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Speciation and Antimycotic Susceptibility Pattern of *Candida* Species Isolated from Various Clinical Specimen by Using Chromogenic Agar and Conventional Method

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A B S T R A C T

A total of 100 isolates of candida species were recovered from various clinical samples. *C. albicans* was the most common isolated species (42%) followed by *C. krusei* (17%) *C. tropicalis* (15%) *C. parapsilosis* (13%) *C. famata* (8%) *C. glabrata* (4%) and *Cryptococcus* species (1%). Non albicans Candida were isolated at a higher rate (58%) than *C. albicans*. Most of the Candida isolate were susceptible to Amphotericin (81%) followed by Nystastin (79%) and Miconazole (58%). Fluconazole was least effective with 79% resistant. So goal of the study was to show there is an increase in the incidence of No nalbicans *Candida* with antifungal resistant strain of *Candida* species underlines the need of early and accurate diagnosis of infecting *Candida* species along with antifungal susceptibility testing for selecting the most appropriate antifungal agent for therapy.

Introduction

Over the last few years, the incidence of mycotic infection has progressively increased. Fungi once considered as non-pathogenic or less virulent are now recognized as primary cause of morbidity and mortality in immune-compromised and severely ill patients Mokaddas *et al.,* (2007).

Candidiasis is the commonest fungal disease found in human. The infection may be acute or chronic, superficial or deep and its clinical spectrum is wide. It is found mainly as secondary infection in individual with some underlying immune compromised condition and very rarely as primary disease.

Non albicans Candida species are emerging pathogens and can also colonize human mucocutaneous surfaces and invades tissues, leading to life threatening disease in patients whose cell mediated immunity is decreased by disease or iatrogenic intervention (Ajello, 1997; Verma, 2003; Akpam, 2002 and Al-Abeid *et al.,* 2004, prolonged use of antimicrobial drugs, diabetes, chemotherapy and catheterization (Ali Zarei, 2013; Anil K Paswan, 2012; Anil, 1997 and Baradkar *et al.,* 1996).

Although *C. albicans* remains the most common cause of human candidiasis, now for the past four decades Non albicans *Candida* species like *C. glabrata*, *C. krusei*, *C. parapsilosis*, *C. tropicalis*, *C. rugosa* are emerging as important opportunistic pathogens which have shown increased resistance to anti-fungal agents Abi-said *et al.*, (1997). These Candida species differ in their expression of putative virulence factors and antifungal susceptibility Baillie and Douglas (1998).

Accurate species identification is therefore important for the treatment of Candida infection as the Non albicans *Candida* species of Candida continued to be increasingly documented as not all the species respond to the same treatment.

This study has been undertaken to isolate and Speciated Candida species from the various clinical specimens and to study the distribution of *Candida albicans* and Non albicans Candida species in clinical specimens and to determine the anti-fungal susceptibility pattern of Candida species.

Materials and Methods

The study was conducted in the Microbiology lab of Mahatma Gandhi Hospital Dr. S.N. Medical College, Jodhpur. A total of 100 Candida species isolated from various clinical specimens including urine, pus, sputum, stool and bronchoalveolar lavage (BAL) were taken up for the study over a period of one year from out patients and in patients admitted into various wards and intensive care units.

Specimen processing

The various clinical specimens were collected and processed as per standard microbiological guidelines. The primary inoculation of specimens was done on Blood agar and MacConkey agar medium. The culture plates were incubated aerobically at 37^0 C for 24-48 hours. The visual growth is stained and one which revealed gram positive budding yeast cells with or without pseudo hyphae were confirmed as yeast. All the isolated candida were inoculated immediately on Sabouraud Dextrose Agar (SDA) and incubated at 37^0 C for 24-48 hours. Cultures were identified by the colony characters and by gram's stain. Once the colonies were confirmed speciation done by the following methods (Segal *et al.*, 2007; Rippon, 1988; Milne, 2007).

Germ Tube Test: (Reynolds Braude Phenomenon) (Milne, 2007; Forbes *et al.*, 2007).

CHROM (HICHROME) Agar Candida- isolated species were inoculated on Hi CHROM Agar plates. These agar plates were incubated at 37^0 C for 24-48 hours. The species were identified by characteristic colony colour as per Hi Media technical data (Sagar *et al.*, 2013; Shyamala K. Shetter *et al.*, 2012).

Corn meal agar inoculation-Formation of chlamydospores was identified by Dalmau plate culture method in Corn meal agar with 1% tween-80 incubated for 2- 3 days at room temperature (Ann P. Koehler *et al.*, 1999) Observed for the presence of true hyphae or pseudohyphae, blastoconidia, arthroconidia and Chlamydospores

Sugar fermentation test (Arunaloke Chakrabarti. *et al.*, 2008)

Sugar Assimilation Test (Arunaloke Chakrabarti *et al.*, 2008; Larone, 2002)

Urease test.

Antimycotic Susceptibility test was done by Kirby- Bauer disc diffusion method as recommended by CLSI M-44A guidelines on methylene blue Mueller Hinton agar using commercially available.

The antifungal discs (Hi Media Mumbai, India) used for disc diffusion method were Fluconazole (10mcq), Voriconazole (1mcq), Itraconazole (10mcq), Amphotericin B

(100mcq), Ketoconazole (10mcq), Nystatin (100mcq) and Miconazloe (50mcq). The quality control test was performed by using *C. albican* (ATCC90028) and *C. parapsilosis* (ATCC22019).

Results and Discussion

A total of 100 Candida species isolated from various clinical specimens like urine, sputum, pus, bronchoalveolar lavage (BAL) and stool were processed during the study period (Table 1).

The study showed that female (51%) were more prone to candida infection than male (49%) (Table 2). Consisted with study of Rizwi *et al.,* (2011) reported female preponderance in their study group with ratio of 0.85:1 (M:F). Female indicating that women are at increased risk to develop UTI than men (Koneman *et al.,* 2006). However in the study by Shanoo *et al.,* (2017) the incidence was found to be higher in male (51%).

In all the 100 specimens included in this study the most common clinical specimen was urine 49 (49%) followed by sputum 39(39%), pus 9(9%), bronchoalveolar lavage (BAL) 2 (2%) and stool 1 (1%) (Table 3). Our observation is similar with the study of Deorukhkar *et al.,* (2014) where urine samples were in majority (34.6%) and Patel *et al.,* (2012) where urine showed the highest number of isolates (34.5%) followed by sputum (28.9%).

In the present study Non albicans Candida were isolated at a higher rate (58%) than *Candida albicans* (42%) similar finding were observed in study by Shanoo *et al.,* (2017) showed Non albicans Candida (58%) and *Candida albicans* (42%), Mokaddas *et al.,* (2007) which also showed the Non albicans candida incidence (60.5%) to be higher than that of *Candida albicans* (39.5%).

Among the 58(58%) Non albicans *Candida* species *C. krusei* 17 (17%) *C. tropicalis* 15 (15%) *C. parapsilosis* 13 (13%) *C. famata* 8 (8%) *C. glabrata* 4 (4%) and Cryptococcus species 1 (1%) were isolated (Table 4). In our study *C. krusei* 17 (17%) was most common Non albicans *Candida* species followed by *C. tropicalis* 15 (15%). The present study is in agreement with study conducted by Nirmladevi *et al.,* (2018) showed *C. krusei* 7 (11%) predominant Non albicans *Candida* followed by *C. tropicalis* 4 (6%). Whereas (Latiff *et al.,* 2004) reported that *C. parapsilosis* was the most common Non albicans *Candida* species accounting for 21%, Shivprakash *et al.,* (2007) (36%) and Enwuru *et al.,* (2008) (18%) documented *C. tropicalis* was the most common Non albicans Candida species.

Sensitivityrate for Amphotericin B, Nyststin, Miconazole, Voriconazole, Itraconazole, Ketoconazole and Fluconazole were 81%, 79%, 75%, 62%, 48%, 28% and 21% respectively (Table 5). We observed that *Candida albicans* was less resistant to antifungal drugs compared to Non albicans Candida. Most of the Candida isolates were susceptible to Amphotericin B (81%) and Nystatin (79%) which is in concordance with Vijaya *et al.,* (2011), Mondal *et al.,* (2013). Fluconazole was least effective with only (21%) susceptible to it which is in concordance with Ragini *et al.,* (2011), Vijaya *et al.,* (2011), Mondal *et al.,* (2011).

All 100 *Candida* species were presumptively identified to species level by their colony morphology & colour using chromogenic medium Mokaddas *et al.,* (2007). 42/100 *Candida* species produced green coloured, 17/100 *Candida* species produced purple fuzzy coloured, 15/100 Candida species produced blue coloured, 13/100 Candida species produced white coloured, 4/100 Candida species produced off white coloured colonies.

Table.1 Gender wise distributions of *Candida* species from various clinical specimens

Gender	No.patients	Percentage
Male	49	49%
Female	51	51%

Table.2 Distribution of *Candida* species in various clinical specimens

S.NO.	Clinical specimens	Total No. of isolates
1	Urine	49 (49%)
2	Sputum	39 (39%)
3	Pus	9 (9%)
4	BAL	2 (2%)
5	Stool	1 (1%)

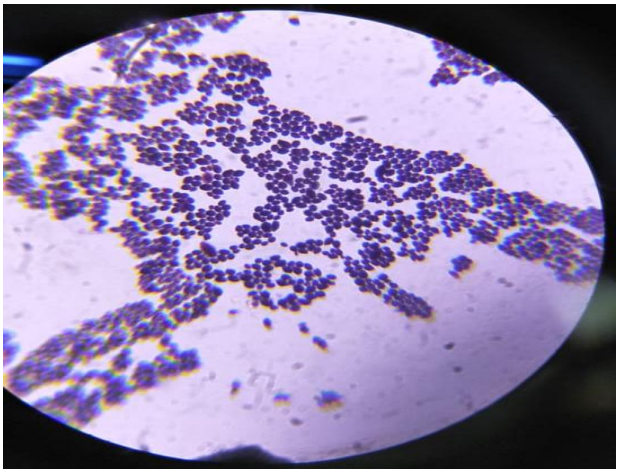
Table.3 Distribution of *Candida* species in various clinical specimens

Species	Urine (49)	Sputum (39)	Pus (9)	BAL (2)	Stool (1)	Total
C.albicans	17 (34.69%)	22 (56.41%)	3 (33.33%)	0 (0%)	0 (0%)	42 (42%)
C.krusei	9 (18.36%)	7 (17.94%)	1 (11.11%)	0 (0%)	0 (0%)	17 (17%)
C.tropicalis	6 (12.24%)	7 (17.94%)	1 (11.11%)	1 (50%)	0 (0%)	15 (15%)
C.parapsilosis	7 (14.28%)	2 (5.12%)	4 (44.44%)	0 (0%)	0 (0%)	13 (13%)
C.famata	6 (12.24%)	0 (0%)	0 (0%)	1 (50%)	1 (100%)	8 (8%)
C.glabrata	4 (8.16%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	4 (4%)
***Cryptococcus* species**	0 (0%)	1 (2.56%)	0 (0%)	0 (0%)	0 (0%)	1 (1%)

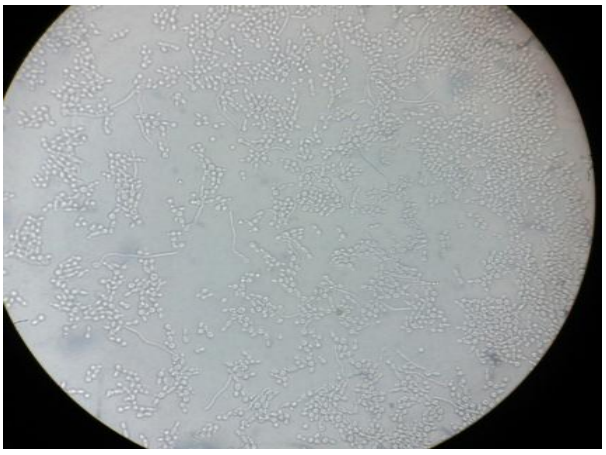
Table.4 Species distribution of the *Candida* isolated from various specimens

S.No.	Species	No. of isolates
1	*C. albicans*	42 (42%)
2	*C. krusei*	17 (17%)
3	*C. tropicalis*	15 (15%)
4	*C. parapsilosis*	13 (13%)
5	*C. famata*	8 (8%)
6	*C. glabrata*	4 (4%)
7	*Cryptococcus* species	1 (1%)

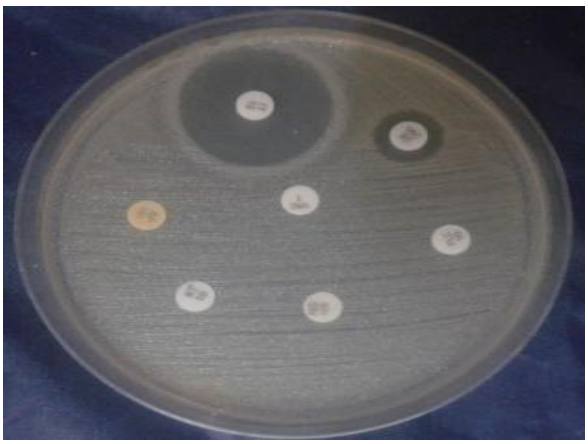
Gram positive budding yeast cells

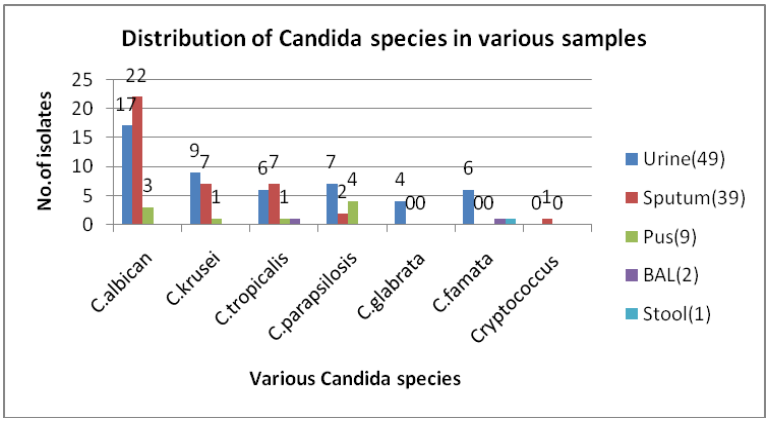


Germ Tube Test for *Candida albicans*



Antimycotic Susceptibility test





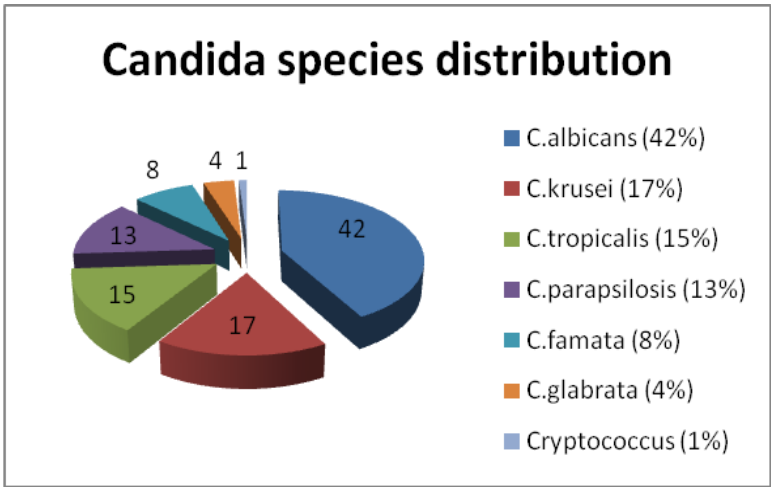
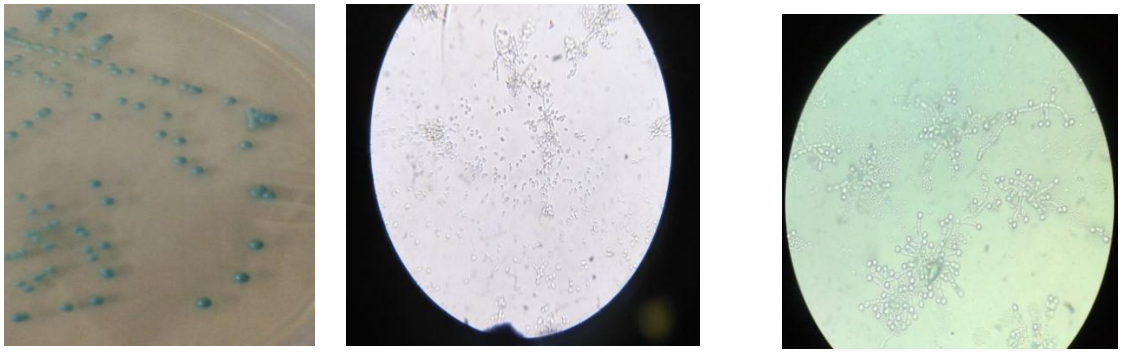


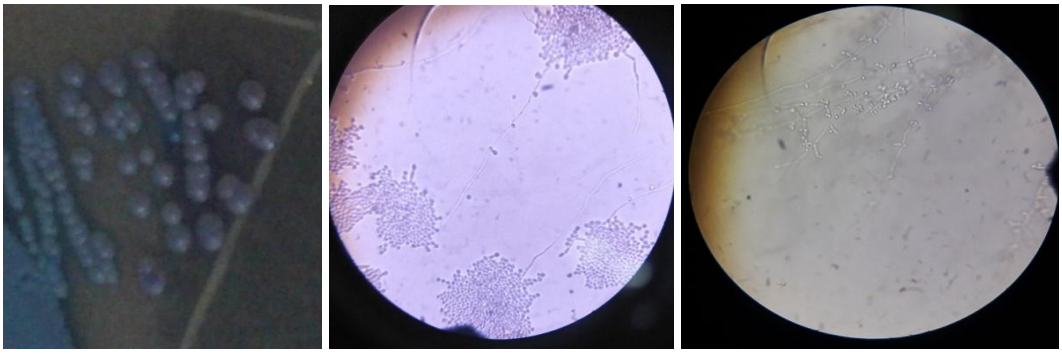
Fig.1 Morphology of *Candida albicans*



(A) **(B)** **(C)**

(A) *Candida albicans* on Hichrom agar- green, smooth colonies
(B) *Candida albicans* (Microscopic picture after 24 hours); Budding yeast and pseudohyphae
(C) *Candida albicans* (Microscopic picture after 48 hours)
Showing Chlamydospores usually terminal

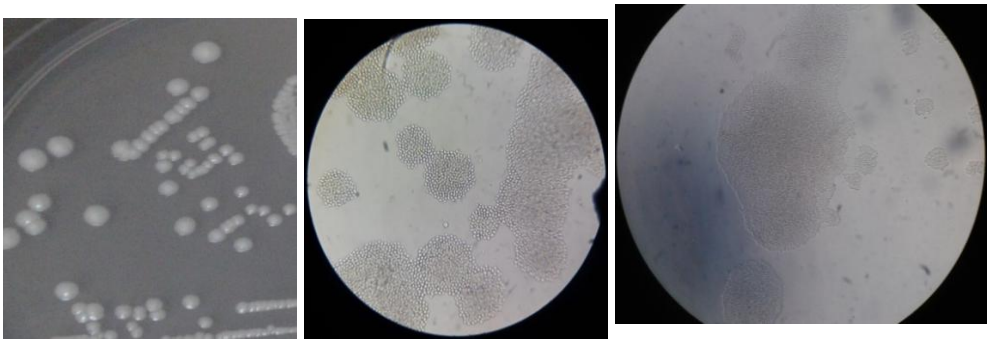
Fig.2 Morphology of *C. tropicalis*



(A) (B) (C)

(A)*Candida* tropicalis on Hichrom agar: mettalic blue colonies.
(B)*Candida* tropicalis showing lateral blastospores (Microscopic picture after 24 hours)
(C) *Candida* tropicalisshowing lateral blastospores(Microscopic picture after 48 hours)

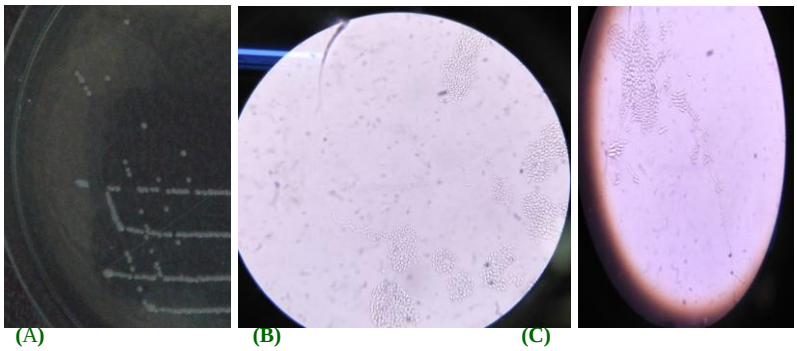
Fig.3 Morphology of C.glabrata



(A) (B) (C)

(A)*Candida* glabrata on Hichrom agar white small glossy colonies
(B)*Candida*glabrata only small budding yeast cell (Microscopic picture after 24 hours)
(C)*Candida* glabrata only yeast cells no pseudo/true hyphae (Microscopic picture after 48 hours)

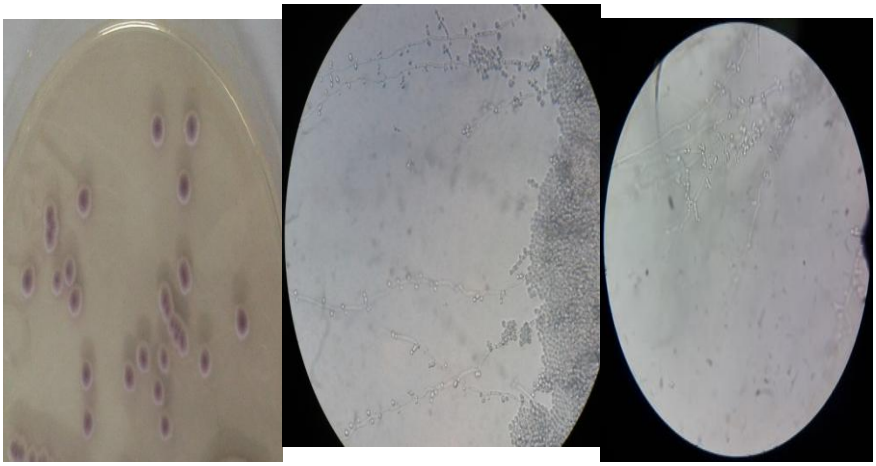
Fig.4 Morphology of C.parapsilosis



(A) (B) (C)

(A)*Candida* parapsilosis on Hichrom agar off-white large colonies;
(B) *Candida*parapsilosisshort, tree like pseudohyphae.
(Microscopic picture after 24 hours)
(C) *Candida*parapsilosis tree like pseudohyphae.
(Microscopic picture after 48 hours)

Fig.5 Morphology of *C.krusei*



(A) (B) (C)

(A)*Candida* krusei on Hichrom agar large, purple fuzzy colonies
(B)*Candida* kruseimatch stick appearance (Microscopic picture after 24 hours)
(C) *Candida* kruseimatch stick appearance (Microscopic picture after 48 hours)

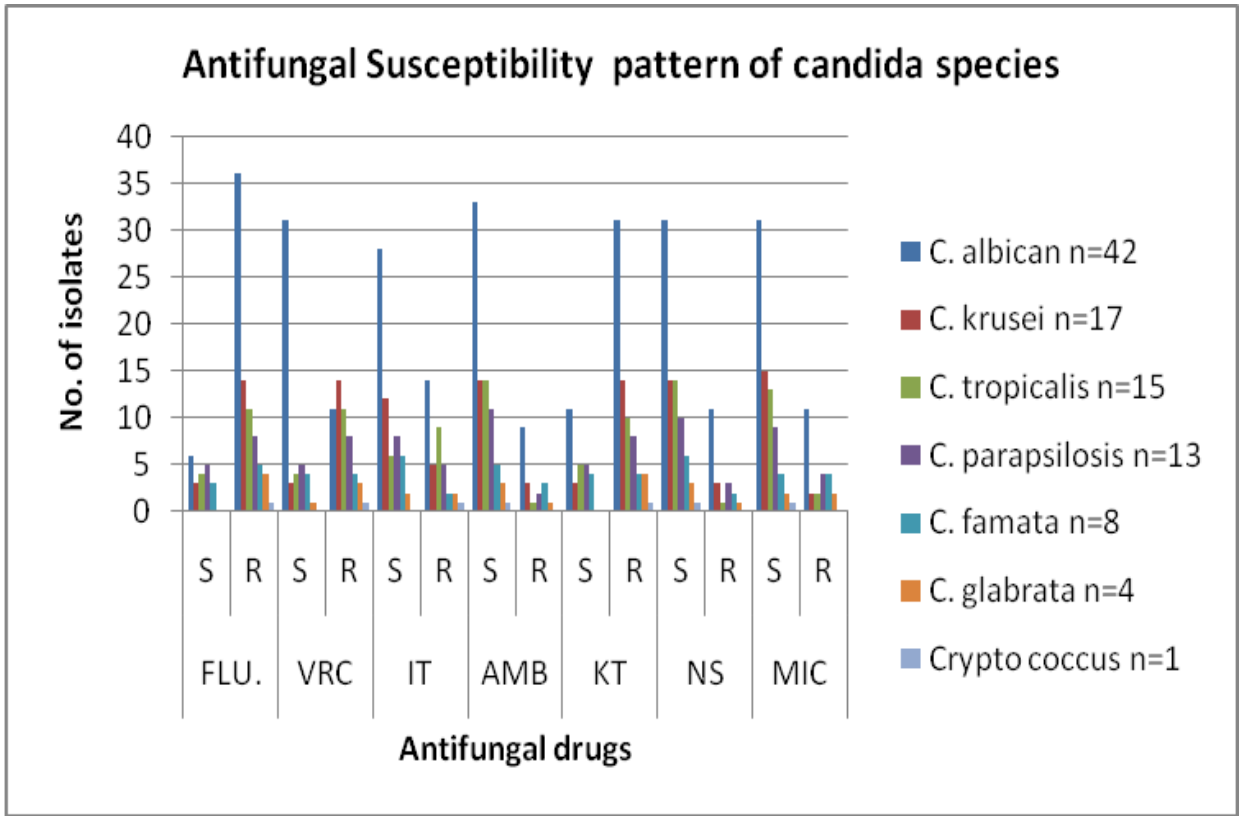


Table.5 Antifungal Susceptibility of *Candida* species

Species	FLU.		VRC		IT		AMB		KT		NS		MIC	
	S	R	S	R	S	R	S	R	S	R	S	R	S	R
C. albicans n=42	6 (14.28%)	36 (85.71%)	31 (73.80%)	11 (26.19%)	28 (66.66%)	14 (33.33%)	33 (78.57%)	9 (21.42%)	11 (26.19%)	31 (73.80%)	31 (73.80%)	11 (26.19%)	31 (73.80%)	11 (26.19%)
***C. krusei* n=17**	3 (17.64%)	14 (82.35%)	3 (17.64%)	14 (82.35%)	12 (70.58%)	5 (29.41%)	14 (82.53%)	3 (16.64%)	3 (16.64%)	14 (82.53%)	14 (82.53%)	3 (17.64%)	15 (88.23%)	2 (11.76%)
***C. tropicalis* n=15**	4 (26.66%)	11 (73.33%)	4 (26.66%)	11 (73.33%)	6 (40%)	9 (60%)	14 (93.33%)	1 (6.66%)	5 (33.33%)	10 (66.66%)	14 (93.33%)	1 (6.66%)	13 (86.66%)	2 (13.33%)
***C. parapsilosis* n=13**	5 (38.46%)	8 (61.53%)	5 (38.46%)	8 (61.53%)	8 (61.53%)	5 (38.46%)	11 (84.61%)	2 (15.38%)	5 (38.46%)	8 (61.53%)	10 (76.92%)	3 (23.075)	9 (69.235)	4 (30.76%)
C. famata n=8	3 (37.5%)	5 (62.5%)	4 (50%)	4 (50%)	6 (75%)	2 (25%)	5 (62.5%)	3 (37.5%)	4 (50%)	4 (50%)	6 (75%)	2 (25%)	4 (50%)	4 (50%)
***C. glabrata* n=4**	0 (0%)	4 (100%)	1 (25%)	3 (75%)	2 (50%)	2 (50%)	3 (75%)	1 (25%)	0 (0%)	4 (100%)	3 (75%)	1 (25%)	2 (50%)	2 (50%)
***Cryptococcus* species n=1**	0 (0%)	1 (100%)	0 (0%)	1 (100%)	0 (0%)	1 (100%)	1 (100%)	0 (0%)	0 (0%)	1 (100%)	1 (100%)	0 (0%)	1 (100%)	0 (0%)
Total n=100	21 (21%)	79 (79%)	48 (48%)	52 (52%)	62 (62%)	38 (38%)	81 (81%)	19 (19%)	28 (28%)	72 (72%)	79 (79%)	21 (21%)	75 (75%)	25 (25%)
x2	0.3613		0.0009		0.3743		0.6927		0.4512		0.7999		0.2963	

These isolates were further confirmed by germ test tube, microscopic examination of Corn Meal Agar growth, Sugar Fermentation Test and Sugar Assimilation Test and other biochemical test and confirmed the identification patterns of *Candida* species (Fig. 1, 2, 3, 4, 5). These indicate that Hichrom agar can be used at field level for rapid presumptive identification. This medium also carries the potential of improving identification of *Candida* from mixed culture.

To conclude the present study showed that *Candida albicans* was the most commonly isolated yeast from various clinical specimens and also the increase in the resistance especially to azole is a major concern.

Therefore the species level identification of *Candida* isolates and its sensitivity profile is must. More importantly this capability will also enable clinician to choose appropriate antifungal agent, this decreasing patient morbidity and mortality.

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