

Original Research Article

<https://doi.org/10.20546/ijcmas.2019.805.133>

Speciation and Antifungal Susceptibility Testing of *Candida* Isolates in Various Clinical Samples in a Doctors' Diagnostic Centre, Trichy, Tamil Nadu, India

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ABSTRACT

Candida species form part of normal flora of human beings. In the presence of predisposing factors, these can cause different infections with varied severity. Over the last few months fungal infection rates have increased and a change is seen in their epidemiology and antifungal susceptibility pattern. Hence this study was conducted to learn the distribution of *Candida* species in various samples and their antifungal susceptibility pattern. A total number of 60 *Candida* isolates were included in the study. Identification was done by colony morphology and Gram stain. Speciation was carried out by Germ tube test, urease test, chlamydoconidia production test, colony characteristics on HiCrome™ *Candida* Differential Agaragar medium, sugar assimilation test, sugar fermentation test and Vitek2 compact (Biomerieux) using ID-YST 21342 cards. Antifungal testing was done on Vitek2 compact using AST YS08 cards which included fluconazole, voriconazole, amphotericin-b, caspofungin, micafungin and flucytosine. 60 *Candida* isolates were included in this study. Samples from which *Candida species* were isolated were urine (62%), vaginal swab (16.5%), pus (11.5%), Ear swab (5%), Endo tracheal (1.5%), and sputum (3.5%). Isolates from males and females were 30% and 70% respectively. Isolates from geriatric age group (>65 years) and adults (18-65 years) were 52% and 48% respectively. Isolates from samples received from In-Patient Department (IPD), Out-Patient Department (OPD) and Intensive Care Unit (ICU) were 58%, 34% and 8% respectively. Out of all isolates, *Candida albicans* was 58%, *Candida tropicalis* 20%, *Candida glabrata* 10%, *Candida parapsilosis* 9% and *Candida krusei* 3%. All *Candida species* (except *Candida glabrata*) showed 100% sensitivity to amphotericin-b and caspofungin. Sensitivity to azole group of drugs was 100% among *Non-Albicans Candida (NAC)* except *C. glabrata* and *C. krusei* and more than 90% among *C. albicans*. *C. albicans* was the commonest isolate followed by *C. tropicalis*. Overall also, *C. albicans* were predominant as compared to *NAC*. All *Candida isolates* except (*C. glabrata*) showed good sensitivity to all antifungals. Antifungal resistance among certain *NAC* is on the rise. The commonest underlying risk factor for *Candida* infection was diabetes mellitus followed by bronchial asthma on steroid treatment.

Keywords

Speciation,
Antifungal
susceptibility,
Resistance

Article Info

Accepted:
12 April 2019
Available Online:
10 May 2019

Introduction

Candida species are ubiquitously present as commensals in the human body. In immunocompromised and hospitalized patients, they can cause various types of infections ranging from cutaneous to bloodstream infections and hence are capable of causing morbidity and mortality in patients. The genus comprises of heterogeneous group of organisms out of which 20 different *Candida species* are known to cause human infections (2). Candidiasis is on the rise due to indiscriminate use of antibiotics and increase in number of patients with AIDS (2). *Candida albicans* has years but indiscriminate use of azole group of drugs has led to increase in NAC infection and resistance to antifungal drugs in *Candida species* (2,3). Hence, infections with NAC and overall resistance to antifungals are on the rise (3). This makes species identification of *Candida* very essential to prevent treatment failures. Hence, this study was undertaken to study the epidemiology and antifungal sensitivity pattern of *Candida* isolates in our institute.

Materials and Methods

Study design

The present study is an observational study carried out at Department of Microbiology during the period of June 2018 to December 2018. 60 *Candida* isolates from various clinical samples of patients from all age groups and both genders from outpatient and inpatient departments were included in the study. The study was approved by the scientific and ethics committee of the institute.

Inclusion criteria

1) All samples collected under strict sterile conditions using aseptic precautions, deeply

expectorated mucoid sputum, urine samples (midstream urine and urine from catheterized patients) collected using standard recommended procedure were included.

2) Non-duplicate *Candida* isolates obtained from samples of Human Immunodeficiency Virus (HIV) positive patients, patients with risk factors like diabetes mellitus, excess antibiotic use, invasive procedures.

3) Non-duplicate isolates recovered from a second sample also, of a patient and isolates showing pure growth.

4) Isolates from samples showing significant number of pus cells.

Exclusion criteria

Isolates of samples not showing pure growth or from patients not having above criteria.

Sample processing

The samples included were sputum, urine (midstream and catheterized), stool, blood, sterile body fluids (pleural, ascitic, cerebrospinal, synovial, peritoneal), pus, tissue, vaginal swab, nail clipping, skin scraping and hair. Direct Potassium Hydroxide Mount ((KOH), 10% or 20% depending on the sample) and Gram stain was done from the sample after inoculation to look for yeast and pus cells.

They were inoculated on Sabouraud Dextrose Agar ((SDA), Himedia), both plain and with antibiotics and incubated at 37⁰C and 25⁰C respectively for 48-72 hours according to standard recommended procedures. For blood culture, 8-10 ml venous blood was collected aseptically and cultured in 50 ml Brain heart infusion (BHI) broth. It was then incubated at 37⁰C for up to 96 hours. Gram stain was done from the growth.

Identification

The growth was identified as *Candida* on the basis of colony morphology (cream coloured, smooth and pasty colonies) and Gram stain. Speciation was done by conventional tests and Vitek 2 compact (Biomeriux). Conventional tests used were germ tube test, urease test, colour change on HiCrome *Candida* Differential Agar (Himedia Pvt Ltd, Mumbai), sugar fermentation and assimilation tests. Identification by Vitek 2 compact (Biomeriux) was done using ID-YST cards.

Antifungal susceptibility

Antifungal susceptibility test was done using AST-YS08 cards. The antifungal agents included were fluconazole, voriconazole, amphotericin-b, flucytosine, caspofungin and micafungin.

Statistical analysis

The results were expressed as percentage analysis. The data was analysed statistically using SPSS statistics version 19.0 (Chicago, IL, USA) and values of $P < 0.05$ were considered statistically significant.

Results and Discussion

60 *Candida* isolates obtained during the study period from different clinical samples were included in the study. Samples from which these isolates were obtained were Urine 37 (62%), Vaginal swab 10 (16.5%) pus 7 (11.5%), Ear swab 3 (5%), endotracheal secretion 1 (1.5%), and sputum 2 (3.5%). Isolates from females were 42 (70%) and males were 18 (30%). Isolates from geriatric age group (>65 years) were 31 (52%) and adults (18-65 years) were 29 (48%). Isolates from IPD samples were 35 (58%), OPD samples 20 (34%) and ICU 5 (8%). Species identification revealed that *Candida albicans*

constituted 35 isolates (58%), *Candida tropicalis* 12 isolates (20%), *Candida glabrata* six isolates (10%) *Candida parapsilosis* five isolates (9%) and *Candida krusei* two isolates (3%). *Non-albicans Candida* constituted 25 isolates (42%) of all (Figure 1).

In urine samples, 33 isolates were of *Candida albicans* followed by three isolates of *C. tropicalis* and one of *C. glabrata*. Among vaginal swabs, 5 isolates were of *Candida albicans* followed by 3 isolates of *C. tropicalis*, one of *C. glabrata* and one of *C. krusei*. Among pus samples, five were *C. parapsilosis* one each was *C. glabrata* and *C. krusei*. Two isolates were of *C. albicans* and one of *C. glabrata* from ear swab. From endotracheal secretion and sputum one isolate each was of *C. albicans* and *C. tropicalis* respectively. Sample wise distribution of *Candida species* is shown in Table 1.

Sensitivity of *C. albicans* to amphotericin-b, flucytosine and echinocandins was 100%, 94% (33 isolates) to fluconazole and 91% (32 isolates) to voriconazole. *C. tropicalis* and *C. parapsilosis* showed 100 % sensitivity toazole group, amphotericin-b and caspofungin. Sensitivity to flucytosine and micafungin was 92% (11 isolates) among *C. tropicalis* and 100% among *C. parapsilosis* and *C. glabrata* isolates showed 100% sensitivity to flucytosine, 67% (four isolates) to azoles and amphotericin-b and 50% (three isolates) to echinocandins. Both isolates of *C. krusei* were resistant to fluconazole, sensitive to azoles and echinocandins and one (50%) was sensitive to flucytosine (Table 2).

Candida species are part of normal human flora and are opportunists capable of causing a wide spectrum of infections (5,1). Colonisation of the mucocutaneous surfaces is the first step towards infection. Alteration in this balance results in growth and subsequent

invasion and is supported by various risk factors leading to immunosuppression (5). Some of these include infection with HIV/AIDS, indiscriminate antibiotic use, use of intravenous catheters, urinary tract catheterisation, hepatic and renal failure, prolonged hospital stay, chemotherapy, organ transplant, leukaemia, diabetes mellitus and Chronic Obstructive Pulmonary Disease (COPD) (1,2,6). Though infection with *C. albicans* is common, infection with drug resistant NAC are on the rise over the last few years (3). This makes *Candida* species identification and susceptibility testing of these isolates mandatory and important. In the present study, 35 isolates (58%) were from samples of IPD patients, 20 isolates (34%) from OPD and 5(8%) from ICU samples which was also seen in a study by Rajeevan *et al.*, in which more samples were from IPD as compared to OPD(1). There was a female predominance among isolates as 42 were from females as compared to 18 from males similar to studies by Mukhia *et al.*, and Pawar *et al.*,(7,8). This may be because maximum samples in the present study were sputum which was more from females. More isolates were from geriatric age group (>65 years) which was comparable to other studies (9,6). This population is more prone to have co-morbid conditions leading to immunosuppression and *Candida* infection. The commonest sample received were Urine (62%) Vaginal swab (16.5%) followed by other less common samples like pus (11.5%), Ear swabs (5%), endotracheal secretion (1.5%), and sputum (3.5%). This was in accordance with other studies (2,6,8-10). The commonest isolate was *C. albicans* (58%) followed by *C. tropicalis* (20%), *C. glabrata* (10%), *C. parapsilosis* (9%) and *C. krusei* (3%). Overall also, *C. albicans* (58%) predominated as compared to NAC (42%). This was also observed in separate studies by Khadka *et al.*, and Khan *et al.*,(10,11). This shows that NAC infections are also gaining

importance as is also documented in another study by Bajwa and Kulshreshtha which showed that NAC rates in India range from 52% to 96% (12). Also, in various countries, significant geographic variations in the etiological pattern of invasive *Candida* species is reported (13). In the present study, commonest NAC species isolated was *C. tropicalis* comparable to other studies (14,15). Among the lower respiratory tract samples, sputum samples grew *C. albicans* (85%), *C. tropicalis* (11%) and *C. glabrata* (4%) and one endotracheal aspirate grew *C. albicans* with significant colony count.

Bathala *et al.*, found that with age and in the presence of certain predisposing factors, *Candida* which is considered a coloniser in the respiratory tract may get converted to pathogen (5). All the urine samples had one or more inclusion criteria required for this study. Most of the patients from whom these samples were received had one or more associated risk factors and the remaining had significant microscopic and culture findings. In vaginal samples also, commonest species was *C. albicans* (45%) followed by *C. tropicalis* (40%), *C. glabrata* (10%) and *C. krusei* (5%). This was also observed by Sumana *et al.*, in their study (18). Diabetes mellitus was the commonest risk factor in patients from whose urine samples these isolates were grown as was seen in other studies also(1,13,19). In diabetics, susceptibility to *Candida* infection increases probably due to increase antibiotic use, associated illnesses and hyperglycaemia (20). Out of 20, 3 urine samples were from ICU and three from catheterized patients. Catheterisation increases chances of urinary tract infection by allowing migration of organisms into the bladder from external peri-urethral surface (21). It is also the commonest risk factor for candiduria in ICU patients (22). Thus, *C. albicans* and *C. tropicalis* were mostly isolated from Urine and vaginal swab.

C. glabrata constituted 10% of the total isolates and grew from urine, pus, ear and vaginal swab. This was in accordance with another study where this isolate also grew mainly from urine and vaginal swab(2) *C. parapsilosis* formed 9% of the total, comparable to other studies where it was 8% and 10% respectively(6,11). Three of these isolates were from patients with recurrent ear discharge not responding to antibiotics and two were obtained by invasive procedure.

Two isolates were of *C. krusei* of which one was from vaginal swab and one from pus. Singh *et al.*, in their study also grew *C. krusei* from vaginal swab (13). All vaginal swabs were from pregnant female patients with vaginal discharge and itching. Guru *et al.*, observed that pregnancy is a risk factor for *Candida* infection and in their study *C.*

albicans was the commonest isolate in this group (19). One sputum sample grew *C. tropicalis*. The patient had complaints of fever and was diagnosed as a case of superior mesenteric artery thrombosis leading to ischaemia of small intestine. Predominance of *C. tropicalis* in sputum has also been observed by other authors (8,9). *C. glabrata* showed significant resistance to all antifungals except flucytosine.

In our study, resistance to fluconazole in *C. glabrata* was 33% comparable to study by Mondal *et al.*, in which it was 29.4% (3). Sandhu *et al.*, found decreased susceptibility to fluconazole in *C. glabrata* and *C. krusei* (23). Guru *et al.*, and Sandhu *et al.*, also found higher rate of antifungal resistance in NAC as compared to *C. albicans* (19,23).

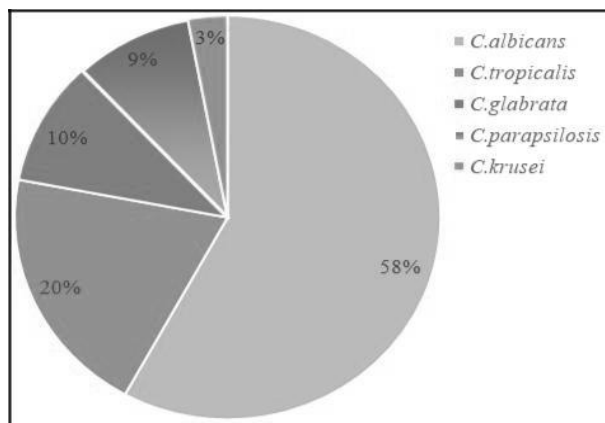
Table.1 Sample wise distribution of *Candida* isolates

	Sample	<i>C. albicans</i>	<i>C. tropicalis</i>	<i>C. glabrata</i>	<i>C. parapsilosis</i>	<i>C. krusei</i>
	(No)	No (%)	No (%)	No (%)	No (%)	No (%)
Urine (37)		33(89)	03(8)	01(3)	-	-
Vaginal swab (10)		05(50)	03(30)	01(10)	-	01(10)
Pus (07)		-	-	01(14)	05(72)	01(14)
Ear swab (03)		02(66)	-	01(34)	-	-
Endotracheal secretion (01)		01(100)	-	-	-	-
Sputum (02)		01(50)	01(50)	-	-	-

Table.2 Antifungal susceptibility of *Candida* species

Species		Amphotericin-b	Caspofungin	Micafungin	Flucytosine	Fluconazole	Voriconazole
(No)		No (%)	No (%)	No (%)	No (%)	No (%)	No (%)
<i>C. albicans</i> (35)		35(100)	35(100)	35(100)	35(100)	33(94)	32(91)
<i>C. tropicalis</i> (12)		12(100)	12(100)	11(92)	11(92)	12(100)	12(100)
<i>C. glabrata</i> (06)		04(67)	03(50)	03(50)	06(100)	04(67)	04(67)
<i>C. parapsilosis</i> (05)		05(100)	05(100)	05(100)	05(100)	05(100)	05(100)
<i>C. krusei</i> (02)		02(100)	02(100)	02(100)	01(50)	0	02(100)

Fig.1 Species distribution of *Candida* isolates



All other *Candida* species showed 100% susceptibility to amphotericin-b comparable to other studies (2,11,24). Sensitivity to flucytosine was 100% in all other species except *C. tropicalis* (92%) and *C. krusei* (50%). Adhikari *et al.*, in their study found similar susceptibility pattern to flucytosine in all *Candida* isolates (2,25). All other *Candida* species were susceptible to echinocandin group. 100% susceptibility was seen to fluconazole and voriconazole in all species except *C. albicans* and *C. glabrata*. Singh *et al.*, observed overall sensitivity of 95.6% and 100% among *Candida* isolates to fluconazole and voriconazole respectively (2). In the present study, *C. albicans* showed 6% and 9% resistance to fluconazole and voriconazole respectively. More resistance to azole derivatives was seen in *C. albicans* according to Rajeevan *et al.*, (1). This is because *C. albicans* is the commonest species isolated and azole group are the commonest antifungals used against them. Both isolates of *C. krusei* were resistant to fluconazole which was in accordance with study by Mondal *et al.*, (3). *C. krusei* exhibits intrinsic resistance to fluconazole, both *in-vivo* and *in-vitro* (26). Also, in the present study results of conventional method (especially HiCrome *Candida* Differential Agar) and automated method for identification of *Candida* species were comparable. An additional advantage of

HiCrome *Candida* Differential Agar is ability to detect mixed cultures though in the present study there were no mixed cultures.

In conclusion, identification of *Candida* isolates up to species level and antifungal susceptibility pattern are indispensable, keeping in mind the changing scenario of epidemiology of these isolates and antifungal susceptibility pattern. Previously considered insignificant, NAC are also gaining significance and the increasing resistance to antifungals among them should not be disregarded. This will help in judicious use of antifungal drugs in patients and help in preventing resistance.

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How to cite this article:

Rengaraj, A. and Bharathidasan, R. 2019. Speciation and Antifungal Susceptibility Testing of *Candida* Isolates in Various Clinical Samples in a Doctors' Diagnostic Centre, Trichy, Tamil Nadu, India. *Int.J.Curr.Microbiol.App.Sci*. 8(05): 1169-1176.
doi: <https://doi.org/10.20546/ijemas.2019.805.133>