

Prevalence and Antifungal Susceptibility of Candida Species Isolated From Patients Attending Tertiary Care Hospital

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Abstract:

Objectives: Study of prevalence, speciation and antifungal susceptibility of Candida species from samples received by Microbiology Laboratory during the period of one year

Methods: A total of 103 Candida isolates were included in the study. Candida was identified from clinical samples by wet mount, gram stain and culture on SDA. The organisms were further speciated by germ tube test, cornmeal agar morphology, sugar assimilation and fermentation tests and CHROM agar. Antifungal sensitivity was performed by using disc diffusion method.

Results: The prevalence of Candida species was found to be 6.1%. The most common isolate found to be Candida albicans (65.0%) followed by non albicans Candida mainly C.tropicalis (24.3%), and C.krusei (10.7%). Highest isolates (40.8%) were from high vaginal swab. The common predisposing factors causing candidiasis were diabetes, pregnancy, prolonged drug intake.. Resistance to commonly used antifungal agents found to be Fluconazole — 12.62%, Clotrimazole - 7.76%, Ketoconazole - 0.97%, Amphotericin-B — 0.97% and No resistance was seen to Nystatin.

Conclusion: C.albicans is the common cause of candidiasis followed by C.tropicalis and C.krusei. Use of CHROMagar is rapid, technically simple and cost effective compared to time consuming technically demanding expensive conventional method. Antifungal susceptibility testing has to be carried out before starting therapy because of varying susceptibility pattern in different species of Candida.

Key words: Antifungal agents; Candida albicans; candidiasis; CHROM agar; .

I. Introduction

The genus Candida comprises about 200 species, of which close to 20 has been associated with pathology in human. Candida species are the most common cause of fungal infections worldwide. They can cause a great variety of infections, including simple mucocutaneous to severe invasive infections that can involve virtually any organ. Blood stream infections by Candida are increasingly common, and often are associated with high mortality rates^{1,2}. Recently, there has been an increment in the frequency of non-albicans species of Candida, such as C.glabrata, C.krusei, C.tropicalis, and C.parapsilosis, as causes of fungemia^{3,4}. With the remarkable modern advances in medicine, there has been an increase in the number of immunocompromised individuals who need extensive care in hospitals.

This has resulted in a rise in the incidence of fungal infections, C.albicans is amongst the most common fungal causative agent in superficial and deep seated candidiasis. However, non-albicans Candida species are also being implicated in recent years⁵.

The overall increase in candidemia in recent years is complicated by the emergence of non-albicans Candida (NAC) species as both colonizers and pathogens causing nosocomial fungal blood stream infection (BSI). The NAC species isolated were C.glabrata, C.krusei, C.tropicalis and C.parapsilosis. other species like C.guilliermondii, C.lusitaniae, C.dubliniensis, C.kefyr, C.lipolytica, and C.pelliculosa were occasionally isolated⁷.

II. Material And Methods

Present study was carried out in the department of Microbiology, Navodaya medical College hospital and Research Centre, Raichur for a period of one year.

Sample size: All the clinical samples (1702) that came to the Department of Microbiology laboratory during the study period constituted the material for the study.

The specimens for laboratory investigation were collected from the clinically suspected cases of candidiasis under strict aseptic precautions. Sterile swabs and bottles were used to collect the appropriate clinical materials. The various clinical specimens collected were oral swabs, ear swabs, vaginal swabs, stool, CSF, sputum, blood, pus, nail scrapings etc. the specimen was subjected to preliminary tests like wet mount, gram stain, culture on SDA and urease test.

Besides these above specimens for microbiological tests; routine investigations were conducted such as hemogram, urine for albumin and sugar, fasting and post prandial blood sugar, screening the chest, VDRL, and tests for HIV.

Mycological examinations for 103 candida species were done.^{6,11,13.}

1) Direct examination:

- a. Wet mount
- b. Gram's stain

a. Wet Mount:

For direct microscopic examination the specimen was placed on the glass slide then 10% KOH (Potassium Hydroxide) was added and coverslip was placed on the specimen. The slide was slightly warmed and gentle pressure was applied over the coverslip to remove the trapped air. Microscopic examination was done, first under the low power then under high power.

In case of nail specimen 40% KOH is added to a small test tube to which nail clipping was added and incubated overnight for dissolving nail keratin. The specimen was then examined under microscope.¹¹ Attempt was made to identify the pseudohyphae and yeast cells.

b. Gram's stain: Smears were made from the clinical sample on a clean and grease free glass slide, then heat fixed by just passing the glass slide over the flame.

c. Then the smear was stained by gram's method and observed under oil immersion objective and looked for the presence of gram-positive oval yeast like budding cells (2-4µm) and / or pseudohyphae.^{9,13}

2) Culture:^{11,14} For culture sabouraud's dextrose Agar (SDA) with chloramphenicol was used. The sample was inoculated on SDA slopes and incubated at 25°C. the slopes were observed regularly daily from 2 days to 3 weeks.

Colonies were identified by the colony characters and by gram's stain. Once the colonies were confirmed speciation was done by following methods.

- a. Germ tube
- b. Corn meal agar inoculation
- c. Sugar fermentation.
- d. Sugar Assimilation
- e. CHROMagar Candida

Antifungal susceptibility testing:

Antifungal susceptibility testing of the yeast isolates was assessed using the agar diffusion method according to CLSI guidelines.

Disc diffusion method was used in the present study. The antifungal agents used for disc diffusion method are:

- 1. Amphotericin B (100 IU)
- 2. Fluconazole (25µg)
- 3. Nystatin (50µg)
- 4. Ketoconazole (50µg)
- 5. Clotrimazole (10µg)

To determine whether the isolates tested against amphotericin B, nystatin, fluconazole, ketoconazole, clotrimazole, were susceptible, intermediate, or resistant; the diameters of zones of inhibition obtained were compared with the standard Zones interpretive break points published by CLSI M44-A2¹⁵ guidelines, ROSCO diagnostica¹⁶

III. Results

Statistical analysis:

Descriptive statistics such as mean, standard deviation (SD), Proportions and graphs were used to describe the data. Differences between proportions were analyzed using χ^2 tests, or by fisher's exact test, if sample sizes were small or unbalanced. A two-tailed p-value <0.05 was considered statistically significant.

Table 1: Prevalence of Candida species in clinical specimens

Clinical specimens	Total No. screened	Total No. of isolates	Percentage
HVS	360	42	11.7
Sputum	300	21	7.0
Oral Swabs	60	12	20.0
Pus	330	6	1.8
Urine	550	6	1.1
Ear Swabs	15	5	33.3
Nail	10	5	50
Stool	40	5	10.0
Conjunctival swabs	5	1	20.0

Blood	32	1	3.1
Total	1702	103	6.1

$\chi^2 = 77.43, p < 0.0001$, Significant

Hundred and three (103) samples out of the 1702 tested positive for candida species giving (6.1%)

Table 2: Different species of candida isolated

Organism	Total isolates (103)	Percentage
C. albicans	67	65.0
C. tropicalis	25	24.3
C. krusei	11	10.7
Total	103	100.0

Candida albicans was the major species accounting for 67(65%) of the total isolates. non albicans candida constituted 25 (24.3%) of C. tropicalis, followed by C. krusei 11(10.7%). All the candida species, isolated from various clinical specimens which were characterized by conventional method, could be identified by CHROM agar.

Table 3: Distribution of isolates among various predisposing factors

Predisposing Factor	Male	Female	Total (%)
DM	18	16	34(33%)
Pregnancy	0	23	32(22.3%)
Prolonged drug intake and secondary to other disease (PD and SD)	12	11	23 (22.3%)
HIV	4	2	6(5.8%)
Prolonged contact with	3	2	5(4.9%)
Others	4	8	12 (11.7%)
Total	41	62	103 (100%)

$\chi^2 = 0.772, p = 0.3$, Not Significant

In the study, diabetes mellitus was the major predisposing factor constituting 34(33%) followed by pregnancy 23(22.3%), prolonged contact with water 5(4.9%). Other 12(11.7%) in predisposing factors include, catheterization, stay in ICU, parenteral nutrition, prematurity and low socio-economic status, poor personal hygiene. Even though there is female predominance as compared to male, it is statistically not significant ($p > 0.05$)

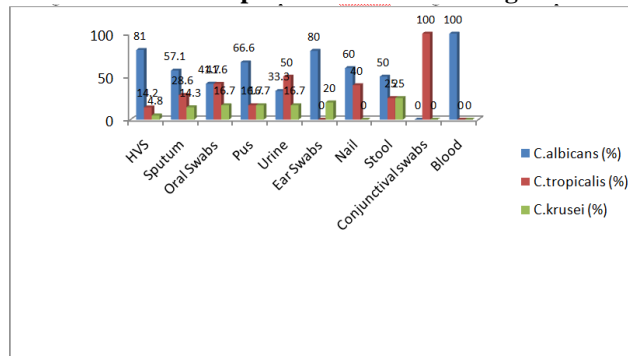
Table-4: Distribution of different species of candida among various clinical specimens

Clinical specimens	C.albicans (%)	C.tropicalis (%)	C.krusei (%)	Total (%)
HVS	34 (81)	6 (14.2)	2 (4.8)	42 (40.8)
Sputum	12 (57.1)	6 (28.6)	3 (14.3)	21 (20.4)
Oral Swabs	5 (41.7)	5 (41.6)	2 (16.7)	12 (11.7)
Pus	4 (66.6)	1 (16.7)	1 (16.7)	6 (5.8)
Urine	2 (33.3)	3 (50)	1 (16.7)	6 (5.8)
Ear Swabs	4 (80)	0	1 (20)	5 (4.9)
Nail	3 (60)	2 (40)	0	5 (4.9)
Stool	2 (50)	1 (25)	1 (25)	4 (3.9)
Conjunctival swabs	0	1 (100)	0	1 (1)
Blood	1 (100)	0	0	1 (1)
Total	67	25	11	103

$X^2 = 8.697, p = 0.013$, significant

The highest number of isolates was from high vaginal swab isolated from vulvovaginitis constituting 42(40.8%). The other major samples were sputum 21(20.4%), oral swabs 12 (11.7%), urine 6(5.8%), pus 6(5.8%), nail scrapings 5(4.9%), ear swabs 5(4.9%), followed by stool 4(3.9%) and blood 1(1.67%).

Figure -1: Distribution of different species of candida among various clinical specimens



Among 6 urine samples, 2(33.3%) were C.albicans, 3(50%) were C.tropicalis and 1(16.7%) was Candida krusei.

Among 5 nail scrapings, 3(60%) were C.albicans and 2(40%) were C.tropicalis.

Among 5 ears swabs, 4(80%) were C.albicans, 1(18.18%) one was C.krusei (20%)

Among 4 samples of stool, 2(50 %) species were C.albicans and one (25%) each of C.tropicalis and C.krusei.

Table-6: Antifungal Susceptibility Pattern.

Species	Amphotericin B			Nystatin			Fluconazole			Ketoconazole			Clotrimazole		
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
C. albicans	66	0	1	66	1	0	62	4	1	66	0	1	62	0	5
C. tropicalis	25	0	0	24	1	0	24	0	1	25	0	0	23	0	2
C. krusei	11	0	0	9	2	0	0	0	11	11	0	0	10	0	1
Total	102	0	1	99	4	0	86	4	13	102	0	1	95	0	8
χ^2 Value	0.526			3.13			11.37			0.526			0.02		
p-value	0.99			0.056			0.001			0.99			0.87		

In the present study, among 103 Candida isolates tested, Ketoconazole and Amphotericin B showed resistance in 0.97% cases.

For Nystatin, no strains showed resistance and 3.88% strains showed intermediate sensitive.

For clotrimazole, 92.23% strains showed sensitive, 7.76% strains were resistant and among them 4.85% were C. albicans, 1.97% for C. tropicalis, and 0.97% for C. krusei.

For fluconazole, 83.49% strains showed sensitive, 12.6% showed resistance and 3.88% showed intermediate sensitivity (or dose dependent susceptibility) (p= 0.001)

Amphotericin B and Ketoconazole are more sensitive followed by Nystatin, Clotrimazole and Fluconazole. Resistance is seen significantly among non-albicans Candida for Fluconazole (p=0.001) and Nystatin showed intermediate sensitive (p=0.056).

IV. Discussion:

The study identified the specific species of Candida that causes infections among patients who attended a tertiary care hospital in North-interior Karnataka, India. The study also determined the in vitro susceptibility of Candida species isolated from the various clinical samples. The antifungal agents tested include Amphotericin B, Nystatin, Fluconazole, Ketoconazole and Clotrimazole.

Candida albicans is by far the most common species causing infection in humans. Candida albicans and non-albicans species are closely related but differ from each other with respect to epidemiology, virulence characteristic and antifungal susceptibility. Distinction between species facilitates the understanding of epidemiology of Candida species particularly regarding the reservoir and mode of transmission which is requirement for the development of effective measures to prevent and control transmission of resistant pathogens.

In the present study it was found that candidiasis can occur at all ages and in both sexes. The youngest in our study was one month old baby while the oldest was 79 years and mean age was 37.3 years.

According to study conducted by Dalal et al¹⁷, Akorth et al,⁷ Puri KJ et al¹⁸, Jayalaxmi et al¹⁹, maximum cases were in the age group of 21-40 years.

In the present study, females are more commonly affected than males with an incidence of 62 (60.2%) and 41 (39.8%) respectively in a ratio of 0.66:1(M:F). In a similar study by Kandhari KC et al²⁰ at AIIMS, New Delhi, the incidence in females was about 61.2% while in males it was only 38.8% with a ratio of 1:1.57(M:F) and Rizvi MW et al^{5also} reported female preponderance in their study group with a ratio of 0.85:1(M:F).

In the present study, Candida species isolated from various clinical samples and the highest was from high vaginal swab (40.8%), followed by sputum (20.4%), oral thrush (11.7%), urine (5.8%), pus (5.8%), nail scrapings (4.9%), ear swabs (4.9%), stool (3.9%), blood (1%) and conjunctival swab (1%).

In this study Candida isolated from 20.4% sputum sample which correlates with the study done by yang et al²³, who isolated Candida from 16% of sputum sample)

Dastjdar et al²², in their study found oral thrush in 12% of patients while in our study 11.6% which is similar to the

The predominant species isolated in our study was Candida albicans constituting 65%. The non-albicans Candida isolated are Candida tropicalis (24.3%) followed by Candida krusei (10.7%).

Yang et al²³ in Taiwan reported C.albicans (69.1%) as the most common species, followed by C.tropicalis (12.9%) and C.krusei was isolated from 0.6% cases. In a study by Basu et al²¹ in Delhi, the C.albicans (45.8%) was the predominant isolate followed by C.tropicalis (24.7%), C.krusei (7%). This study is similar to above studies.

Vijaya D et al²⁵ isolated 102 clinical isolates of Candida species from various clinical specimens. The candida species isolated include candida albicans (45.9%), C.tropicalis (35.29%) and C.krusei (10.78%). In this study C.albicans isolated in 65% cases, a proportion similar to that found by Yong et al²³.

In the present study, the results of CHROM agar were exactly paralleled to that of conventional method.

In this study, diabetes mellitus was the most important predisposing factor, accounting for 33% of the total cases, followed by pregnancy (22.3%), prolonged drug intake and secondary to other disease infections which accounted for 22.3% of cases, HIV (5.8%), prolonged contact with water (4.9%) and other predisposing factors constitutes for 11.7% cases.

In our study, candida species were isolated from patients on prolonged corticosteroids, broad-spectrum antibiotic use and secondary to other diseases accounted for 22.3% of cases. Among the candida species isolated, 69.7% were C. albicans, 17% were C.krusei, and 13 were C.tropicalis.

Arora D et al² reported that, 35% of candidiasis was due to prolonged use of antibiotics. Pitter D et al²⁶, in his study, showed that 19 of 29 patients (66%) developed candida infection, who received more than two antibiotics before candida infection or some time during their stay within the critical care units.

In present study 6 cases are attributed to HIV Infection, C.tropicalis was isolated from 4 patients and C.albicans isolated from 2 cases.

In a study done by Kapoor et al²⁴ isolated candida species from various clinical samples from 6 AIDS patients, 3 (50%) were C.albicans and 3 (50%) were C.tropicalis. but Baradkar VP et al¹² found C.albicans (70%) as predominant species followed by C.tropicalis in HIV patients with oral candidiasis.

In the present study, candida isolated from 4.8% cases of onychomycosis.

Sehgal et al²⁸ had observed in their study that nail plate can be involved secondarily in candidiasis and the incidence to be more common in housewives and in professions such as fruit sorters, canners, bartenders and dishwashers.

Other comprised of 11.7% in predisposing factors which include, catheterization, stay in ICU, parenteral nutrition, prematurity, poor socioeconomic status, poor personal hygiene and oral contraceptive.

Antifungal susceptibility testing:

Monitoring antifungal resistance among Candida is useful because apart from tracking and detection of resistance, it also gives clues to emerging threats of new resistant strains. This helps in assessing empirical treatment recommendation.

In present study, among 103 candida isolates tested, Ketoconazole and Amphotericin B showed resistance in 0.97% cases. For Nystatin, no strains showed resistance and 3.88% strains showed intermediate sensitive, 12.6% showed resistance and 3.88% strains showed intermediate sensitive. For clotrimazole, 92.23% strains showed sensitive, 7.76% strains were resistant and among them 4.85% were C.albicans, 1.97% for C.tropicalis, and 0.97% for C.krusei. for fluconazole, 83.49% strains showed sensitive, 12.6% showed resistance and 3.88% showed intermediate sensitivity (or dose dependent susceptibility) (p=0.001)

Sobel et al²⁹ reported no fluconazole resistance among the 401 Candida albicans recovered from candidal vaginitis in the USA reported fluconazole resistance to be 3.6% among Candida albicans.³⁰

Finding from this study indicates that all the isolates of Candida krusei were resistant to fluconazole. It is well established that Candida krusei is intrinsically resistant to fluconazole. This was also confirmed by Hamza et al³¹ who reported in Tanzania that all isolates of Candida krusei tested were resistant to fluconazole.

Vijaya et al²⁵ reported 100% sensitive to Amphotericin B, Clotrimazole, Nystatin and Ketoconazole and 25% of *C.krusei*, 28% *C.tropicalis* showed resistance to fluconazole.

To conclude, vulvovaginal candidiasis was the most common clinical type of candidiasis. Commonest age group involved was 20- 49 years and females are more affected than males.

Candida albicans was the most frequently encountered species in the present study, there is rise in the non-*Candida albicans* isolates from various clinical conditions. the results of CHROM agar were exactly paralleled to that of conventional method. Even though most of the yeasts tested were susceptible to the antifungal agents, periodic antifungal susceptibility testing should be carried out according to CLSI guidelines.

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