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Research Article

Isolation, Speciation and Antifungal Susceptibility Testing of Candida from Clinical Specimens at a Tertiary Care Hospital

Dr. Jayalakshmi L*[1], Dr G. RatnaKumari[2], Dr S.H. Samson[3]
[1]Associate Professor of Microbiology, Osmania Medical College, Hyderabad, India
[2]Associate Professor of Microbiology, Andhra Medical College, Visakhapatnam, Andhra Pradesh, India
[3]Post Graduate in Microbiology, Andhra Medical College, Visakhapatnam, Andhra Pradesh, India

***Corresponding author**
Dr. Jayalakshmi L
Email: jayalingam12@yahoo.com

Abstract: The occurrence of infections due to various species of Candida has increased as a result of increase in favourable factors for opportunistic infections. Identification of species of Candida and their susceptibility patterns to antimycotic drugs can be helpful in the management of these infections. The aim of the study is to speciate Candida isolates from various clinical specimens at a tertiary care hospital and to study their susceptibility to antifungal agents. The materials for the study include one hundred and five isolates of Candida in hundred different clinical specimens from hospitalized chronic ill patients. Speciation of Candida isolates was done by germ tube test, morphotyping using cornmeal agar & HICHROME Candida agar and biotyping using a battery of sugar assimilation and fermentation tests. The antifungal susceptibility testing was performed using disc diffusion method. Results showed that among the hundred specimens double species of Candida were isolated from five specimens. Twenty six isolates were from HIV reactive patients and eighteen were from diabetic patients. Most common isolate among all species of Candida was *C. albicans* (31.42%) followed by *C. tropicalis* (26.66%). Except *C. kefyr* all other isolated Candida species were susceptible to Amphotericin – B and Nystatin. Ketoconazole was the next effective drug with 83.8% susceptibility. 34.2% resistance was observed with fluconazole. To conclude resistance to antifungal drugs was observed in various isolated species of Candida and highest resistance was to fluconazole.
Keywords: Candida, Speciation, Morphotyping, Biotyping, Non albicans Candida

INTRODUCTION

Candidiasis is caused by various species of yeast like fungi belonging to the genus Candida with C.albicans as the representative species. It is found mainly as secondary infection in individuals with some underlying immunocompromised condition and very rarely as the primary disease [1, 2]. The favourable factors for opportunistic Candidial infection are physiological (age, pregnancy), endocrinological (diabetes), immunological and iatrogenic factors like the advancements in medical management of patients like long term intravenous therapy, long term antibiotic use, immunosuppressive therapy, inadequate catheter care etc. [3, 4].

Recovery of yeasts from normally sterile body fluids (blood, cerebrospinal fluid, etc), recovery from patients whose defenses were compromised from chronic diseases and repeated recovery from multiple specimens certainly indicates infection with the yeasts [4]. Thus identification of yeasts isolated from clinical specimens up to species level and their antifungal susceptibility testing has become increasingly important and can be helpful for the management of these infections.

The objective of the present study was to speciate the isolates of Candida from various clinical specimens of hospitalized chronic ill patients received at a tertiary care hospital and to determine their antifungal susceptibility.

MATERIALS AND METHODS

The material for study included one hundred and five isolates of Candida from one hundred different clinical specimens collected from patients in intensive care units with intravenous therapy, chronic respiratory tract infections including tuberculosis, patients with post surgery, sepsis, dialysis, PUO on long term therapy, leucorrheic patients. The associated condition like HIV was seen in 26 cases and diabetes mellitus in 18 patients.

The specimens showing Candida isolation in two consecutive samples were included in the study and the specimens included were blood, pus, wound swabs, vaginal/ cervical swabs, sputum, corneal ulcer, urine, stool, throat swabs.

The yeast like colony growth on culture showing budding yeast cells in Gram's staining method were confirmed as Candida by negative urease test. Germ tube test was performed on all urease negative yeast isolates for presumptive identification of *C. albicans* [3]. Acid & gas production in sugar fermentation tests using Glucose, Maltose, Sucrose and Lactose in 2% concentration with Andred's indicator and Durham's tube were noted [3, 5].

For sugar assimilation test, discs with 4% sugars were prepared and placed on inoculated yeast nitrogen base agar and incubated at 30°C for 48 hours. Presence of growth around the disc indicates assimilation of that carbohydrate. The sugars used for assimilation testing were glucose, sucrose, lactose, maltose, trehalose, raffinose, galactose, cellobiose, melibiose and dulcitol (Fig. 1). The pattern of sugar assimilation and fermentation were used for species identification of Candida[3,4].

Morphotyping of Candida was performed using cornmeal agar inoculated by Dalmau plate method, incubated at 30°C for 2-5 days and studied microscopically for the presence of pseudohyphae, chlamydospores&blastospores (Fig. 2).

Inoculated chromogenic HICHROME Candida agar was studied after 48 - 72 hrs incubation at 30°C and the colour of the growth was used for speciation of Candida according to the manufacturer instructions (Fig. 3).

Antifungal susceptibility testing was performed for all the isolates of Candida using disc diffusion method on Mueller Hinton agar supplemented with 2% glucose and 0.5 µg / ml of methylene blue [6] (Fig. 4). The commercially available antifungal discs were used and zones of inhibition were measured after 24 – 48 hours incubation at 37°C. The antifungal discs used were Amphotericin – B (20 µg), Clotrimazole (10 µg), Fluconazole (10 µg), Itraconazole (10 µg), Ketoconazole (10 µg) and Nystatin (100 units). *C. albicans* MTCC3017, equivalent to ATCC90028 was used as quality control strain.

RESULTS

In the present study 105 isolates of Candida were obtained from 100 specimens as five specimens yielded double isolates of Candida that included three sputum specimens, one specimen each of urine and vaginal swab (Table 1). The sputum specimens from various respiratory tract infections (tuberculosis, pneumonia, chronic bronchitis, pleural effusion) yielded highest number of Candida isolates (38) followed by throat swab & swabs from apthous ulcers (26), vaginal swabs from leucorrhea cases (12). All these patients were on prolonged antibacterial use and intravenous therapy.

Table 1: Distribution of specimens and Candida isolates

Sl. No	Specimen	Male	Female	No. of Candida Isolates
1	Sputum – 33	17	16	36
2	Urine – 29	11	18	30
3	Blood – 13	06	07	13
4	Pus – 07	02	05	07
5	Vaginal swab- 06	--	06	07
6	Throat / oral swab – 09	06	03	09
7	Corneal scrapings – 02	--	02	02
8	Stool – 01	01	--	01
	Total – 100	43	57	105

Non albicans Candida (NAC) isolation was higher (68.57%) than *C. albicans* (31.42%). Among all species of Candida commonest isolate was *C. albicans* (31.42%) followed by *C. tropicalis* (26.66%). Other species isolated were *C. glabrata* (19.04%), *C. parapsilosis* (10.47%), *C. krusei* (5.71%), *C. kefyr* (4.76%) and *C. guilliermondii* (1.9%) (Table 2).

Table 2: Species distribution of Candida isolates (n=105)

Candida species	No. of isolates	Percentage
Candida albicans	33	31.42%
C. tropicalis	28	26.66%
C. glabrata	20	19.04%
C. parapsilosos	11	10.47%
C. krusei	06	5.71%
C. kefyr	05	4.76%
C. guilleirmondii	02	1.9%
Total	105	

Species identification was done by biotyping (sugar assimilation and sugar fermentation methods) and cornmeal agar morphology. The colour of growth on Hichrome Candida agar helped in identifying double isolates in the primary growth.

All the species of Candida except *C.kefyr* were susceptible to Amphotericin B and Nystatin (Table 3). Ketoconazole was the next effective drug with 83.8% susceptibility followed by itraconazole 75.23% and clotrimazole 71.42%. According to the zone interpretative chart for Candida isolates[6] the commonly used antifungal drug fluconazole showed only 54.2 % susceptibility, 34.2% resistance and 5.7 % dose dependent susceptibility.

Table 3: Antifungal susceptibility pattern of the isolates of Candida(n=105)

Species of Candida	Amphotericin B		Clotrimazole		Fluconazole			Itraconazole		Ketoconazole		Nystatin	
	S	R	S	R	S	SDD	R	S	R	S	R	S	R
C. albicans (n= 33)	33 (100%)	--	30 (90.9%	3 (9.0%)	28 (84.8%	1 (3.0%)	4 (12.1%	31 (93.9%	2 (6.06%	32 (96.9%	1 (3.03%	33 (100%)	--
C. tropicalis (n= 28)	28 (100%)	--	15 (53.5%	13 (46.4%	8 (28.6%	2 (7.1%)	18 (64.3%	19 (67.9%	9 (32.1%	23 (82.1%	5 (17.9%	28 (100%)	--
C. glabrata (n= 20)	20 (100%)	--	13 (65%)	7 (35%)	10 (50%)	1 (5%)	9 (45%)	12 (60%)	8 (40%)	17 (85%)	3 (15%)	20 (100%)	--
C. parapsilosis (n=11)	11 (100%)	--	10 (90.9%	1 (9.09%	7 (63.6%	1 (9.1%)	3 (27.3%	10 (90.9%	1 (9.09%	10 (90.9%	1 (9.09%	11 (100%)	--
C. krusei (n= 6)	6 (100%)	--	--	6 (100%)	---	---	---	1 (16.7%	5 (83.3%	1 (16.7%	5 (83.3%	6 (100%)	--
C. kefyr (n= 5)	3 (60%)	2 (40 %)	5 (100%)	--	2 (40%)	1 (20%)	2 (40%)	4 (80%)	1 (20%)	3 (60%)	2 (40%)	4 (80%)	1 (20%
C. guilliermondii (n= 2)	2 (100%)	--	2 (100%)	--	2 (100%)	--	--	2 (100%)	--	2 (100%)	--	2 (100%)	--
Total	103	2	75	30	57	6	36	79	26	88	17	104	1
Percentage	97.1	2.8	71.4	28.5	54.2	5.7	34.2	75.2	24.7	83.8	16.2	99	0.95

S = sensitive, R = resistant, SDD = susceptible dose dependent

The predominant isolate among non albicans Candida group, *C. tropicalis* was showing least susceptibility to fluconazole (28.6%). Susceptibility of C.kefyr isolates to fluconazole was 40%, *C. glabrata* was 50% and *C. parapsilosis* was 63.6%.

In the present study both the isolates of *C. guilliermondii* were susceptible to all the antifungal agents tested. *C. parapsilosis* was showing 100% susceptibility to Amphotericin – B & Nystatin and 90.9% susceptibility to clotrimazole, itraconazole& ketoconazole, but only 63.63% susceptibility to Fluconazole.

All the 6 isolates of *C. krusei* were resistant to clotrimazole and 83.33% were showing resistance to both itraconazole and ketoconazole. *C. kefyr* isolates were showing 40% resistance to Amphotericin – B, fluconazole and ketoconazole.

In the present study highest resistance of *C. albicans* and *non albicans Candida* was towards Fluconazole which was 12.12% and 44.44% respectively (Table 4).

Table 4: Showing susceptibility pattern among C.albicans and Non albicans Candida

Isolate	AP		CC		FLC			IT		KT		NY	
	S	R	S	R	S	Sdd	R	S	R	S	R	S	R
C.albicans (n=33)	33	--	30	3	28	1	4	31	2	32	1	33	--
Percentage	100		90.9	9.0	84.8	3.0	12.1	93.9	6.1	96.9	3.0	100	--
Nonalbicans Candida (n=72)	69	3	51	21	29	5	32	48	24	56	16	71	1
Percentage	95.8	4.2	70.8	29.2	40.3	6.9	44.4	66.7	33.3	77.8	22.2	98.6	1.4

AP= Amphotericin – B, CC= Clotrimazole, FLC= Fluconazole, IT= Itraconazole, KT= Ketoconazole, NY= Nystatin, S = sensitive, R = resistant, Sdd = susceptible dose dependent.

Fig. 1: Carbohydrate assimilation test on Yeast Nitrogen Agar

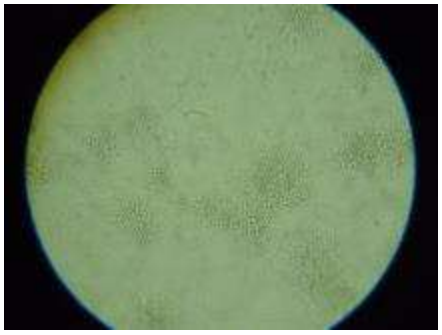

Fig. 2c: Short, fine pseudohyphae with small blastospores – *C. guilliermondii*

Fig. 2a:Dalmau plate culture on corn meal agar

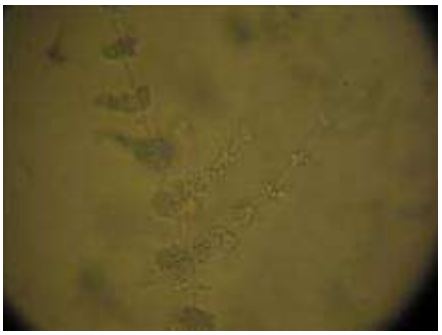

Fig. 2d: Long branching pseudohyphae, clusters of blastospores – *C. tropicalis*

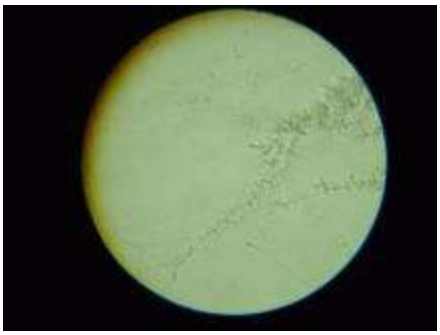

Fig. 2b: Many pseudohyphae, blastospores and few terminal chlamydospores – *C. albicans*

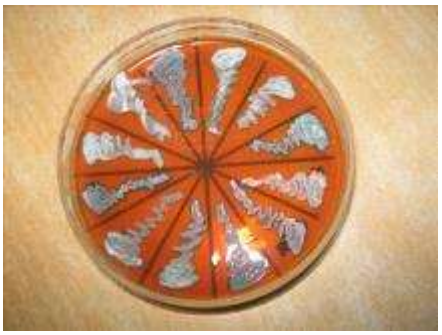

Fig. 3: Growth on HICHROME Candida agar

Fig. 4: Antifungal susceptibility testing of Candida isolates

DISCUSSION

Candida species colonise the mucosal surfaces of all humans soon after birth [3]. Increasing incidence of iatrogenic Candida infections and the infections in immunocompromised and immunosuppressed individuals was due to the collective role of the fungal virulence factors and host susceptibility. In the immunocompetent host several conditions predispose to fungal infections like prolonged antibacterial therapy, corticosteroid use, integumentary breach as in intravenous or intra arterial catheters, surgical procedures, poor nutritional status and metabolic derangements [3]. The extensive use of antimycotic drugs for prolonged therapeutic courses led to change in the relative prevalence of various species of Candida [1, 7]. Many studies in the past decade showed the isolation of various species of Candida and increase in isolation of *non albicans Candida* in these situations and similar results were obtained in the present study (Table 5).

Antifungal susceptibility testing by disc diffusion method for yeasts was established in 2003 according to the CLSI document M44-A [6] and since then many studies were undertaken to find the development of resistance among Candida species and in the present study 34.2% resistance to fluconazole was observed (Table 6).

Table 5: *C.albicans* and *Non-albicans Candida* spp. isolation in different studies

Author	Year	*C. albicans*	*Non-albicans Candida*
Kaviarasan *et al.* [8]	2002	60.5 %	39.5 %
Resende *et al.* [9]	2002	51 %	49 %
M A Pfaller *et al.* [10]	2003	57.7 %	42.3 %
Shaheen MA *et al.* [11]	2006	56 %	44 %
Vaishali Wabale *et al.* [12]	2008	76 %	24 %
R Adhikary *et al.* [13]	2011	26.4 %	73.6 %
Present study	2011-12	31.42 %	68.57 %

Table 6: Comparative study of Fluconazole resistance

Author	Year	Fluconazole resistance
MA Pfaller *et al.* [14]	2007	9.9 %
Parisa Badiee *et al.* [15]	2010	8.4 %
Shivanand Dharwad *et al.* [16]	2011	4.3 %
R Adhikary *et al.* [13]	2011	25 %
Present study	2011-12	34.2 %

CONCLUSION

Identification of yeasts isolated from clinical specimens up to species level has become increasingly important for the diagnostic laboratory as the changing epidemiology of Candida infections highlights the need for monitoring of species distribution and susceptibility of Candida in order to optimize therapy.

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