

## Species Distribution and Antifungal Susceptibility Pattern of *Candida* Isolated from Various Clinical Sample Obtained in a Tertiary Care Hospital in Southern India A

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*Candida* species are the fourth most common cause of blood stream infection and in recent years a distinct increase has been noted in non-albicans *Candida* (NAC) cases with decreased susceptibility to antifungal treatment. Therefore species identification and antifungal susceptibility testing helps in successful treatment of candidiasis. To speciate the isolates obtained from clinical samples and to carry out antifungal susceptibility by disc diffusion method and also to detect the common *Candida* species causing blood stream infection in our hospital. A total of 120 samples were collected from clinically suspected cases of candidiasis visiting a tertiary care hospital in southern India over a period of 1 year (May 2013 to June 2014) and were subjected to culture on Sabouraud's Dextrose Agar (SDA) with Chloramphenicol, CHROM agar, Germ tube test, Corn meal agar inoculation and their antifungal susceptibility test for fluconazole and amphotericin B. A total of 85 isolates were obtained from 120 samples ( $P < .01$ ). *C.albicans* was the most common isolates from all the clinical samples including blood, although statistically NOT significant. Sensitivity rate for Amphotericin B was 91.76% and for Fluconazole was 71.76%. Prolonged medication appeared to be the commonest predisposing factor for *Candida* infection followed by *Diabetes mellitus*, the difference was significant ( $P < .05$ ).

**Key words:** *Candida albicans*, Non-albicans candida, CHROM agar, Speciation, Antifungal susceptibility.

Over the last three decades, *Candida* species has emerged as an important cause of health care associated opportunistic infections. The increased use of intravenous catheter, total parenteral nutrition, broad spectrum antibiotic and cytotoxic chemotherapy and increase in the population of immunocompromised patients have contributed to the increase of these infection.<sup>1</sup> *Candida albicans* is the most common cause of candidiasis accounting for 60-80% of infections,

although the frequency of isolation of Non-albicans *Candida* (NAC) species is increasing gradually. NAC such as *C.glabrata*, *C.krusei* and *C.tropicalis* are emerging as important opportunistic pathogens and this transition has significant clinical impact due to decreased susceptibility of these NAC to antifungal agent.<sup>2,3</sup> *Candida* species are the fourth most common cause of blood stream infection in the hospitalized patients and a leading cause of invasive fungal infection in the ICU setting.<sup>4</sup> Candidemia in critically ill patient is usually a severe and life threatening condition with mortality ranging between 20-49%.<sup>5</sup> So the isolation and prompt species identification is important for early antifungal therapy.

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## MATERIALS AND METHODS

A total of 85 culture positive isolates were collected from clinically suspected cases of candidiasis attending to our tertiary care hospital over a period of one year (May 2013 to June 2014).

Specimens were collected with all aseptic precautions and were subjected for direct examination (KOH wet mount / Gram staining), culture on Sabouraud's Dextrose agar.<sup>6</sup> Once the colonies were confirmed, speciation was done by Germ tube test, and inoculation on cornmeal agar and chromogenic media (CHROM agar)

Germ tube test is rapid method for identifying *C. albicans* and *C. dubliniensis*. To perform this test a colony was emulsified in 0.5 ml of human serum and were examined after 2 hours for germ tube. Germ tube is a elongated daughter cells from mother cell without constriction at their origin.<sup>7,8</sup> Cornmeal agar with Tween 80 (Hi-Media, Mumbai) was used for demonstration of chlamydospores, blastospores and pseudohyphae. This test was performed by inoculating a colony by cutting the media, spreading the inoculum by making 2-3 streaks and was covered with sterile coverslip. The plate was incubated at 22-25°C for 48 to 72 hours. After the incubation, the plate was examined by placing it on microscope stage without lid by using 40x objective.<sup>9,10,11</sup>

CHROM agar (Hi-Media, Mumbai) has chromogenic substance which reacts with specific enzymes of different species and yields colonies of different colour and thus helps in the rapid identification of *Candida* species.<sup>12,13,14</sup> All culture positive isolates were subjected for antifungal susceptibility test by disc diffusion method as per CLSI M44 guideline. Mueller-Hinton agar supplemented with 2% glucose (to speed up the growth) and 0.5 µg/ml of methylene blue (to enhance edge of zones), Amphotericin B (100 units/disc) and Fluconazole (10 mcg/disc) supplied by Hi-Media, Mumbai were used.<sup>9,15,16</sup>

## RESULTS

A total of 85 clinical isolates of *Candida* from various clinical specimen were processed during the study period. A total 40 isolates were

**Table 1.** Distribution of *Candida* species isolated from various clinical specimens.

S. No.	Specimen	Total No.	<i>C. albican</i>	<i>C. tropicalis</i>	<i>C. parapsilosis</i>	<i>C. glabrata</i>	<i>C. guilliermondii</i>	<i>C. krusei</i>	<i>C. dubliniensis</i>
1	Candiduria(patient with risk factor)	40	16	3	3	3	1	1	-
2	Blood (patient with risk factor)	14	9	11	5	2	1	-	1
3	Oral swab (Oral thrush)	14	6	4	1	1	-	-	1
4	Vaginal swab(Vulvovaginitis)	6	4	2	1	1	-	1	-
5	Ear swab (CSOM)	4	2	-	-	-	-	-	-
6	Nail scrapping (Onychomycosis)	3	1	-	-	-	3	-	-
7	Bronchoalveolar lavage	2	-	1	-	-	-	-	-
8	Pus for diabetic foot	2	1	1	1	-	-	-	-
	Total	85	38	21	11	7	4	2	2

obtained from urine followed by blood (14), oral swab (14), high vaginal swab (6), others (11). [Table -1]

*Candida albicans* was the commonest species (44.70%), followed by *C.tropicalis* (24.7%), *C.parapsilosis* (12.94%), *C.glabrata* (8.23%), other

less isolated species were *C.guilliermondii*(3.52%) *C.krusei* (2.35%) and *C.dublinsiensis* (2.35%). A higher incidence of *C.albicans* were found in urine followed by blood and oral swab. Whereas higher incidence of *C.tropicalis* was found in blood followed by oral swab. [Table -1]But, in either case

**Table 2.** Gender wise distribution of patients with Candidiasis

S. No.	Gender	Total	<i>C.albican</i>	<i>C.tropicalis</i>	<i>C.parapsilosis</i>	<i>C.glabrata</i>
1	Male	37	24	13	8	4
2	Female	48	14	8	3	5
	Total	85	38	21	11	7

**Table 3.** Age wise distribution of patients from whom Candida species were isolated

S. No.	Age in years	Total	<i>C.albican</i>	<i>C.tropicalis</i>	<i>C.parapsilosis</i>	<i>C.glabrata</i>
1	≤ 1	12	3	2	6	1
2	>1-£18	7	2	2	2	1
3	>18-£45	36	21	11	1	3
4	>45-£60	9	4	3	1	1
5	>60	14	8	4	1	1
	Total		38	21	11	7

**Table 4.** Distribution of pre-disposing factors in patients with Candidiasis

Predisposing factor	No.of cases	Percentage
Prolonged medication	31	36.47
Diabetes mellitus	18	21.17
Pregnancy	10	11.76
Preterm/ Low birth weight	9	10.58
Sepsis	7	8.23
HIV positive cases	5	5.88
Prolonged contact with water	5	5.88
Total	85	100

**Table 5.** Antifungal susceptibility testing pattern

Species	Total	Fluconazole			Amphotericin B		
		S	I	R	S	I	R
<i>C.albican</i>	38	28	7	3	36	2	-
<i>C.tropicalis</i>	21	12	1	8	16	3	2
<i>C.parapsilosis</i>	11	7	3	1	11	-	-
<i>C.glabrata</i>	7	2	-	5	7	-	-
<i>C.guilliermondii</i>	5	2	1	2	5	-	-
<i>C.krusei</i>	2	-	1	1	2	-	-
<i>C.dublinsiensis</i>	1	1	-	-	1	-	-
Total		58	11	17	78	5	2

there was no significant difference found ( $P=0.752$ ;  $P=0.655$ \* respectively). *Candida albicans* was NOT significantly higher ( $P=0.22$ ), than NAC counterparts. Again, out of 120 samples, 85 samples were positive for fungal infection, this was statistically significant ( $P<0.01$ ).[ \*multiple response]

It was observed that 85 culture positive cases, 56.47% were females and 43.52% were male. But, there was no significant difference in incidence amongst either sex ( $P=0.126$ ). [Table -2]

Candidiasis was most common in age group of greater than 18 years upto 45 years (44.7%) followed by the age group greater than 60 years. This has been found to be significantly higher ( $P<0.01$ )[Table -3]

An analysis of predisposing factor showed that 36.47% were on prolonged medication (Antibiotic / Steroid / Oral contraceptive pills) and 21.17% were diabetic, others 42.36%. The proportion of cases resulting from effects of medication were significantly higher ( $P<0.05$ ), in comparison to those suffering from diabetes [Table -4].

All the isolates were subjected to antifungal susceptibility testing [Table-5]. 68.23% of isolates were sensitive to fluconazole whereas 91.76% of isolates were sensitive to Amphotericin-B.

Data collected were analyzed using the statistical package for social sciences (SPSS) 20.0 Software (IBM Corp; New York, USA), and Program for Epidemiologist version 4. Statistical test of significance like Z test and ANOVA test were applied wherever necessary. A p-value  $<0.05$  was considered statistically significant.

## DISCUSSION

Candidiasis is a primary or secondary infection which involves a member of genus *Candida*. It may be acute, chronic, superficial or deep. It may be localized to the mouth, lungs or the gastrointestinal tract or may become systemic as in septicemia, endocarditis and meningitis.

In the present study, a total of 120 clinically suspected cases of *Candida* infection was taken for study, among them 85 cases were culture positive accounting for 71% incidence rate. Jarvis in 1995 reported incidence rate of 72%. This

result can be correlated with the present study.

According to study conducted by PJ Dalal and SS Kelkar in 1980,<sup>17</sup> the greatest incidence of candidiasis was in the age group of 21-40 years (35%). In our study youngest was 1 day old while the oldest was 70 year old. It was found that the highest incidence was in the age group of 18-45 years (44.70%).

In a study of candidiasis conducted by KC Kandhari *et al* at AIIMS, New Delhi, the incidence among female was found to be 61.2% and male to female ratio was 1:1.57. The present study also confirms higher incidence of candidiasis among female (56.47%), although statistically insignificant. The female preponderance may be due to increased number of pregnancy cases in the study, prolonged contact with water, use of contraceptive.

Out of total 85 *Candida* isolates from various clinical specimen, urine showed the highest number of isolates (47.05%) followed by blood (16.47%) and oral swab (16.47%). According to study done by Pfaller, *et al*<sup>18</sup> *Candida* species were the seventh most common nosocomial pathogen in a hospital and were the cause of 25% of all the urinary tract infections.

In this study *C.albicans* was the most commonly isolated species (44.70%) followed by *C.tropicalis* (24.7%), *C.parapsilosis* (12.94%), *C.glabrata* (8.23%). Studies by different researchers showed that the isolation of *C.albican* was the highest in all of them except in Chkrabarti A,<sup>19,20</sup> study which showed that the isolation of *C.tropicalis* was the highest (42%) followed by *C.albican* (25%). In a study done by Mokaddar, *et al*<sup>21</sup> *C.albican* (39.5%) was the commonest isolate.

In the present study, prolonged medication contributed to 36.47% of all culture positive cases followed by diabetes mellitus, the difference was found to be significant ( $P<0.05$ ). Winner and Hurley in 1964, have found considerable evidence in support of the enhancement of the candidiasis by use of systemic antibiotic and corticosteroids. In a study done by Kandhari KC *et al* found diabetes as commonest risk factor. According to Rippon,<sup>22</sup> there is some effect of the antibiotics on the host tissue, which predisposes it to invasion by the organism, and the antibiotics itself may stimulate the growth of *Candida*.

In a study by Chakrabarti *et al* in 1995, 2-5% of *Candida* strains were resistant to Amphotericin B, 13-20% were resistant to fluconazole. In the present study 2.35% of isolates were resistant to amphotericin B, 20% were resistant to fluconazole.

It can be seen in the present study that resistance to fluconazole was higher than amphotericin B, this might be due to widespread and indiscriminate use of fluconazole for prolonged period.

### CONCLUSION

CHROM agar gives an excellent results within short time when used for speciation of *C.albicans* and non-albicans *Candida*. Hence CHROM agar can be routinely used. If this is corroborated with addition tests like germ tube, cornmeal agar inoculation, identification becomes more accurate and their susceptibility pattern will help clinician to start suitable antifungal drugs thereby avoiding any treatment failure.

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