"To Study the In Vitro Antifungal Susceptibility of Candida Albicans and Non-Albicans Candida Species Isolated from Different Clinical Samples at a Tertiary Care Setup, Kanpur"

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Abstract:

Introduction: Candida albicans are the most common species in humans however, increasing of Non Candida albicans is on rise. This changing epidemiology and increasingly resistance to antifungal agents makes it important to identify Candida spp up to the species level and study its antifungal susceptibility pattern.

Aim and Objective: To study the invitro antifungal susceptibility of Candida albicans and non-albicans candida species isolated from different clinical samples at a Tertiary care setup, Kanpur".

Material and Methods: The present study was conducted in the Department of Microbiology at RMCH&RC, Kanpur over a period of one year from August 2021 to August 2022. Ethical clearance was duly obtained from the Institute Ethical Committee for conducting the study. Clinical samples collected from different sites were cultured on Sabouraud's dextrose agar and incubated and Candida isolates were identified using standard microbiological procedures and speciation was done following conventional and HiChrome differential media. Antifungal susceptibility testing (AST) was determined by using Clinical and Laboratory Standards Institute (CLSI) disk diffusion method.

Results: A total of 204 Clinical samples collected from different sites were cultured on Sabouraud dextrose agar and incubated which were identified using Standard microbiological procedures. A total of isolates collected from Outpatient was 35 and 169 from Inpatient Department. Out of 204 isolates the ratio of Females (62.7%) was more as compared to Males (37%) with different clinical specimens 61 (29.9%) was from sputum and 52 (25.4%) from urine. The maximum number of cases was reported in the age group of more than 60 years of age and least reported in the age group of 0- 20 years. Candida albicans comprised of 37.2% of the total isolates whereas the NAC spp. comprised of 62.7% of the total isolates. The maximum isolates was found in Sputum samples of Candida albicans., whereas for NAC Candida tropicalis was the most common isolate present in urine specimen. Voriconazole was the most sensitive drug with (88%) followed by Amphotericin B (80%) and Itraconazole (62%) and least with Ketonazole (51%) and Fluconazole (48%).

Conclusion: The increasing incidence of candida infections and the emergence of antifungal resistance have emphasized the need for updated laboratory data to guide clinicians in selecting appropriate antifungal therapy.

Keywords: Candida, NAC, CLSI, AST

Introduction

Candida continues to be a leading cause of morbidity and mortality in large population of immune compromised and hospitalized patients. Candida species are the most common cause of fungal infections causing wide range of life-threatening diseases such as blood stream candidiasis to non-life-threatening mucocutaneous candidiasis such as genitourinary candidiasis, vulvovaginal candidacies, and oropharyngeal candidacies [1]. Among fungal infections, invasive candidiasis is commonly associated with high morbidity and mortality rate. Until recently, C. albicans was recognized as the commonest species causing most of the cases of candidiasis.

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However, in the last few decades, several studies reported that there has been a progressive shift from a predominance of C. albicans to non-albicans Candida species (NAC) such as C. tropicalis, C. glabrata and C. krusei [2, 3].

The epidemiology of non-albicans candida has been on the rise in last few years [4]. Five species of Candida namely, C. albicans, C. glabrata, C. parapsilosis, C. tropicalis and C. krusei are reported to cause more than 90% of invasive infections, although the relative distribution of the species depends on the geographical area, patient population and predisposing conditions, local hospital related factors, and the types of antifungal agents received[5]. The extensive use of antifungals for prophylaxis became the leading cause of colonization of non-albicans Candida (NAC) species and increasing resistance to antifungal drugs [6]. Therefore this study was undertaken to study the distribution of Candida spp. isolated from various clinical specimens at our tertiary hospital, their susceptibility to antifungal agents and associated risk factors.

Material and Methods

This was a cross-sectional study carried out in the Department of Microbiology, RMCH&RC over a period of 1 year i.e., August 2021 to August 2022. A total of 204 Clinical suspected samples from patients of various age groups being treated for suspected Candidiasis at different departments of our tertiary care hospital in Uttar Pradesh was included in our study. Detailed clinical and treatment history of patients was recorded. Yeast cultures obtained from blood, urine, sputum, bronchoalveolar lavage, cerebrospinal fluid, pus, peritoneal fluid, high vaginal/ cervical swab were included in the study.

The isolation and identification of fungi were performed using standard CLSI methods in the microbiology laboratory [7]. Samples were subjected to Gram's stain to look for presence of Gram positive yeast like budding cells with pseudohyphae and KOH mount. The samples were inoculated on Sabouraud's dextrose agar (SDA) with choramphenicol & Blood agar and incubated at 37 º C and 25 º C [8]. Identification & Speciation Suspected colonies of Candida on SDA were confirmed by Gram's stain. Further species identification was done by a) germ tube test b) Dalmauplate culture for Chlamydospore production c) inoculation on chromogenic medium d) carbohydrate utilization patterns by Sugar Assimilation Tests.

Antifungal susceptibility testing was performed for all the isolates of Candida using disc diffusion method on Mueller Hinton agar supplemented with 2% glucose and 0.5 µg / ml of methylene blue as per the procedure described in the Clinical and Laboratory Standard Institute (CLSI, 2020) against two antifungal agents [7]. Cell suspensions of individual Candida strains were prepared in 5 ml saline solution. The turbidity was adjusted to yield 0.5 McFarland standards. The commercially available antifungal discs of Fluconazole (25 µg), Voriconazole (1µg),Ketaconazole(10 µg), Amphotericin B(30 µg/ml), were used and zones of inhibition were measured after 20- 24 hours of incubation at 37°C [8].

Results

The present study was conducted in the Department of Microbiology at RMCH&RC, Kanpur over a period of one year from July 2021 to July 2022. Ethical clearance was duly obtained from the Institute Ethical Committee for conducting the study.

A total of 204 Clinical samples collected from different sites were cultured on Sabouraud dextrose agar and incubate. Candida isolates were identified using Standard microbiological procedures and speciation was done following conventional and HiChrome differential media. Antifungal susceptibility testing (AST) was determined by using Clinical and Laboratory Standards Institute (CLSI) disk diffusion method [7]

During the study period, 204 isolates were identified from different clinical specimens, in which the rate of Females (62.7%) was more as compared to the Males (37%) [Table no. 1] in which more samples were from 61 (29.9%) from sputum and 52 (25.4%) from urine as shown in [Table No. 2]. A total of isolates were collected from Outpatient was 35 and 169 from Inpatient Department.

Table no.1: Gender wise distribution of patients from the study

S. No.	Gender	Number of Isolates	Percentage
1.	Male	76	37%
2.	Female	128	62.7%

Table No 2: Sample wise: Distribution of Cases

S.No.	Samples	No. of Isolates	Percentage
1	Urine	52	25.4%
2	Sputum	61	29.9%
3	Blood	36	17.6%
4	Oral swab	21	10.2%
5	Nail clipping	6	2.9%
6	Catheter tip	13	6.3%
7	Skin scrapping	9	4.4%
8	Vaginal swab	6	2.9%
	Total	**204**	**100%**

In our Study the maximum number of cases was reported in the age group of more than 60 years of age followed by 31-40 years. The minimum number of cases was reported in the age group of 0- 20 years of age, which is illustrated in the below Table no. 3.

Table No 3: Age wise Distribution of patients from the study

S.No.	Age (in years)	No. of Patients	Percentage
1.	0-10	4	1.9%
2.	11-20	8	3.9%
3.	21-30	10	4.9%
4.	31-40	16	7.8%
5.	41-50	14	6.8%
6.	51-60	9	4.4%
7.	≥61	82	40.1%

In our study *Candida albicans* comprised of 37.2% of the total isolates whereas the NAC spp. comprised of 62.7% of the total isolates. Among the NAC, *Candida tropicalis* (31.3%) was the most predominant species followed by *Candida dubliniensis* (9.8%) and least was *Candida glabrata* (3.9%)

Maximum isolate of *Candida albicans* were 37.2%. Among the NAC there was a total of 62% isolated. The maximum number of isolates was found for *Candida*

tropicalis (31.3%) and least for *Candida glabrata* with 3.9% [Table No. 4].

Table No 4: Distribution of Candida sp. from the study

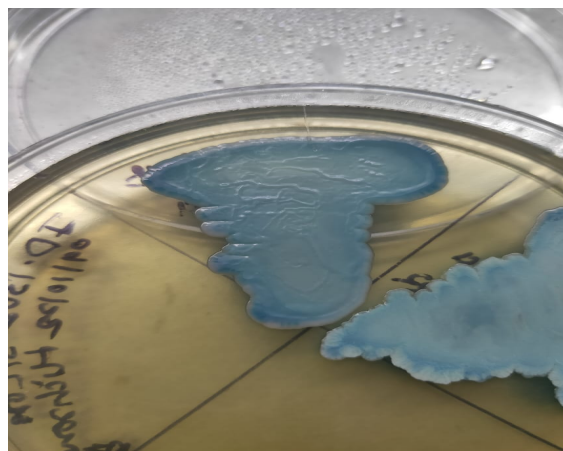| S.No.	Candida species	No. of Isolates	Percentage
1	*Candida albicans*	76	37.2%
2	*Candida tropicalis*	64	31.3%
3	*Candida krusei*	36	17.6%
4	*Candida dubliniensis*	20	9.8%
5	*Candida glabrata*	8	3.9%
	Total		**100%**

Figure No 1: Candida Tropicalis Isolated on Hi Chrome

Table No. 5: Distribution of different species of *Candida* among various clinical specimens.

S.No.	Candida species	SAMPLES							
		Urine	Sputum	Blood	Oral swab	Nail clipping	Catheter tip	Skin scrapping	Vaginal swab
1	*Candida albicans*	18	32	4	2	3	3	11	3
2	*Candida tropicalis*	28	15	15	2	4	-	-	-
3	*Candida krusei*	12	10	8	6	-	-	-	-
4	*Candida dubliniensis*	12	8	-	-	-			
5	*Candida glabrata*	5	3	-	-	-	-	-	-
	Total								

Among the distribution of different species of *Candida* among various clinical specimens, the maximum isolates was found in Sputum samples of *Candida albicans.,* whereas f*or* NAC *the Candida tropicalis* was the most common isolate with urine specimen containing 28 number of isolates.

In the antifungal, the most sensitive Antifungal agent was voriconazole with (88%) followed by Amphotericin B (80%) and Itraconazole (62%) and least with Ketonazole (51%) and Fluconazole (48%).

Discussion

Candidiasis is an opportunistic infection occurring in presence of predisposing factors like extensive and prolonged administration of broad-spectrum antimicrobials, corticosteroids, immunosuppressive agents and cytotoxic drugs, diabetes mellitus, HIV, chronic renal failure, haemodialysis, renal transplantation or indwelling urinary catheter

Candida spp. has been increasingly emerged as principal pathogens of opportunistic infections in healthcare settings. In our study the Majority of the isolates were obtained from sputum followed by urine. This might be due to the fact that the presence of fungi.

There were other studies performed by other authors where the maximum cases were from the sputum and urine. Gopi A and Murthy NS observed that predominant isolates were from sputum (41.6%) and urine samples (20.4%) [9]. However, study by Shaik N et al., and Joseph K et al., recovered maximum number of isolates from urine (60% and 46.9%, respectively) followed by respiratory samples (17.3% and 20.4%, respectively) [10]. In our Study the maximum number of cases was reported in the age group of more than 60 years of age followed by 31-40 years. The minimum number of cases was reported in the age group of 0- 20 years of age. Similar findings were found by Joseph K et al., and Goel R et al., [11]. Predominance of *Candida* spp. in elderly group in current study might be due to the presence of significant co-morbid conditions like

diabetes, chronic obstructive pulmonary disease and prolonged antibiotic therapy.

In our study *Candida albicans* comprised of 37.2% of the total isolates whereas the NAC spp. comprised of 62.7% of the total isolates. Among the NAC, *Candida tropicalis* (31.3%) was the most predominant species and least was *Candida glabrata* (3.9%) followed by *Candida dubliniensis* (9.8%). Our study was in support with the study conducted by the other author where NAC was more isolated [12, 13]. There were few studies performed by other authors which was in contrast to our study, where *Candida albicans* was predominant [14, 15]

In the antifungals, the most sensitive Antifungal agent was voriconazole with (88%) followed by Amphotericin B (80%) and Itraconazole (62%) and least with Ketonazole (51%) and Fluconazole (48%), this was parallel to other studies where similar susceptibility patterns have been observed by Bhaskaran R et al., and UrvashiChongtham et al.,, [16,17]where among the azoles, voriconazole showed the maximum sensitivity of 86%, was the most sensitive and least in ketoconazole (56%). It has also been observed that all the *C. krusei* were resistant to fluconazole as they are intrinsically resistant to fluconazole. It was also noted that the rate of Females in our study ws more as compared to the Males [17].

Our present study is focused on the speciation and antifungal susceptibility of clinical isolates of *Candida* has gained significance in the management of *Candida* infections in and around Kanpur region. This study was undertaken with the objective of generating data on different species of *Candida*, their characterization up to the species level and to determine their antifungal susceptibility patterns.

Conclusion

There is increasing incidence of NAC over Candida albicans. An increase in the predisposing conditions in the recent years has resulted in an increasing incidence of Candida infections. Therefore early speciation of Candida isolates not only will restrict the empirical use of antifungal agent but also greatly influence the treatment options for the clinicians and thus will be beneficial for the patients as some Candida species are intrinsically resistant to some antifungal.

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