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Original Research Article

Species Distribution and Antifungal Susceptibility Profile of Candida Isolated in Various Clinical Samples at a Tertiary Care Centre

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ABSTRACT

Introduction: Fungal infections are a major cause of morbidity and mortality in immune compromised individuals and Candida are among the most common pathogens in these patients. Candida species belong to the normal microbiota of an individual and are responsible for various clinical manifestations from mucocutaneous overgrowth to blood stream infections. The potential clinical importance of species level identification has been recognized as candida species differ in the expression of putative virulence factors and antibiotic susceptibility.
Aims &Objectives: The aim of the present study is to identify the spectrum of candida species in clinical infections and evaluation of the Antifungal Susceptibility profile to initiate prompt, early and definitive treatment.
Materials and Methods: A total 112 Isolates of Candida obtained were recruited for the study. Gram staining, wet mount was performed. Culture on Blood agar and SDA was done. Growth on this media was subjected to species identification test. The Kirby Bauer disk diffusion method was used to test the susceptibility of Candida isolates following the Clinical and Laboratory Standards Institute (CLSI) guidelines M44-A.
Results: A total of 112 isolates, the highest Candida isolates were observed to be from the age group above 60 years (38.39%). Candida was highest in patients with Chronic Obstructive Pulmonary Disease (36.63%). 75(66.96%) isolates were predominantly from male patients. Candida albicans 53(47.32%) were the maximum isolated species followed by Candida tropicalis 40(35.71%). Isolation of Candida non albicans species was more. Maximum Antifungal resistance was observed for Fluconazole.
Conclusion: As various underlying risk factors are responsible for the Candida infection, most commonly being Chronic Obstructive pulmonary disease (COPD) as reported in our study, it should always be evaluated clinically by the treating physician. The present study showed that prevalence of Non Candida albicans was higher from various clinical specimens.

Keywords: Candida albicans; Candida non albicans species; Antifungal Susceptibility.

INTRODUCTION

Fungal infections are a major cause of morbidity and mortality in immune compromised individuals and Candida are among the most common pathogens in these patients. [1] Candida species are important nosocomial pathogens in critically ill patients and are associated with substantial mortality and prolonged hospitalization in the intensive care unit. [2] Candida species belong to the normal microbiota of an individual's mucosal oral cavity, gastrointestinal tract and vagina and are responsible for various clinical manifestations from mucocutaneous overgrowth to blood stream infections. [3]

The potential clinical importance of species level identification has been recognized as candida species differ in the expression of putative virulence factors and antibiotic susceptibility. [4] Rapid identification of yeast species also guides early appropriate antifungal therapy. The aim of the present study is to identify the spectrum of candida species in clinical infections and to initiate prompt and early treatment.

MATERIALS AND METHODS

A total 112 Isolates of Candida obtained during the period of January 2014-December 2014 from different clinical specimens submitted to the Department of Microbiology of a Tertiary Care Centre at Nanded were recruited for the study. Gram staining, wet mount was performed. Culture on Blood agar and SDA was done. Growth on this media was subjected to species identification test like germ tube test, sugar assimilation test, chlamydospore formation and pigment production on chrome agar .The isolates were tested for the antifungal susceptibility. The Kirby Bauer disk diffusion method was used to test the susceptibility of Candida isolates to Fluconazole (25 µg), Voriconazole (1 µg), Amphotericin B (20 µg), Itraconazole (10 µg), Clotrimazole (10 µg) and Ketoconazole (30 µg) following the Clinical and Laboratory Standards Institute (CLSI) guidelines M44-A for antifungal disk diffusion susceptibility testing of yeasts. The final inoculation was prepared at 5 x 10^5 CFU/mL using 0.5 McFarland turbidity standards. Plates were inoculated for 24 hours.

RESULTS

A total of 112 isolates of Candida species were obtained from different clinical specimens of patients visiting the Outpatient department (OPD), In-Patient Department (IPD), and admitted to ICU during the year January 2014-December 2014.

The highest Candida isolates were observed to be from the age group above 60 years(38.39%) followed by age group between 18 to 45 years(31%) [Table 1].

Table 1: Age Distribution among which Candida Species Isolated.

E	No. of Isolates	% of Isolates
<1	2	1.78%
1 to 18	4	3.57%
18 to 45	35	31%
45 to 60	28	25.00%
>60	43	38.39%
Total	112	100%

In the present study, detailed examination of the medical records of each patient was done to evaluate the main risk factors associated. The main risk factors which were found in this study were Chronic Obstructive pulmonary disease (COPD), smoking, tuberculosis, malnutrition, malignancy, diabetes mellitus, HIV infection, and prolonged use of antibiotics. Isolation of Candida was highest in patients with Chronic Obstructive Pulmonary Disease (36.63%), followed by other risk factors [Figure 1].

Of the total 112 isolates, 75(66.96%) were from male patients and 37(33.03%) were from female patients [Figure 2].

Of the total samples, 54 (48.21%) were from Sputum, 19 (16.96%) from voided urine, 13 (11.60%) from blood, 7 (6.25%) from urine of patients with in dwelling urinary catheter, 6 (5.35%) from vaginal swab, 5 (4, 46%) from Pus, 5 (4.46%) from Endotracheal Secretion and 3 (1.3%) from stool. [Table 2].

Candida albicans 53(47.32%) were the maximum isolated species followed by Candida tropicalis 40(35.71%), Candida parapsilosis 9(8.04%), Candida krusei 8(7.14%) and Candida glabrata 2(1.79%) [Table 3].

The Antifungal Susceptibility profile of C. albicans was 30.19% resistant to Fluconazole,11.32% resistant to Voriconazole, 7.55% resistant to Ketoconazole, 5.66% resistant to Itraconazole, 3.77% resistant to Clotrimazole and 1.89% resistant to Amphotericin B. While Antifungal Susceptibility profile of Non Candida albicans was 25.42% resistant to

Fluconazole,15.25% resistant to Itraconazole, 13.56% resistant to Voriconazole, 16.95% resistant to Clotrimazole and 1.69% resistant to Ketoconazole, 13.56% resistant to Amphotericin B. [Figure 3 and Figure 4].

Table 2: Distribution of the Candida species in various clinical samples

Organism	Sputum	Urine	Urine from catheter	Blood	Vaginal swab	Pus	Endotracheal Secretion	Stool	Total
Candida albicans	33	8	3	1	4	2	1	1	53
Candida tropicalis	21	8	2	3	1	1	2	2	40
Candida parapsilosis	0	1	1	3	1	2	1	0	9
Candida krusei	0	1	0	6	0	0	1	0	8
Candida glabrata	0	1	1	0	0	0	0	0	2
Total	54	19	7	13	6	5	5	3	112

Table 3: Distribution of Candida species

Candida isolate	Number of Isolates	Percentage
Candida albicans	53	47.32%
Candida tropicalis	40	35.71%
Candida parapsilosis	9	8.04%
Candida krusei	8	7.14%
Candida glabrata	2	1.79%
Total	112	100%

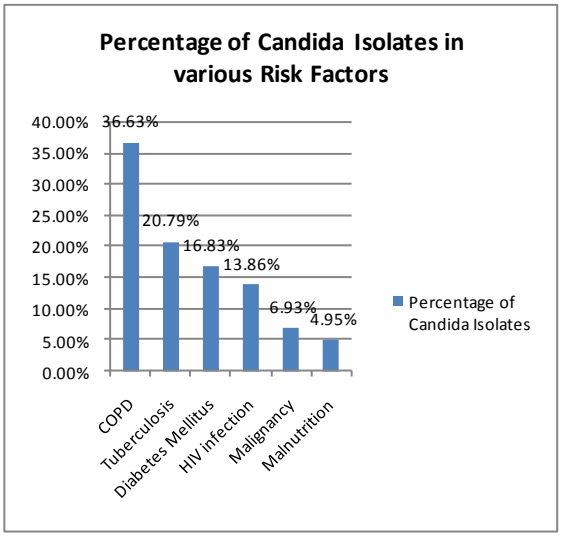

Figure 1: Percentage of distribution of Candida Isolates in the various risk factors observed.

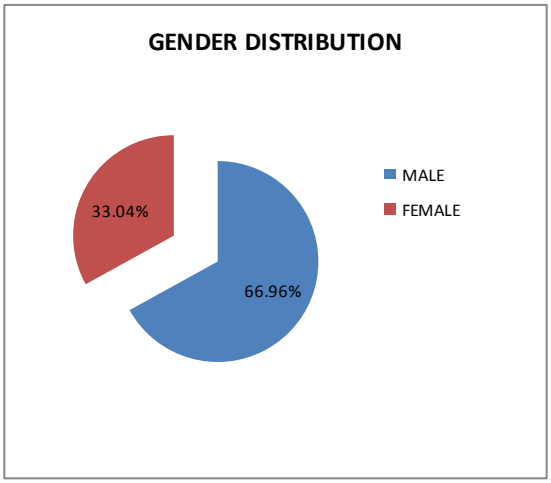

Figure 2: Gender distribution

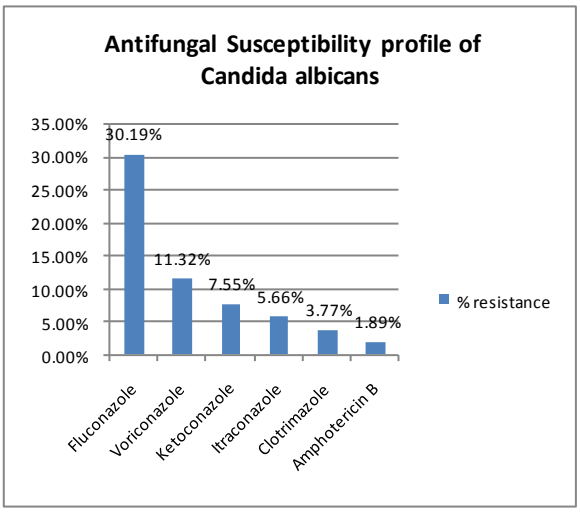

Figure 3: Antifungal Susceptibility profile of Candida albicans.

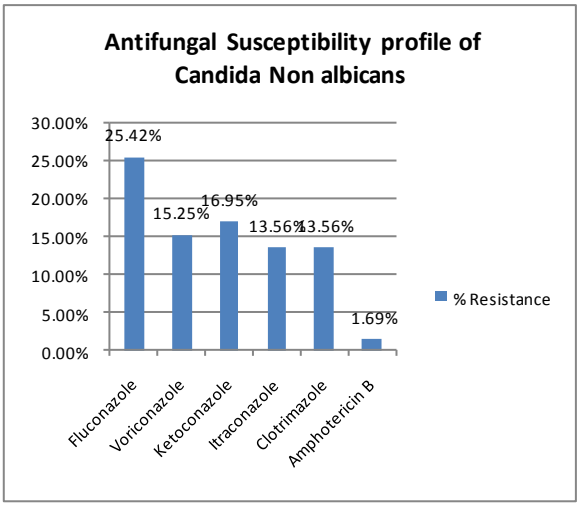

Figure 4: Antifungal Susceptibility profile of Candida Non albicans.

DISCUSSION

A total of 112 isolates of Candida species were studied during January 2014 to December 2014.

Though Candida infection can occur in any age group, in our study it was found to be highest in the age group above 60

years (38.39%) which is similar to the findings of RensedePinho J C et al. [5] followed by the age group 18 to 45 years (31%).

In our study, of the various risk factors which were found, Chronic Obstructive pulmonary disease (COPD) was the most frequently associated risk factor responsible in the isolation of Candida species, which is in concurrence with the study conducted by Jha B K et al. [6]

Our study reveals that Male (66.96%) were the predominant population involved in the Candida infection which is in concurrence with the study conducted by Latha et al. [7] This can be attributed to the fact that smoking and chewing tobacco which leads to various obstructive lung diseases is more common in males.

In this study, the most frequently isolated species was C. albicans, accounting for 47.33% of the infections, followed by C. tropicalis (35.72%), C. parapsilosis (8.03%), Candida krusei (7.14%) and C. glabrata (1.78%) respectively.

Comparative studies on different Candida species which were isolated in their studies by different researchers showed that the isolation of C. albicans was the highest in each of them, T Jaggi et al (44%), Dastidher (72.8%), Gupta D (64%) and Mokaddas et al (39.5%) found C. albicans to be the commonest isolate. [8,9]except in Chakrabarti et al, [10] which showed that the isolation of C. tropicalis was the highest (42%) and that the isolation of C. albicans was 25%.

C. albicans was the predominant species recovered from all the specimens except for blood, which yielded C. krusei as the most frequent species.

In sputum sample, Candida albicans was the most frequently isolated species accounting for about 61.11% of the total which is in concurrence with the study conducted by Lata Patel et al which documented the isolation of Candida albicans in sputum to be 67.77%. [11] The maximum number of Candida albicans isolation in Sputum was seen in age group above 60 years. The population above 60 years of age has co-morbid conditions leading to immunosuppression and Candida infection.

The different blood isolates were recovered from the patients admitted in intensive care unit with high fever and respiratory distress. In these blood samples, Candida krusei was the maximum isolated species with 46.15%, which correlates with the study conducted by Basu S et al. The emergence of C. krusei as a common etiological agent of Candidemia has important clinical implication due to its innate resistance to fluconazole. [12]The isolation of non-albicans Candida has been frequently encountered from candidemic patients in the past few decades as shown in the study conducted by Kothari et al. [13]

The maximum number of isolates from urine was from the Candida non albicans group accounting for about 57.89%. Candida albicans accounted for about 42.10% of the isolates in urine which correlates with the results of Bobade et al, where Candida albicans was isolated in 36.6% of the isolates. [14]

The most common species isolated in urine from catheter were Candida albicans. Two species of Candida glabrata were isolated in urine and urine from catheter. C. glabrata currently ranks second or third as the causative agent of superficial (oral, esophageal, vaginal, or urinary) or systemic Candidal infections, which are often nosocomial, and our study correlates with the findings of Paul L Fidel et al. [15] Candida glabrata has the ability to form sticky “biofilms” that adhere to living and non-living surfaces (such as catheters) thus forming microbial mats, making treatment more difficult.

Numerous worldwide studies showed that Candida albicans are responsible for the greatest number of symptoms associated with the vaginal candidiasis. Our findings have also showed 66.67% of C .albicans positive cases from the vaginal swabs. Similar findings are reported from the studies of Babin D et al

[16] Mahmoudi et al, Uma et al, S.R. Fan et al, Ahamedet al. [17]

Various studies over the years have shown that there is a considerable shift towards the non-albicans Candida isolates. Our study also showed that non-albicans Candida were isolated at a higher rate (52.67%) than C. albicans (47.33%), which was in agreement with the findings of the studies by Ragini Ananth Kashidet al, [18] who also showed the non candida albicans incidence (70.7%) to be higher than that of C. albicans (29.2%).

The in vitro susceptibility testing of antifungal agents is becoming increasingly important because of the introduction of new antifungal agents and the recovery of clinical isolates that exhibit inherent or developed resistance to Amphotericin B, the Azole group of drugs during chemotherapy. In our study, the resistance of yeast isolates against antifungal drugs was most commonly seen against Fluconazole and most of the species were sensitive to Amphotericin B which is in concurrence with the study conducted by Parvez AK et al. [19]

CONCLUSION

As various underlying risk factors are responsible for the Candida infection, most commonly being Chronic Obstructive pulmonary disease (COPD) as reported in our study, it should always be evaluated clinically by the treating physician. The present study showed that prevalence of Non Candida albicans was higher from various clinical specimens. Therefore,, the species level identification will be helpful to laboratories for rapid identification of clinically important Candida spp. More importantly this will also enable clinicians to choose appropriate antifungal agents, as shown in our study for different candida isolates, thus decreasing patient's morbidity and mortality.

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