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RESEARCH ARTICLE

SPECIATION OF CANDIDA AND ANTIFUNGAL SUSCEPTIBILITY TESTING FROM CLINICAL SPECIMENS IN A TERTIARY CARE HOSPITAL, BANGALORE

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ABSTRACT

Objective: Candida albicans is generally considered the major pathogen among the Candida species. An increase in the prevalence of non-albicans Candida species has been noted during the last decades and also azole resistance is seen more commonly in non-albicans Candida species compared to Candida albicans. The objective of the study was to identify, isolate and speciate Candida and perform antifungal susceptibility testing from various clinical specimens which has a direct impact on choice of empirical antifungal treatment.

Methodology: A total of 100 Candida isolates from various clinical specimens were processed for speciation using standard mycology methods. Antifungal susceptibility testing was performed by disc diffusion method according to CLSI guidelines M44-A2.

Results: The present study had a male preponderance, with an overall male: female ratio being 1.4:1. Isolation of Candida was highest among the extremes of age group i.e., neonates followed by 50-70 yrs. The various species of Candida isolated in the study were C.*tropicalis* (39%), C.*albicans* (35%), C.*krusei* (13%), C.*glabrata* (7%) and C.*parapsilosis* (6%). There was variation in the susceptibility pattern of Candida spp. to frequently used antifungal drugs. The Candida species showed highest sensitivity to Nystatin (98.92%) and Amphotericin B (84.95%) followed by Fluconazole (42.53%), Clotrimazole (30.12%) and Ketoconazole (25.8%). C.*tropicalis*, C.albicans and C.*parapsilosis* were 100% sensitive to Nystatin. C.krusei was 100% sensitive to Clotrimazole and 92.3% sensitive to Amphotericin B and Nystatin. The susceptibility of C.albicans to fluconazole was only 60%.

Conclusion: Non-albicans Candida is gaining clinical significance. Hence identification of species will be helpful in selection of antifungals for the earlier and cost effective treatment. CHROM agar serves as a primary isolation and differentiation medium for clinical specimens that could allow laboratories to rapidly identify Candida spp, enabling clinicians to choose appropriate antifungal agents, thus decreasing patient's morbidity and mortality.

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INTRODUCTION

Candida species are the members of normal flora of the skin, mucous membrane and gastrointestinal tract and cause secondary infection in individuals with some underlying immunocompromised conditions. *Candida albicans* is generally considered the major pathogen among *Candida* species. An increase in prevalence of non-*albicans* species has been noted during the last decades. Patients admitted at tertiary care hospitals have access to very intensive management modalities. This, along with increasing number of immunocompromised patients have lead to rise in infections caused by *Candida* especially by non-*albicans Candida* (Chander, 2009; Jawetz *et al.,* 1978; Shaheen *et al.,* 2006).

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The growing number of immunocompromised individuals can be attributable to HIV pandemic, use of long-term immunosuppressive therapy in cancer and organ transplant patients, use of intravascular catheters, invasive surgical procedure and long duration of hospital stay. *Candida* can cause simple mucocutaneous lesion to life-threatening systemic infections. They may be acute or chronic, superficial or deep. *Candida albicans* and non-*albicans* species are closely related but differ from each other with respect to epidemiology, virulence characteristics and antifungal susceptibility[1]. Azole resistance is seen more commonly in non-*albicans Candida* species compared to *Candida albicans*, therefore species level identification and antifungal susceptibility testing has a direct impact on choice of empirical antifungal treatment. The aim of the study was to isolate and speciate *Candida* spp. from various clinical specimens and to detect antifungal susceptibility pattern. It also helps to understand the epidemiology of *Candida* species particularly the source and mode of

Table 1.*Candida* species isolated from clinical samples tested

Candida isolate	Blood	Urine	Nail	Sputum	Pus	Fluids	Cornea	Skin	Total
C.tropicalis	25(48.07%)	10(37.03%)	2(22.22%)	1(20%)	-	-	1(100%)	-	39
C.albicans	10(19.23%)	13(48.15%)	5(55.56%)	4(80%)	2(66.67%)	1(50%)	-	-	35
C.krusei	12(23.06%)	-	-	-	1(33.33%)	-	-	-	13
C.glabrata	3(5.8%)	3(11.12%)	-	-	-	1(50%)	-	-	7
C.parapsilosis	2(3.84%)	1(3.7%)	2(22.22%)	-	-	-	-	1(100%)	6
Total	52	27	9	5	3	2	1	1	100

Table 2. Antifungal sensitivity pattern

Candida species	Isolates	No.(%) sensitive				
		Fluconazole	Ketoconazole	Amphotericin B	Clotrimazole	Nystatin
C.tropicalis	39	12(30.8%)	1(2.56%)	31(79.49%)	1(2.56%)	39(100%)
C.albicans	35	21(60%)	9(25.71%)	31(88.57%)	11(31.43%)	35(100%)
C.krusei	13	-	12(92.3%)	12(92.3%)	13(100%)	12(92.3%)
C.parapsilosis	6	4(66.7%)	2(33.33%)	5(83.33%)	3(50%)	6(100%)
Total		37(42.53%)	24(25.8%)	79(84.95%)	28(30.12%)	92(98.92%)

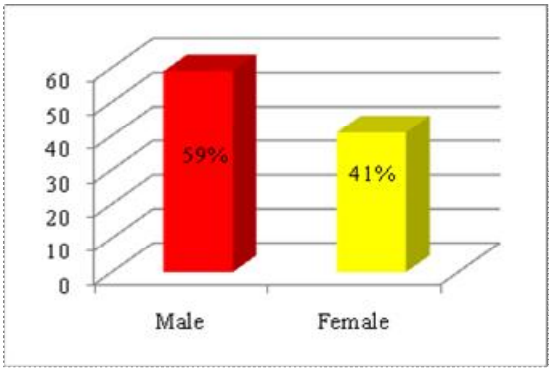


Figure 1. Male: Female distribution

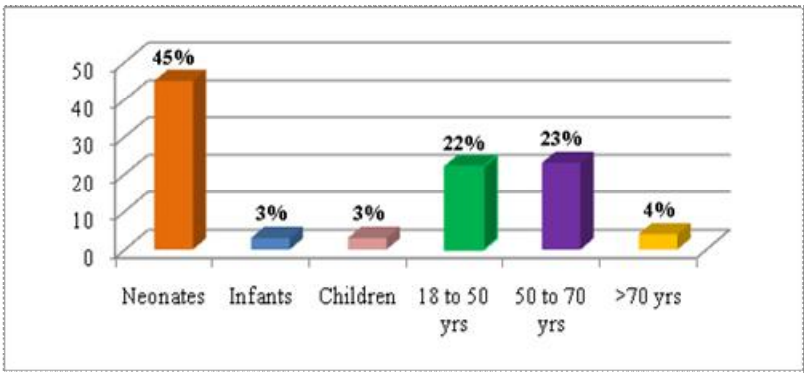
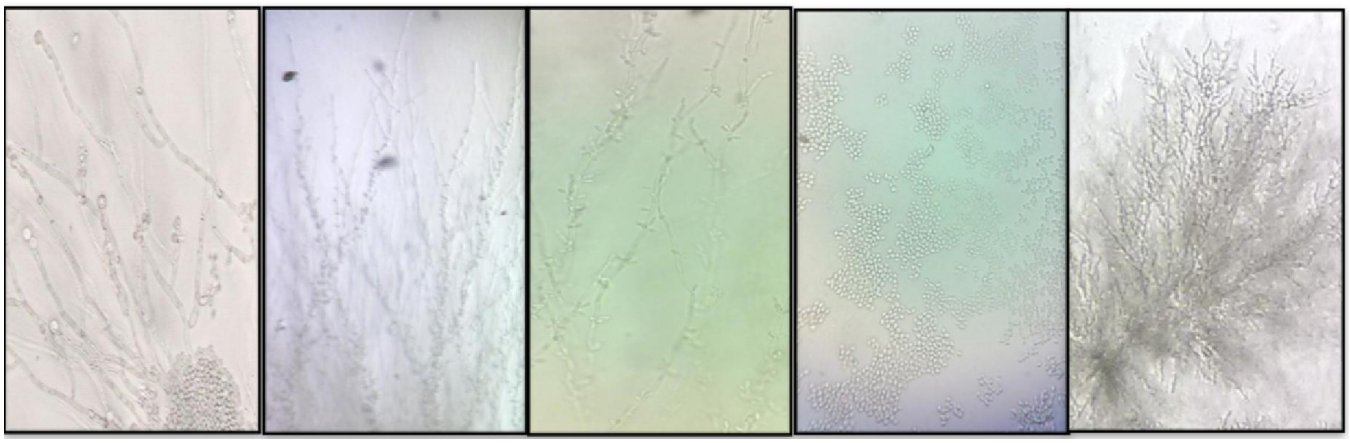


Figure 2. Age distribution



C.albicans ***C.tropicalis*** ***C.parapsilosis*** ***C.glabrata*** ***C.krusei***

Figure 3. Morphology on corn meal agar

transmission which in turn facilitates the development of effective measures to prevent and control transmission of resistant pathogens.

MATERIALS AND METHODS

Total 100 *Candida* species isolated from various clinical samples including urine, sputum, pus, body fluids, blood, ear swab, high vaginal swab, skin, nail and corneal scrapings, intravascular devices and medical implants were taken up for the study. Duration of the study was from January 2016 to December 2016 conducted at the Department of Microbiology, Bangalore Medical College and Research Institute.

The isolates were processed for the identification and speciation using standard mycology methods (Fisher and Cook, 1998). The specimens were inoculated on Sabouraud's dextrose agar, incubated at 37^{o}C for 24 hrs. Speciation of *Candida* was done by germ tube test, Chlamydospore formation on corn meal agar (Dalmau technique), colour of colony on HiCHROM *Candida* agar, carbohydrate fermentation and carbohydrate utilization pattern by Sugar assimilation tests (Dye pour plate method) (Milne *et al.,* 1996) Figure 3,4,5. Antifungal susceptibility testing was performed by disk diffusion test on Mueller-Hinton agar with 2% glucose and methylene blue. The susceptibility pattern was determined for Fluconazole 25µg using the National Committee for Clinical Laboratory Standards 2011 method for antifungal disc diffusion susceptibility for yeasts with approved

Table 3. *Candida* species isolated by various workers (In %)

	Present study 2016	Shah *et al*. 2016	Tejashree *et al*. 2014	Jaggi T *et al*. 2014	BineshLal Y *et al*. 2011	Madhavan P *et al*. 2010	Vijaya D *et al*. 2009	Capoor *et al*. 2007	Shiva Prakash *et al*.2007
C.tropicalis	39	35	50.9	26.4	54.3	24	35.29	39	36
C.albicans	35	41	30.37	44	37.8	17	45.9	26	3
C.krusei	13	-	2.8	2.4	-	15	10.07	3	-
C.glabrata	7	14	10.28	11.2	2.4	7	-	6	12
C.parapsilosis	6	3	3.7	12.8	5.5	15	7.84	26	29
Other species	-	7	1.82	3.2	-	22	0.9	-	20

Table 4. Antifungal susceptibility as reported by various workers (% sensitivity)

	Fluconazole	Ketoconazole	Amphotericin B	Clotrimazole	Nystatin
Present study 2016	42.53%	25.8%	84.95%	30.12%	98.92%
Shah SR *et al*. 2016	85%	36%	82%	-	-
Patel LR *et al*. 2012	36%	-	85.35%	-	-
Antony B *et al*. 2011	44.7%	52%	70%	84.7%	64.7%
BineshLal Y *et al*. 2011	74%	81.9%	100%	-	-
Pethani JD *et al*. 2011	50%	-	-	-	-

Figure 4. Growth on CHROM agar

guideline M-44 A2. Whereas, for Ketoconazole 10µg, Amphotericin B 100U, Clotrimazole 10µg and Nystatin 50µg sensitivity was determined as per Quality Control Limits for ATCC strains of *C.albicans, C.parapsilosis, C.tropicalis and C.krusei* provided in the manufacturer's product insert (HiMedia).

RESULTS

The present study had a male preponderance, with an overall male: female ratio being 1.4:1 (Figure 1). The highest no. of isolates were among the neonates followed by the age group 50-70 yrs (Figure 2). Among the 100 samples, non-*albicans Candida* was the most common causative agent comprising of *C.tropicalis* (39%), *C.krusei* (13%), *C.glabrata* (7%) and *C.parapsilosis* (6%) whereas *Candida albicans* showed a distribution of 35% (Figure 6, Table 1). Majority of isolates were from blood (52%). Antifungal susceptibility pattern: Overall, majority of strains were susceptible to Nystatin (98.92%) and Amphotericin B (84.95%). Only 42.53% of *Candida* species were susceptible to Fluconazole. Sensitivity to Ketoconazole and Clotrimazole were 25.8% and 30.12% respectively. All the strains of *C.glabrata* tested to fluconazole alone were resistant to it. Species-wise distribution pattern of antifungal susceptibility pattern to each drug is shown in Table 2.

DISCUSSION

A total of 100 isolates from various clinical specimens were included in our study, of which blood showed the highest number of isolates (52%), which is similar to the study by Jaggi *et al.* (2014), followed by urine (27%), nail (9%), sputum (5%) and remaining 10% being pus, fluids, cornea and skin. Data from surveillance and control of pathogens of epidemiological importance (SCOPE) surveillance system confirms that *Candida* species have become the fourth leading cause of blood stream infections. A study by Chowta *et al.* (2007) shows that Candidemia is associated with increased cost and attributable mortality of 38% (Chowta *et al.,* 2007). Out of 100 isolates, 59 were from malesi.e a male preponderance which is similar to the study of Patel *et al.* (2012) and Jaggi *et al.,* (2014). Candidiasis was most common among neonates (45%), followed by 50-70yrs age group (23%), 18-50yrs age group (22%), remaining 10% being infants, children and >70yrs age group. Of the neonates, majority of samples in which *Candida* was isolated was from blood (97.77%). Thus, stating that neonates are at higher risk of developing candidemia in consistent with study by Pethani *et al.* (2011). In the present study non-*albicans Candida* (65%) was isolated at a higher rate than *C.albicans* as reported by other workers. Non-*albicans Candida* included *C.tropicalis* (39%), *C.krusei* (13%), *C.glabrata* (7%) and *C.parapsilosis* (6%) whereas

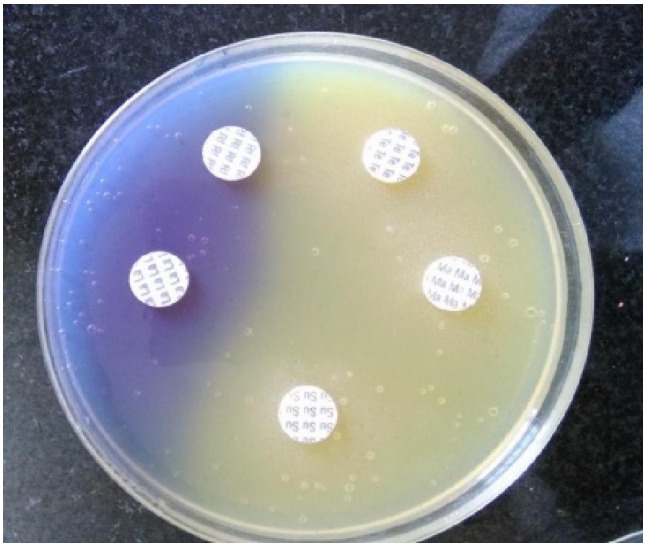


Figure 5. Carbohydrate assimilation pattern by dye pour plate method

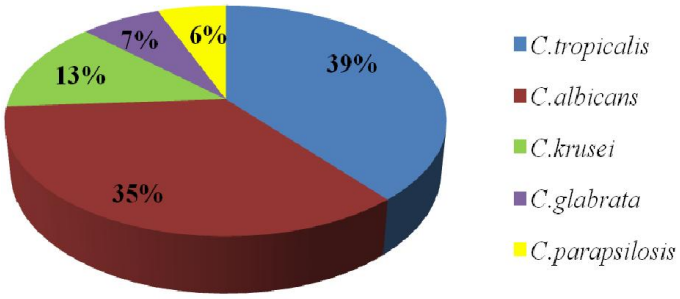


Figure 6: Distribution of Candida species

Candida albicans constituted 35% of the isolates. *Candida spp.* isolated by various workers is shown in Table 3. *C.tropicalis* (48.07%) was the predominant species causing candidemia followed by *C.krusei* (23.06%), *C.albicans* (19.23%), *C.glabrata* (5.8%) and *C.parapsilosis* (3.84%). Among the urine isolates, *C.albicans* was prevalent in 48.15%, followed by *C.tropicalis* (37.03%), *C.glabrata* (11.12%) and *C.parapsilosis* (3.7%). *C.albicans* was isolated in 55.56% of nail samples, followed by *C.tropicalis* and *C.parapsilosis* (22.22% each). Among the sputum and pus samples, *C.albicans* (80% and 66.67% respectively) was more prevalent compared to non - *albicans Candida*. *Candida* species found in fluids were *C.albicans* and *C.glabrata* 50% each. *C.tropicalis* and *C.parapsilosis* were singly isolated in cornea and skin respectively. There was variation in the susceptibility pattern of *Candida* spp. to frequently used antifungal drugs. The *Candida* species showed highest sensitivity to Nystatin (98.92%) and Amphotericin B (84.95%) followed by Fluconazole (42.53%), Clotrimazole (30.12%) and Ketoconazole (25.8%). *C.tropicalis*, *C.albicans* and *C.parapsilosis* were 100% sensitive to Nystatin. *C.krusei* was 100% sensitive to Clotrimazole and 92.3% sensitive to Amphotericin B and Nystatin. The susceptibility of fluconazole which is the most commonly used empirical drug was only 60% to *C.albicans* which is similar to studies conducted by Ravinder Sandhu *et al.* (2015), Bhaskar *et al.,* (2015) and Saleem *et al.* (2016). The drug of choice from the present study appeared to be Amphotericin B and Nystatin which was also in accordance with the previous findings. Overall, there is a great variation in the antifungal susceptibility pattern among different studies, some of which are shown in the Table 4.

Conclusion

Candidiasis is the most common fungal disease in humans affecting skin, nails, mucosa and internal organs of the body. Non-*albicans Candida* is gaining clinical significance in the recent years. The present study also shows the predominance of non-*albicans Candida* species over *Candida albicans*. They differ from each other with respect to epidemiology, virulence characteristics and antifungal susceptibility pattern. The identification of species helps in the choice of antifungal therapy as azole resistance is seen more commonly with non-*albicans Candida* compared to *Candida albicans*. There is also a need for periodic surveillance of the antifungal susceptibility pattern of the prevalent *Candida* species which will help in the choice of empirical region for that particular institution. The results of *Candida* CHROM agar was consistent with that of conventional methods. It has the advantage of being technically simple, rapid and cost effective as compared to time consuming, technically demanding conventional methods. CHROM agar serves as a primary isolation and differentiation medium for clinical specimens that could allow laboratories to rapidly identify *Candida* spp, enabling clinicians to choose appropriate antifungal agents, thus decreasing patient's morbidity and mortality.

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