

Prevalence of Non-albicans candida and its drug susceptibility pattern isolated from a tertiary care hospital, Karnataka.

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Abstract

Background: *Candida* infections have increased over the past few decades. *Candida* species are opportunistic yeast infections which are also common commensal of human gastro-intestinal tract. It causes spectrum of diseases extending from Mucocutaneous infections to systemic infection with dissemination. The increased incidence of life threatening infections in immunocompromised hosts and the emergence of non *albicans* candida (NAC) have made the treatment options difficult. Over to it, rampant use of azoles as prophylaxis has led to the emergence of azole resistant strains which are of great concern for clinical and therapeutic aspects. The present study was taken up with the aim to isolate and identify the candida species from clinical samples using a chromogenic media and to derive anti-fungal susceptibility pattern.

Methods: Total 484 samples was processed using Hichrome agar media and identified using standard identification methods up to species level. Antifungal susceptibility tests were performed according to CLSI guidelines for Fluconazole, Voriconazole, Ketoconazole and Itraconazole.

Results: Total 484 clinical specimens was collected from all the department. Out of which 176 candida species was isolated. Predominant isolate was *C. tropicalis* followed by *C. guilliermondi* and *C. parapsilosis*. Hichrome agar showed good sensitivity and specificity. Voriconazole (77.5%) showed good sensitivity over Fluconazole (41.4%) sensitivity.

Conclusion: present study highlights there is need to identify NAC and give sensitivity pattern.

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Introduction

Invasive candidiasis has become an important infection especially among immunocompromised patients and critically ill patient. Recent reviews shows candida as the fourth common pathogen causing blood stream infections, other than causing genito-urinary infections and oral lesions. Candida is known commensal in gut, genital tract and also in tracheo-bronchial tree; they can lead to serious life threatening complications among risk groups like HIV patients, cancer patients, diabetic persons, pregnancy, newborn and old age.^[1, 2]

Historically *C. albicans* has been the predominant cause of Candidiasis. World-wide Non- *albicans* candida has become the prominent problem since the last decade mainly due to increase in the immunocompromised patients and also due the selection by the use of azole drugs. They pose a major threat for treatment as most of the species are inherently resistant to the common anti-fungal drugs. Accurate and rapid diagnosis of these new species is needed to initiate treatment strategies.^[1]

Ideally laboratories should isolate and speciate not only *C. albicans* but also other major NAC from the clinical specimens, due to resource constraints or time limitations most of the isolates are unidentified up to their species level. In our study we used a selective as well as differential medium (Hichrome agar candida) to selectively isolate Candida spp. and able to identify them by their distinctive color. It facilitates the detection of candida spp. from mixed cultures and provides results within two days.^[3]

This study was undertaken with the aim to isolate and identify the various candida species from clinical specimens using Hichrome agar and to derive anti-fungal susceptibility pattern.

Materials and Methods

Study was done in Department of Microbiology, in the year December 2010- December 2012. It is cross sectional study. Various clinical samples (Oral swabs, Pus, Urine and blood) were collected for processing, after taking written informed consent. Study was approved by the Institute Ethical committee.

Inclusion criteria: Oral swabs (Oral thrush) from old age people/ chemotherapy patients/patients on steroids and Pus swabs which showed budding yeast cells with/without pseudohyphae were included. Urine samples with pure growth of candida isolates

or mixed with bacterial colonies showing $>10^5$ CFU/ml were included. Blood cultures from neonates delivered by vaginal route to the mother with vaginal candidiasis were included.

Exclusion criteria: All swabs which were negative for candida on microscopy was rejected. Urine samples with $<10^5$ CFU/ml were of no clinical significance. Blood cultures other than neonates/ caesarean delivery/ mother without vaginal candidiasis were excluded. Those patients who received antifungal treatment within one month duration were also excluded.

Microbiology: Total 484 samples was directly inoculated on the Hicrome agar plate. Hicrome agar was prepared according to the manufacturer's instructions. Simultaneously samples were subjected for identification by conventional methods on Emmon's modified Sabourauds Dextrose Agar (SDA) supplemented with antibiotics (Gentamycin 5µg/dl and Chloramphenicol 50µg/dl) incubated at 37°C.^[4] Hicrome agar plates were checked for growth at 24hrs, 72 hrs and followed up to 7 days to check for colonial growth, characteristic color, and color intensification and for variation in colony morphology. Yeast colonies were subjected to Germ Tube Test, morphology on Corn meal agar (Dalmau Plate Culture method) read after 48 hours and Auxonographic sugar assimilation test for identification of yeasts up to species level.

Anti fungal susceptibility: Done by Kirby Bauer's disk diffusion method according to CLSI guidelines M24-A2 on Mueller-Hinton agar plate added with 1% glucose and 10µl/100ml Methylene blue to cooled molten agar at 45-55°C.^[5, 6] Antibiotic disks Fluconazole (25µg), Voriconazole (10µg), Ketconazole (10µg) and Itraconazole (10µg) were tested results noted after 24hrs incubation. Zone size noted and interpreted as sensitive/ resistant according to the manufacturer list.

Statistical analysis: Parameters like sensitivity (true positive/true positive + false positive), specificity (true negative/true negative+ false positive) were determined.

Result

Out of 484 samples 176 Candida species were obtained as shown Table 1. In our study *C. tropicalis* was the more prevalent species followed by *C. albicans* as depicted in table 1. All the isolates showed growth on Hicrome within 24-36 hours, of 1-3mm Colony size. Well differentiated color and colony

morphology were appreciated between 24-36 hrs. But species like *C. pelliculosa*, *C. fomata*, *C. glabrata* and few strains of *C. tropicalis* were observed with well differentiated color only after 72 hours. There was statistical difference ($P < 0.01$) between time of growth and time for intensification of color. Hicrome agar-Candida was able to distinguish green, blue, pink and cream color clearly after incubation up to 48hrs, shown Fig 1.

Table 1: Species distribution

Species	Oral swabs (100)	Urine (306)	Pus (21)	Blood (57)	Total (484)
<i>C. albicans</i>	12	12	4	5	33
<i>C. tropicalis</i>	25	18	3	6	52
<i>C. guilliermondi</i>	21	5	-	-	26
<i>C. parapsilosis</i>	13	-	-	-	13
<i>C. kefyr</i>	13	7	-	-	20
<i>C. krusei</i>	8	6	-	-	14
<i>C. glabrata</i>	6	8	-	-	14
<i>C. fomata</i>	3	-	-	-	3
<i>C. pelliculosa</i>	1	-	-	-	1
Total	102	56	7	11	176



Figure 1: Growth of Mixed colonies

Sensitivity and Specificity on Hicrome agar of each species is represented in Fig 2. No batch- batch variation seen, as tested by *C. albicans* ATCC 90028.

Five mixed cultures having ten isolates were identified based on colony color, size and texture. Six isolates of mixed culture were obvious with their

characters and they could be easily identified. While on SDA it demanded experience to identify mixed growth, except for colony size, there were no other defining variations with different isolates.

Table 2: Distribution of the susceptibility pattern among the Candida isolates by disk diffusion method.

AFST	Resistance	Sensitive
Fluconazole	58.6%	41.4%
Voriconazole	22.5%	77.5%
Ketoconazole	35.5%	64.5%
Itraconazole	60.2%	39.8%

Discussion

Fungal infections are important infections worldwide; among which candidiasis is the common infection seen as community as well as hospital associated infections. It is the major problem affecting mainly immunocompromised patients and newborn. Although it was known to cause muco-cutaneous infections, nowadays there is rise in septicemia and other life threatening infections.^[6]

Candida fungaemia trends have been changing with time. NAC incidence started replacing *C. albicans* since 1970. NAC were not considered as obligate human pathogen, as they were commonly isolated from non-human sources like soil, marine, insects and domestic animals. These were transient colonizers of human integument but their isolation started rising from patients with hematological malignancies, neutropenic patients, transplant recipients and steroid therapy.^[6]

In the present study, the striking fact is the predominance of *C. tropicalis* in all the clinical specimens. Most of the published reports show *C. albicans* as the common etiological agent but fungal epidemiology is constantly changing and evolving, hence species other than *albicans* can be expected to have a role in causing candidiasis. Many studies^[6, 7] credits *C. parapsilosis* as the common species isolated from blood. Our study worth notifying that none of the *C. parapsilosis* were isolated in blood, indeed *C. tropicalis* remained the common spp. None of our patients were on IV hyperalimentation. Significant number of *C. guilliermondi* was isolated in our study which correlates with other authors.^[7]

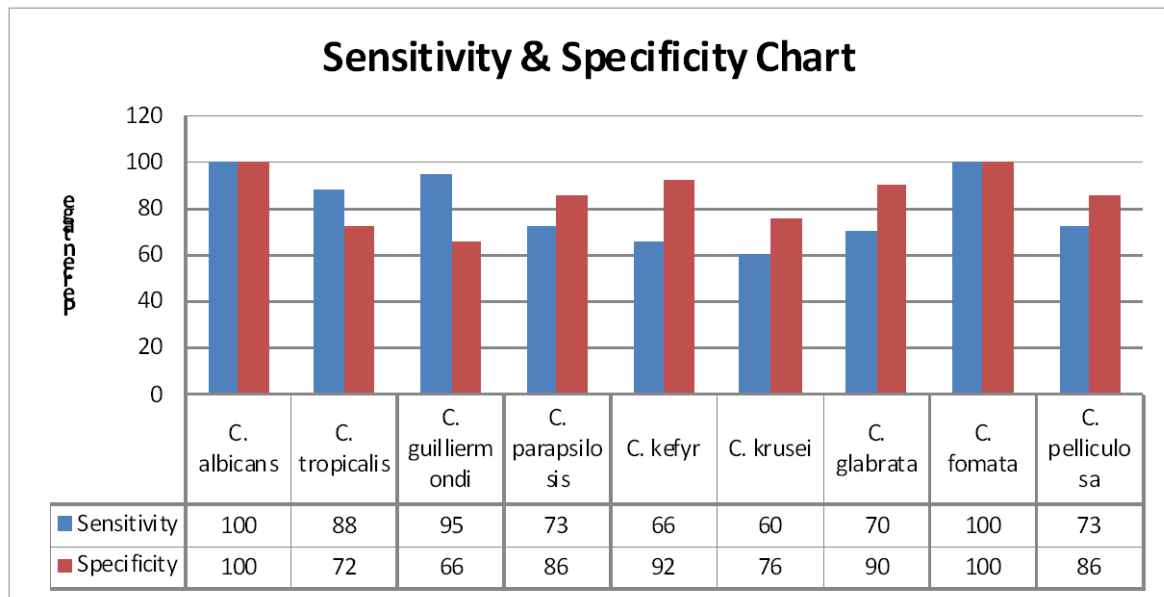


Figure 2: Sensitivity and Specificity of Hicrome Agar

Rapid and accurate diagnosis is necessary to optimize the treatment strategy. We tested a new chromogen media and appreciated its use. Use of chromogen media potentially saved our time and expense of performing various biochemical reaction. Routine use of chromogenic media proves cost savings in the clinical laboratories. Being a selective medium, it readily showed pure growth of *Candida* spp. Although its efficiency to culture other new spp. is unproved, in our study Hichrome media could culture some of the rare species. Good sensitivity and specificity was seen for *C. albicans* which goes in agreement with other authors.^[3, 8] Other spp. showed relatively good sensitivity and specificity.

In recent years use of Fluconazole as prophylaxis has been remarkably increased, which is one of the reason for the high rate of resistance seen. Patients with intermittent exposure to Fluconazole harbor strains which shows acquired resistance to azoles or patients with recurrent infection who carry single resistant strain. Also frequent use of azoles has led to selection, which made the intrinsically azole resistant strains like *C. glabrata* to emerge as pathogen.^[2, 5]

Our study showed good sensitivity to Voriconazole, whereas Fluconazole showed relatively high resistance correlated with previous studies.^[5,9] Indeed use of Itraconazole for therapy is less, but we got high resistance pattern, reason may be its low solubility of

the drug or may be cross resistance. As CLSI doesn't describe methods to test Itraconazole, we followed the same method as Fluconazole described by CLSI. Certainty of the method yet to be evaluated for testing Itraconazole.

Routine anti-fungal susceptibility testing is needed to test all these resistant strains and to note their susceptibility pattern. Most of the resistance are chromosomally mediated, nowadays formation of biofilms are common especially with indwelling catheters and prosthetic valves. The cells embedded within the matrix have low susceptibility to anti-fungals and the regular MIC tested for planktonic cells will be different from the embedded sessile cells. Biofilms are common among NAC.^[1, 10] The other important mechanism of resistance is the active efflux of these drugs which leads to reduced accumulation of drug within the cell, resulting in sub lethal dose for the pathogen.^[5] In the present study there appears to be significant raise in reduced susceptibility to azoles, correlates with other studies, attributed to exposure to several short courses of short or long term suppressive therapies.

Conclusion

This study documents the scenario in accordance to the changing trends of causative role of NAC in causing candidiasis which are resistant to azoles. There is need to emphasis that all the clinical labor-

atories have to take up identification of candida up to species level and derive susceptibility pattern as their routine.

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Competing Interests

None declared

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