66%)
ORAL SWAB (16)	11 (15.94%)	5 (6.17%)	1 (2.43%)	3 (12.5%)	1 (10%)	-
PUS (14)	7 (10.14%)	7 (8.64%)	4 (9.75%)	-	2 (20%)	1 (16.66%)
BLOOD (8)	3 (4.34%)	5 (6.17%)	2 (4.87%)	2 (8.33%)	1 (10%)	-
OTHER (7)	2 (2.89%)	5 (6.17%)	-	2 (8.33%)	1 (10%)	2 (33.33%)
TOTAL (150)	69	81	41	24	10	6

Overall, antifungal susceptibility profile of *Candida* species to fluconazole was found to be 70.67% susceptible (S), 8.66% susceptible dose dependent (SDD), 20.67% resistant (R). In similar way, antifungal profile (S, SDD, R) to voriconazole was (92%, 1.33%, 6.67% resp.) and to ketoconazole (80.67%, 10.67%, 8.66% resp.) as depicted in Table 3.

Table 3: Antifungal susceptibility testing of various Candida spp.

ANTIFUNGALS	CANDIDA SPECIES					
	C.ALBICANS (n= 69)	C.TROPICALIS (n= 41)	C.GLABRATA (n= 24)	C.PARAPSILOSIS (n= 10)	C.KRUSEI (n= 6)	TOTAL (n= 150)
FLUCONAZOLE						
S	58	24	14	10	-	106 (70.67%)
SDD	6	6	1	-	-	13 (8.66%)
R	5	11	9	-	6	31 (20.67%)
VORICONAZOLE						
S	64	39	20	9	6	138 (92%)
SDD	-	-	1	1	-	2 (1.33%)
R	5	2	3	-	-	10 (6.67%)
KETOCONAZOLE						

S	59	32	19	7	4	121 (80.67%)
SDD	7	3	3	3	-	16 (10.67%)
R	3	6	2	-	2	13 (8.66%)

Amongst the three antifungal agents, fluconazole (20.67%) showed highest level of resistance whereas highest level of susceptibility was observed in voriconazole (92%) followed by ketoconazole (80.67%) and fluconazole (70.67%).

In this study, *C.tropicalis* (7.33%) showed more resistant to fluconazole in comparison with C.albicans (3.33%). Voriconazole showed 6.67% resistance while ketoconazole showed 8.66% resistance.

Of the total 25 *Candida* isolates which were resistant to fluconazole by disk diffusion (excluding 6 *C.krusei* isolates which are inherently resistant to fluconazole), 4 isolates showed MIC between 16-32µg/ml (SDD) and 21 showed MIC ≥64µg/ml (resistant) depicted in Table 4.

Table 4: Minimum inhibitory concentration (MIC) of fluconazole for *Candida* by Etest method

Sensitivity pattern	MIC range	Number
S	≤ 8 µg/ml	0
SDD	16-32 µg/ml	4
R	≥ 64 µg/ml	21
Total		25

DISCUSSION

Candida spp. are the most common cause of invasive yeast infections. At least 15 distinct species of *Candida* cause human diseases, although 95% of infections are caused by the five most common pathogens, *C. albicans*, *C. glabrata*, *C. tropicalis*, *C. parapsilosis*, *and C. krusei*.[17] Majority of the isolates in this study were obtained from urine(44%) followed by high vaginal swab (26%). Studies by Shaik N et al.,[23] and Joseph K et al.,[33] recovered maximum number of isolates from urine (60% and 46.9%, respectively) followed by respiratory samples (17.3% and 20.4%, respectively).

In this study, *C.albicans* (46%) was the predominant species isolated followed by *C.tropicalis* (27.33%) similar to other studies in literature.[18, 22-25, 28, 34, 35] Non albicans Candida (NAC) were isolated at a higher rate (54%) than *C.albicans* in our study which is in concordance with different studies from India suggesting that NAC are emerging microbial trend in yeast infections.[18- 21] However, some authors in their studies also observed a significant predominance of *C.albicans* over NAC spp.[25, 28]

When demographically distributed about 56.67% of isolates were from male patients and 40% of *Candida* were isolated from age group of >60 years. Predominance of *Candida* species in elderly group in current study might be due to presence of significant co- morbid conditions like diabetes, prolonged antibiotic therapy due to increasing infections due to increasing age. Similar findings were observed by Urvashi Chongtham et al, [34] Bhaskaran et al [22].

In the present study, it was observed that Immunosuppression and chronic drug therapy were the most frequently associated risk factors followed by Diabetes mellitus and pregnancy. Other significant risk factors were, preterm/low birth weight, sepsis and indwelling devices. Urvashi Chongtham et al [34] & Shaik N et al [23] et al also observed higher rate of *Candida* infections in those patients.

In our study resistance of *Candida* against fluconazole was more (20.67%) in comparison to other antifungals used in this study. Different studies had also reported higher resistance to fluconazole.[19, 20, 24, 25, 34] While Pandita I et al [18] & Joseph K et al [33] reported lower resistance of 1.25% & 8.3% resp. to fluconazole. Indiscriminate use of fluconazole & intrinsic resistance of few NAC species contribute to fluconazole resistance. In our study all isolates of C.krusei were resistant to fluconazole. The difference in susceptibility of C.albicans and C.nonalbicans to fluconazole was found statistically significant (p=0.000179).

In our study, voriconazole was found to be susceptible to 92% *Candida* isolates. Studies by different authors [34,19,33,26] reported 86%, 100%, 90.2%, 100% susceptibility to voriconazole respectively. The susceptibility pattern of *Candida* to voriconazole in present study was in contrast with More SR et al [27] who reported 73.43% susceptibility to voriconazole by disk diffusion method.

The antifungal ketoconazole was found to be susceptible to 80.67% isolates. Studies by Joseph K et al [33], Shaik N et al [23] reported 90.2%, 90% susceptibility to ketoconazole resp. However, Khadka et al [28] & Urvashi et al [34] reported higher resistance of 86%, 39% in their study.

Of total 25 *Candida* isolates which were resistant to fluconazole by disk diffusion, 21 (84%) were resistant by Etest method. Badiee P et al,[29] Deepak Kumar et al,[30] Prasanna S et al,[31] D Cretella et al [32] reported 27.56%, 11%, 48%, 14% isolates were resistant to fluconazole by E- test method.

CONCLUSION

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. Present study highlights increase in cases of fungal infections in elderly and immunocompromised patients and there is increasing resistance seen to commonly used antifungals. Over the last few decades, increasing fungal infections are great challenge to health-care professionals.

Candida albicans is by far the most common species causing infections in humans. It is important to monitor the resistance trends and distribution of *Candida* spp. in the face of increasing usage of potent, broad-spectrum antibacterial agents in hospitals across India. Local guidelines on treatment based on the epidemiology of infection should be developed. Continued surveillance will be important to document changes in epidemiological features of candidiasis and antifungal susceptibilities. Therefore, the identification of *Candida* infections at the species level 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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