Identification of *Candida* Species from Clinical Isolates and Their Antifungal Susceptibility Pattern

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ABSTRACT

BACKGROUND

With increasing use of antibacterial and cytotoxic drugs, lethal invasive Candidiasis is on the rise, with almost half of the cases being caused by non *albicans Candida* species (NAC). Frequent use of azoles for empirical therapy has also led to their increased resistance. We wanted to characterise *Candida* species isolated from various clinical specimens and assess their susceptibility pattern to Fluconazole and Voriconazole.

METHODS

A total of 100 consecutive *Candida* species isolated from various clinical specimens in our institute from January 2016 to December 2016 were included in the study. Standard yeast identification protocol and CHROM agar were used for speciation and their antifungal susceptibility pattern was found by disc diffusion method.

RESULTS

Out of the 100 isolates, *C. tropicalis* was the predominant isolate (47%), followed by *C. albicans* (31%), *C. parapsilosis* (16%) and *C. krusei* (6%). Females (57%) were more affected and maximum number of patients was above 60 years (24%). Diabetes mellitus (21%) was the major predisposing factor for *Candida*, followed by broad spectrum antibiotic therapy (14%). Isolates were more susceptible to Voriconazole (99%) than Fluconazole (87%). NAC spp. showed more resistance to Fluconazole (17.4%) than *C. albicans* (3.3%). Only one isolate of *C. krusei* (16.6%) showed resistance to Voriconazole.

CONCLUSIONS

Due to the increasing incidence of azole resistant NAC spp., the species level identification of *Candida* species, along with their anti-fungal susceptibility patterns can help the clinicians in formulating a treatment protocol and can help in decreasing the mortality and morbidity.

KEY WORDS

Candida albicans, Non-*albicans Candida*, CHROMagar, Antifungal Susceptibility Testing, Fluconazole, Voriconazole

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BACKGROUND

Candida, a genus of yeast are ubiquitous organisms found on many plants and are a part of normal flora of the alimentary tract of mammals and the mucocutaneous membranes of humans. *Candida* causes a diverse range of opportunistic human infections from mild superficial to the life-threatening invasive ones. Adherence to host tissues, medical devices, biofilm formation, and secretion of extracellular hydrolytic enzymes has facilitated the switch of *Candida spp.* from commensal to a potent pathogen.[1] The increasing incidence of HIV infections, use of steroids and broad spectrum antibiotics, organ transplantations, advanced life supports and prosthetic devices, all have led to the increasing incidence of *Candida* infections. *Candida albicans* is generally considered as the most common and the pathogenic member of the genus.[2]

More recently, there has been a significant shift towards non-*albicans Candida* (NAC) infections, which have shown variable resistance to azoles. In developed countries, 40-60% of isolates are C *albicans*; whereas in India there is a preponderance of NAC spp.[3] NAC is a heterogeneous group, of which 19 species are implicated in human infections. *C. tropicalis*, *C. glabrata*, *C. krusei* and *C. parapsilosis* are the most commonly reported NAC spp. The NAC spp. may represent selection of less susceptible species like C *glabrata* and C *krusei* which is intrinsically resistant to Fluconazole. Even though *C. tropicalis* is generally considered as a Fluconazole-susceptible species, recent studies have shown emergence of Fluconazole resistance.[4]

Invasive *Candida* infections are one of major causes of morbidity and mortality in immunocompromised patients. Therefore, in patients with risk factors for Candidemia, clinicians empirically treat the infection with antifungal agents, most commonly azoles. To avoid selection of less susceptible NAC spp. by empirical azole treatment or prophylaxis, identification of the infecting species of *Candida* isolate is essential for initiation of early and effective therapy. Although there are rapid and reliable commercial systems and molecular diagnostic methods available for species identification of *Candida* isolates, in a resource poor country like ours conventional techniques remain the mainstay in most clinical microbiology laboratories.[4] In such setting, usage of CHROMagar which contains chromogenic (hexosaminidase) substrates that react with species-specific enzymes secreted by yeast cells, for species identification would be of benefit for easy and rapid speciation.[3]

As the prevalence of NACs *spp.* significantly vary according to countries and health care facilities, species identification also play a part in formulating local therapeutic guidelines.[4] So the present study was undertaken to characterise the *Candida* isolates in our geographic area and to determine the antifungal susceptibility pattern of the isolates to Fluconazole and Voriconazole so as to formulate an empirical treatment policy.

METHODS

A descriptive study was undertaken in the department of Microbiology, Govt. Medical College, Thrissur, Kerala for a period of 1 year from January 2016 to December 2016. All pure isolates of *Candida spp.* from various clinical specimen during the period of study were included in the study. (based on the previous 3 years hospital records, a sample size of 80-100 was calculated). A total of 100 consecutive and non-repetitive pure cultures of *Candida* from various clinical specimens like blood, urine, sputum, swabs, nail clippings, endotracheal tube tip, skin scrapings, exudates etc. received in the Diagnostic Microbiology section of central laboratory were studied. All the yeast isolates (white to cream-coloured, pasty and smooth colonies) grown on Blood agar were confirmed by Gram staining which showed Gram positive budding cells with or without pseudohyphae. Single colony from the Blood agar was inoculated on Sabouraud dextrose agar and incubated at 37°C for 24 hours. Germ tube test was done for all the isolates and the positives were identified as either *C. albicans* or *C.* dubliniensis. Species identification was done by using Corn meal agar, Sabouraud's Dextrose broth, Christensen's urea agar, Sugar assimilation and sugar fermentation. The isolates were further processed for *Candida* speciation on CHROMagar (HiMedia, Mumbai, India). After incubation at 37 °C for 24–48 hrs, *Candida* species were identified by type and colour of the colonies as per manufacturer's instructions. Type of the growth and colour of isolates on CHROMagar *Candida* plates; *C. albicans* light green, *C. parapsilosis* off white to pink, *Candida krusei* purple, fuzzy and *C. tropicalis* blue to purple.

Antifungal susceptibility testing by disc diffusion method to Fluconazole and Voriconazole were studied for all the isolates of *Candida* as recommended by Clinical and Laboratory Standards Institute (CLSI) M44-A2 document guidelines using Fluconazole disc (25 µg) and Voriconazole disc (1 µg) (HiMedia, Mumbai, India).[5]

Statistical Analysis

Data was entered in Microsoft Excel. Qualitative data was described using proportion and percentages.

RESULTS

A total of 100 *Candida spp.* was isolated from various clinical samples (table 1). Majority of the isolates were from Urine (46%), blood culture (13%) and sputum (12%). Gender-wise distribution showed that 57% *Candida* isolates were from females and 43% from males. Maximum number of patients were above 60 years (24%) followed by reproductive age group (21%) and newborn (21%). Out of the 100 isolates of *Candida*, Non-*albicans Candida* species predominated with 69 % and *Candida albicans* 31%. Among the NAC spp., *C. tropicalis* (47%) was the most common isolate followed by *C. parapsilosis* (16%) and *C. krusei* (6%). *C. albicans* was the predominant isolate from blood, high vaginal swab, exudates and pus swab. *C. tropicalis* was the predominant isolate from urine and sputum. Out of the 100 isolates, 67 were high risk groups, of which diabetes mellitus constituted 21%, followed by prolonged antibiotic therapy (table 2).

Among the total 100 *Candida* isolates, 87% were sensitive to Fluconazole and 99% to Voriconazole. In this study, only 3.3% of *C. albicans* was found to be resistant to Fluconazole.

Among the NAC spp., *C. tropicalis* and. C *parapsilosis* showed 8.5% and 12.5% Fluconazole resistance respectively whereas 100% of C *krusei* were resistant to Fluconazole. All the isolates of *C. albicans*, *C. tropicalis* and *C. parapsilosis* were sensitive to Voriconazole. One isolate of *C. krusei* (16.6%) showed resistance to Voriconazole (table 3).

Specimens	*Candida albicans*	*Candida tropicalis*	*Candida parapsilosis*	*Candida krusei*	Total
Urine	8	31	7	0	46
Blood	5	4	1	3	13
Sputum	4	6	2	0	12
Exudates	3	2	2	2	9
High vaginal swab	6	0	2	0	8
Pus swab	2	1	1	1	5
Skin scraping	1	1	1	0	3
Nail clipping	1	1	0	0	2
Catheter tip	1	1	0	0	2
Total	**31 (31%)**	**47**	**16**	**6**	**100**

Table 1. Species Distribution of Candida Isolates in Various Specimens

Predisposing Factors	*Candida albicans*	*Candida tropicalis*	*Candida parapsilosis*	*Candida krusei*	Total
Diabetes mellitus	12	7	2	0	21
Antibiotic therapy	6	5	3	0	14
Pregnancy	4	4	1	0	9
Sepsis	3	3	1	2	9
Devices in situ	3	3	1	0	7
Malignancy	1	2	0	1	4
Immuno suppression	1	1	0	1	3
Total	**30**	**25**	**8**	**4**	**67**

Table 2. Species Distribution According to Predisposing Factors

Antifungal	*Candida albicans* (n=31)	*Candida tropicalis* (n=47)	*Candida parapsilosis* (n=16)	*Candida krusei* (n=6)
Fluconazole sensitive	30 (96.7%)	43 (91.5%)	14 (87.5%)	0
Voriconazole sensitive	31 (100%)	47 (100%)	16 (100%)	5 (83.3%)

Table 3. Azole Susceptibility Pattern of Candida Species

DISCUSSION

Out of the 100 isolates obtained from various clinical specimens, the majority of *Candida* species were from urine (46%), blood (13%) and sputum (12%). Of these >80% of urinary *Candida* isolates belonged to NAC spp. Our observation is similar to that of Arasi Samyuktha A et al[3] and Khadka S et al,[1] where more than half of the urinary *Candida* isolates belonged to NAC spp. In the most recent study conducted by Pfaller et al for the SENTRY Antimicrobial Surveillance Programme, *Candida* species was found out to be the seventh most common nosocomial pathogen and caused 25% of all urinary tract infections.[6]

In the present study, the isolation rate of *Candida spp.* was highest in the age group more than 60 years (24%) followed by reproductive age group (21%) and newborn (21%). This emphasises the fact that *Candida spp.* is the most important cause of opportunistic infections worldwide in extremes of age as shown in other studies of Arasi Samyuktha A et al[3] and Dharwad S et al[7] *Candida* isolates were found to be highest in female (57%) with a female to male ratio of 1.3:1. Although in majority of studies, male preponderance is noted, female predominance was noted in study by Dharwad S et al [7] with female to male ratio 1.7:1 respectively. The reason for this disparity may be due to the higher number of female samples selected for the study.

CHROMagar offered a rapid and reliable method for identification of clinically important *Candida* species when compared with the traditional techniques. Out of the 100 isolates of *Candida*, Non-*albicans Candida* species predominated with 69 % isolation. Among the NAC spp., *C. tropicalis* (47%) was the most common isolate irrespective of the nature of the specimen, followed by *C. parapsilosis* (16%) and *C. krusei* (6%). This result is consistent with various studies conducted worldwide including India where NAC spp. Outnumbered *C. albicans* with incidence ranging from 54-74% which suggest the emergence of NAC spp. as important pathogens.[3,7-11] The possible reason for this may be the indiscriminate use of antifungals which eliminates more sensitive *C. albicans* and selects azole resistant NAC spp. However, higher incidence of *C. albicans* has been seen in numerous studies.[1,12-14] Among the Non-*albicans Candida*, various studies have reported *C. tropicalis* as the most predominant species in India.[3,7.10.11,13] Variation in predominance of *Candida* species observed in different studies may be due to the change in the environmental conditions, diversity in the study population, or the institutional based protocol for the usage of antifungal agents. In our study, *C. albicans* was the predominant isolate from blood, high vaginal swab, exudates and pus swab. *C. tropicalis* was the predominant isolate from urine. *C. glabrata* has emerged as an important opportunistic pathogen worldwide with concern over the increase in azole resistance. But in the present study, there was no isolate of the species.

Candidiasis occurs mostly in the presence of risk factors which promotes its overgrowth. In this study, Type II diabetes mellitus was the leading risk factor (21%) along with antibiotics, immunosuppressants, malignancy, pregnancy and catheters. A study by Dharwad S et al also showed Diabetes as a frequent risk factor with incidence of 32 %.[7] An increase of glucose concentration can promote the growth of *Candida*; Diabetes mellitus decreases the chemotactic factors and impairs phagocytosis. *C. albicans* was the predominant species in all the predisposing factors. Broad spectrum antibiotic usage was the second most frequently associated risk factor (14%). The most important effect of antibiotics is the elimination and alteration of the normal bacterial flora. It is an established fact that high hormone level leads to a proportional increase in the glycogen content of the vagina increasing prevalence of genital Candidiasis in pregnancy. In the present study, 9 pregnant women had infection.

Sepsis (9%) and urinary and indwelling catheters (7%) were the other significant risk factors, which can cause an increased risk of blood stream infections by *Candida*. In our study, the second most common specimen was blood (13%). In studies by Adhikary et al and Dharwad S et al, the rate of Candidemia was 19% and 16% respectively.[7,10] Dismissal of the isolation of the *Candida spp.* from a single blood culture as a skin contaminant could lead to a delay in administering potentially lifesaving therapy. It is said to consider all blood cultures that yield *Candida spp.* as significant until proven otherwise.[7]

Azoles are safe and effective agents for the treatment of Candidiasis and have gradually replaced Amphotericin B. However, resistance to azoles is now on the rise. According to the SENTRY Program findings, aside from intrinsically

Fluconazole-resistant species, such as *C. krusei* and *C. auris*, increasing rates of acquired resistance to Fluconazole have been noted in other NAC spp. including *C. glabrata*, *C. parapsilosis*, and *C. tropicalis* in India. Voriconazole has been demonstrated to have useful clinical activity in treating mucosal and invasive forms of Candidiasis. The clinical indication for using Voriconazole in invasive Candidiasis has been for oral step-down therapy in patients with *C. krusei* and Fluconazole-resistant, Voriconazole-susceptible *C. glabrata*.[6]

Among the total 100 *Candida* isolates in the present study, 13 % of the total isolates were resistant to Fluconazole and only 1 % to Voriconazole. The rate of Fluconazole resistance was highest in *C. parapsilosis* (12.5%) and *C. tropicalis* (8.5%) with only 3.2% of *C. albicans* showing Fluconazole resistance. All the 6 isolates of *C. krusei* (100%), which are considered to be intrinsically resistant to Fluconazole, were resistant to it. Various studies from the Indian subcontinent shows Fluconazole resistance ranging from 0 to 37% for *C. albicans*.[1,7,9,11,15] In studies by Adhikary et al, Khadka S et al and Dharward S et al, 19%, 20 % and 25% of Fluconazole resistance was noted for *C. tropicalis* respectively.[1.7.10] *C. parapsilosis* has become more significant and prevalent over the past two decades and is now one of the leading causes of invasive Candidiasis.[6] In a multi-centre laboratory based surveillance study conducted in India on Clonal Fluconazole resistant *C. parapsilosis*, over a period of 3 years from 2015-17, 32% were found to be non-susceptible to Fluconazole.[16] These were considered as hospital strains as indiscriminate use of Fluconazole for fungal infections have led to the emergence and subsequent nosocomial transmission of Fluconazole-resistant strains.

All the isolates of *C. albicans*, *C. tropicalis* and *C. parapsilosis* were sensitive to Voriconazole. One isolate of *C. krusei* (16.6%) showed resistance to Voriconazole. In most of the Indian studies, the *C. albicans* was 100% sensitive to Voriconazole.[7,10,11,17] But in a study by Dharward S et al *C. tropicalis* showed 12.5% and *C. krusei* showed 20% Voriconazole resistance and in another study by Kaur R et al *C. tropicalis* and C *parapsilosis* showed 70 % and 30 % Voriconazole resistance respectively.[7,15] In our study, *Candida* isolates displaying resistance to Fluconazole were susceptible to Voriconazole which beyond doubt proves it to be an effective in the treatment of Candidemia caused by Fluconazole resistant strains.

CONCLUSIONS

The shift in epidemiology from *C. albicans* to NAC spp. with increased azole resistance emphasizes the need for constant monitoring of *Candida* species distribution and continuously monitoring antifungal susceptibility patterns. Use of azoles especially Fluconazole should be controlled by good clinical-microbiological correlation to prevent resistance by selective pressure. This will help in the optimum management of the patients, helping in decreasing the overall morbidity and mortality.

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