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Species identification and antifungal susceptibility profile of *Candida* isolates from various clinical specimen: A three years study at a tertiary care teaching hospital in Coimbatore, TN

Dr Aswathy D[1]
Assistant professor, Department of Microbiology, KFMSR, Coimbatore.

Corresponding Author
Dr Aswathy D

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Abstract

Background: In recent years, the incidence of fungal infections in general and candidiasis in particular has increased.Although traditionally, *C. albicans* is considered as the most pathogenic species, recent studies have highlighted emergence of non *albicans Candida* (NAC) spp. These 'cryptic' NAC spp. often develop resistance during course of therapy or are innately less susceptible to commonly prescribed antifungal drugs. The present study was conducted with an aim to identify *Candida* isolates upto species and to study their antifungal susceptibility profile.

Material and methods: *Candida* isolates were identified upto species level by standard mycological protocol. Antifungal susceptibility testing of isolates was performed by disc diffusion method as outlined in Clinical Laboratory Standards Institute's (CLSI) M44-A guidelines.

Results: A total of 207 isolates were *Candida* spp. Predominance of NAC spp. was noted. *C. tropicalis* was the predominant *Candida* spp. Fluconazole resistance was observed in 25.6% of *Candida* isolates. All isolates of *C. krusei* were resistant to fluconazole.Amphotericin B resistance was noted 4 (1.9%) isolates which included 2 isolates of *C. krusei* and single isolate each of *C. tropicalis* and *C. glabrata*.

Conclusion: Non *albicans Candida* species have emerged as an important cause of infections. *C. tropicalis*, *C. krusei* and *C. glabrata*are important NAC spp. As different *Candida* spp. varies widely in their susceptibility to commonly prescribed antifungal drugs, antifungal susceptibility testing often guides clinicians in selection of most appropriate antifungal therapeutic agent.

Keywords: Antifungal susceptibility testing, *Candida*, fluconazole, Non *albicans Candida* species.

INTRODUCTION

Health and diseases have always been the most important matter of concern to human beings.The ever escalating want of better health resulted in discovery of various novel techniques for diagnosing and treating diseases. However, even in the golden era of medicine, where diagnostic and therapeutic modalities have progressed by leaps and bounds, infectious diseases still continue to be one of the major causes of morbidity and mortality in clinical setups worldwide.

Infectious diseases are attributed to bacteria, fungi, parasites and viruses.[1] As compared to other counterparts, bacteria are commonly implicated in human infections. However in recent years, the incidence of fungal infections in general and candidiasis in particular has increased.[2] Various factors like use of broad spectrum antibiotics and immunosuppressive drugs along with increase in immunocompromised individuals are considered important for increased incidence of fungal infections.[3]

Candida spp. is probably the only fungal pathogens that causes broad spectrum of clinical manifestation ranging from mucosal overgrowth to life threatening disseminated infections.[4]Although traditionally, *C. albicans* is considered as the most pathogenic species, recent studies have highlighted emergence of non *albicans Candida* (NAC) spp.[5] These 'cryptic' NAC spp. often develop resistance during course of therapy or are innately less susceptible to commonly prescribed antifungal drugs.[6] Therefore the present study was conducted at a tertiary care teaching hospital with an aim to identify *Candida* isolates upto species and to study their antifungal susceptibility profile.

MATERIAL AND METHODS

The present descriptive cross-sectional study was conducted in the Department of Microbiologyin a private medical college in Tamil Nadu for a period of three years (January 2018 to December 2020). *Candida* spp. isolated from various clinical specimens were included in the study.

Candida isolates were identified upto species level by standard mycological protocol for identification of yeast and yeast like fungi.[7] Germ tube technique, carbohydrate assimilation test and colony color on Hichrom Candida agar (Himedia Laboratories Pvt Ltd Mumbai) were used for identification of *Candida* spp.

Antifungal susceptibility testing of isolates was performed by disc diffusion method as outlined Clinical Laboratory Standards Institute's (CLSI) M44-A guidelines. Disc diffusion test was performed by Kirby-Bauer disc diffusion method on Mueller Hinton agar supplemented with 0.2% glucose and 0.5µg/mL methylene blue agar (MH-GMB).[8]

Isolates were tested for susceptibility against antifungal drugs like amphotericin B (20 µg), fluconazole (25 µg), itraconazole (10 µg) and ketoconazole (10 µg). All antifungal discs were procured from Himedia Laboratories Pvt Ltd. Mumbai.

Statistical analysis was done using statistical packages SPSS 19.0 (SPSS Inc. Chicago, IL). A *P* value <0.05 was considered to be significant. Pictorial presentation of the key findings was done by using appropriate statistical graph.

Results.

During the study period, a total of 4853 clinical specimens were received in the Department of Microbiology. Out of these, 1432 (29.5%) were culture positive. The rates of isolation of bacteria and fungi were 79.2% (n=1134) and 20.8% (n=298) respectively. Among fungal pathogens, a total of 207 isolates were *Candida* spp. (Figure 1). Isolation of *Candida* spp. was significantly high among various fungal pathogens (Fisher Exact test P value <0.05)

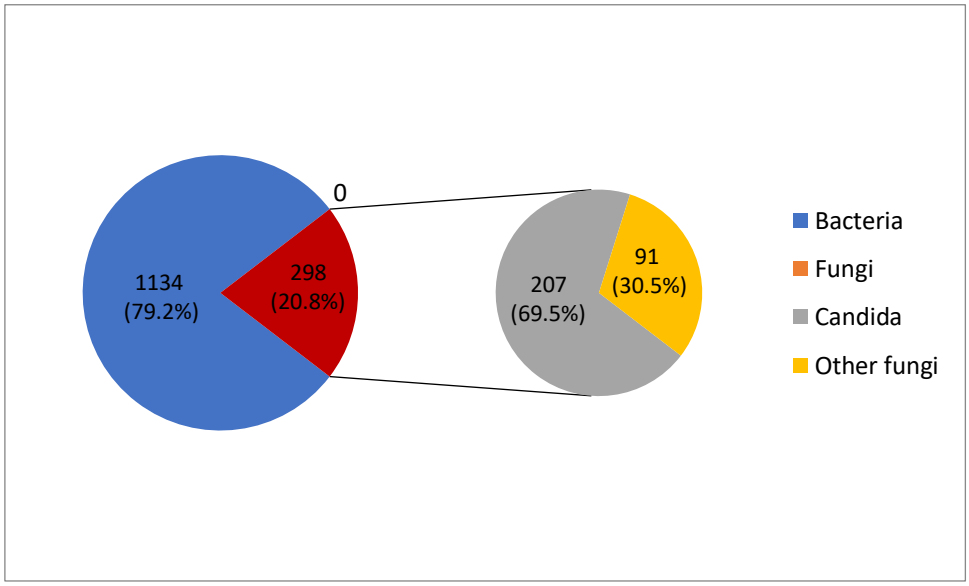

Figure 1: Bacterial and fungal isolates from various clinical specimens.

The year distribution of *Candida* spp. is shown in figure 2. There increasing isolation trend observed for *Candida* spp.

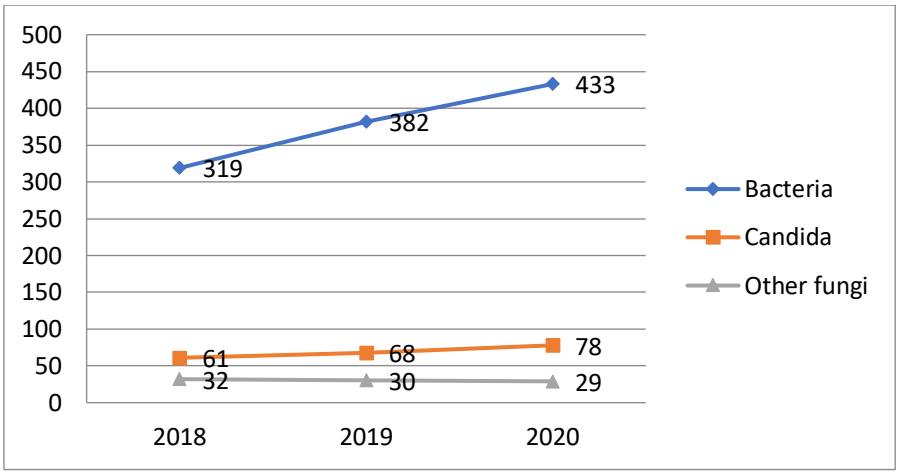

Figure 2: Year wise distribution of *Candida* and other isolates from clinical specimens.

The clinical specimen wise distribution of *Candida* spp. is shown in figure 3. The maximum number of *Candida* isolates were isolated from urine samples (37.7%) followed by vaginal swabs (25.6%). A total 25 (12.1%)*Candida* spp. were isolated from blood cultures.

Female gender, advanced age group, diabetes, use of broad spectrum antibiotics and presence of urinary catheterwere important risk factors observed for isolation of *Candida* spp. from urine samples. Pregnancy, HIV infection and antibiotic therapy were major risk factors for vulvovaginal candidiasis whereas HIV infection and diabetes were the only risk factors noted in patients with oropharyngeal*Candida* infection. ICU stay, mechanical ventilator support, indwelling urinary catheter and central venous catheterization were risk factors for *Candida* blood stream infection (BSI).

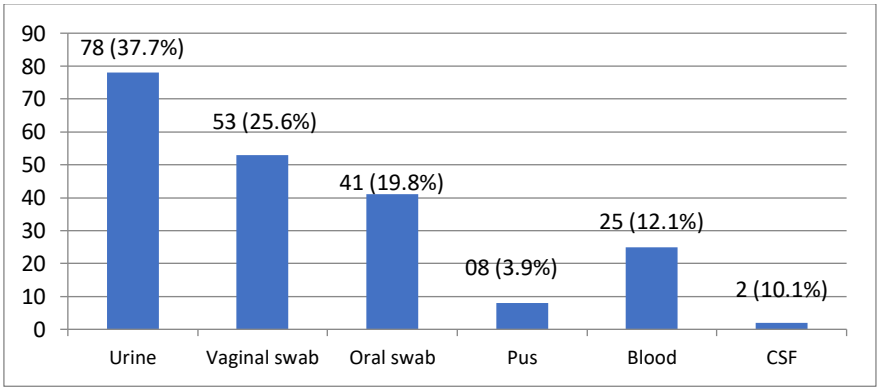

Figure 3: The clinical specimen wise distribution of *Candida* spp.

In the present study, a total of 62 (29.9%) isolates were identified as *C. albicans* whereas 145 (70.1%) isolates belonged to NAC spp. Isolation of NAC spp. was significantly high (Fisher Exact test P value <0.05).

The species wise distribution of *Candida* isolates is shown table 1. *C. tropicalis* (29.9%) was the predominant isolate in the present study. *C. krusei* was isolated from 25 (12.1%) clinical specimens.

Table 1: The species wise distribution of *Candida* isolates.

***Candida* spp.**	**Number (%)**
C. albicans	62 (29.9)
C. tropicalis	75 (36.2)
C. krusei	25 (12.1)
C. glabrata	21 (10.1)
C. kefyr	15 (7.2)
C. guilliermondii	09 (4.3)
Total	207

Antifungal susceptibility profile of *Candida* spp. by disc diffusion method is shown in table 2. Fluconazole resistance was observed in 25.6% of *Candida* isolates. All isolates of *C. krusei* were resistant to fluconazole.

Itraconazole resistance was observed in 10 (4.8%) isolates. A total of 7 (28%) isolates of *C. krusei* were resistant to itraconazole. Amphotericin B resistance was noted 4 (1.9%) isolates which included 2 isolates of *C. krusei* and single isolate each of *C. tropicalis* and *C. glabrata*.

Table 2: Antifungal susceptibility profile of *Candida* spp. by disc diffusion method.

Candida spp.	**Fluconazole**		**Itraconazole**		**Ketoconazole**		**Amphotericin B**	
	S (%)	R(%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)
C. albicans (62)	51 (82.2)	11 (17.8)	61 (98.4)	01 (1.6)	50 (80.6)	12 (19.4)	62 (100)	-
C. tropicalis (75)	63 (84)	12 (16)	73 (97.3)	02 (2.7)	68 (90.7)	07 (9.3)	74 (98.7)	01 (1.3)
C. krusei(25)	-	25 (100)	18 (72)	07 (28)	12 (48)	13 (52)	23 (92)	02 (8)
C. glabrata(21)	16 (76.2)	05 (23.8)	21 (100)	-	18 (85.7)	03 (14.3)	20 (95.2)	01 (4.8)
C. kefyr(15)	15 (100)	-	15 (100)	-	14 (93.3)	01 (6.7)	15 (100)	-

C. guilliermondii(09)	09 (100)	-	09 (100)	-	09 (100)	-	09 (100)	-
Total (207)	154 (74.4)	53 (25.6)	197 (95.2)	10 (4.8)	171 (82.6)	36 (17.4)	203 (98.1)	04 (1.9)

Discussion

In recent years, fungi, once studied in microbiology only as 'microbiological curiosities' without any clinical significance have emerged as an important cause of infections.[7, 9]Researchers from various parts of the world have reported increase in the incidence of fungal infections. Similarly in the present study, the rate of isolation of fungi from various clinical specimens was 20.8% and there was increasing trend observed for isolation of *Candida* spp.*Candida* spp. was the predominant fungal pathogen. Risk factors for *Candida* infections are similar to those for other fungal infections but may vary in cause and are mostly related to medical intervention and immune status of the patient.[9]

In the present study, 37.7% of *Candida* isolates were isolated from urine samples. As *Candida* is often found as commensal of genitourinary tract, its isolation from urine sample usually presents as a challenge to both clinicians and microbiologists as to whether ignore it as a mere colonization or, consider it as an indicator of lower or upper urinary tract infection including ascending pyelonephritis and renal candidiasis with sepsis.[11] However, isolation of urine should never be overlooked as this condition may be the only and often the first indication of systemic *Candida* infection. [12]

In clinical practice, candiduria is a relatively rare finding in a structurally normal urinary tract, and in healthy individuals.[13]Female gender, advanced age group, diabetes, use of broad spectrum antibiotics and presence of urinary catheter were important risk factors of candiduria in the present study. Similar findings were reported by other researchers like Kauffmann (2005)[14], Bukhary (2008)[12] and Achker*et al.* (2010).[15]The waning of host immune defenses in geriatric age group may explain the high incidence of candiduria. As in females the *Candida*colonization of vulvo vestibular area with *Candida* spp. is frequent, they are more at risk of developing candiduria due to ascending infection.[16] In diabetes glycosuria, impaired host defence mechanism, stasis of urine in neurogenic bladder enhance candiduria. As antibiotics suppress and change endogenous bacterial flora of the perineum, it favours both colonization and infection of urinary tract by *Candida* spp.[12]

In the present study, a total of 25 Candida spp. were isolated from blood cultures. In the United States, *Candida* spp. is the third most common cause of hospital associated blood stream infections.Candidemia is associated with high morbidity and mortality in both immunosuppressed and critically ill immunocompetent patients.[17, 18]it also significantly increases the duration of hospital stay and requirement of mechanical ventilation.

In the present study, predominance of NAC spp. over *C. albicans* was noted. Several research studies have documented emergence of NAC spp. Although various factors like increased empirical use of azoles are cited for emergence of NAC spp., improvement in diagnostic mycological methods like use of chromogenic media with an ability to differentiate *Candida* spp. as well as introduction of molecular diagnostic techniques appears to be more important. [19, 20]

C. tropicalis was the predominant species. Similar to our observation, the predominance of *C. tropicalis* among various *Candida* isolates was also reported by Pahwa*et al.* (2014)[21] and Deorukhkar et al (2016).[22]*C. tropicalis* alone, or in association with other species, is more frequently implicated in human infections.[23]It is more frequently isolated from patients admitted to ICU, especially those requiring prolonged catheterization, treated with broad-spectrum antibiotics or with malignancies.[21]

*C. krusei*was isolated 25 (12.1%) clinical specimens. It is considered as an emerging NAC spp.*C. krusei*infections are highly uncommon outside clinical settings involving exposure to azoles and immunocompromised status.[24]

In the present study, antifungal susceptibility profile of *Candida* spp. was studied by disc diffusion method. [25]Compared to the Clinical and Laboratory Standard Institute (CLSI) standardized microbroth dilution method, disc diffusion susceptibility testing is convenient and economical.[26]

In this study, fluconazole resistance was observed in 25.6% of *Candida* isolates. Of various antifungal drugs, resistance to fluconazole is of concern because it is one of the most widely used first line antifungal agents for treatment and prophylaxis of all forms of candidiasis.[27]All isolates of *C. krusei* were resistant to fluconazole.Various national and international studies have reported total fluconazole resistance in *C. krusei* isolates.

In the present study, *Candida* spp. were more susceptible to amphotericin B. Amphotericin B is generally regarded to have the broadest spectrum of antifungal activity and used in life-threatening disseminated infections.[28] Although, amphotericin B resistance was low in this study, high cost and associated toxicity restricts its use.[28]

Conclusion.

Non *albicansCandida* species have emerged as an important cause of infections. *C. tropicalis*, *C. krusei* and *C. glabrata*are important NAC spp. As different *Candida* spp. varies widely in their susceptibility to commonly prescribed antifungal drugs, antifungal susceptibility testing often guides clinicians in selection of most appropriate antifungal therapeutic agent.

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