Section: Microbiology

Original Research Article

DISTRIBUTION OF CANDIDA INFECTION IN CLINICAL SAMPLES AND THEIR ANTIFUNGAL SUSCEPTIBILITY PATTERN IN HOSPITAL OF WESTERN U.P.

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Abstract

Background: Infection due to Candida species ranges from superficial, mucosal infections, opportunistic infection and systemic infections like endocarditis, peritonitis, candidemia, systemic candidiasis etc. There has been an epidemiological shift towards the predominance of candida other than C.albicans such as C.parapsilosis, C.glabrata, C.tropicalis, C.krusei, C.dubliensis etc. accounting for more than half of cases. The rampant misuse of antifungals for various infections has increased the risk of resistance developing against antifungal agents among candida species giving serious challenge for proper treatment of candida infections. This study was aimed to understand isolation of various Candida spp. in different clinical specimens of patients attending a tertiary care centre in western U.P. and to analyse its association with sex and admission in the hospital. This study also extends to determine susceptibility pattern of the isolated candida spp. against different antifungal agents. **Materials and Methods:** A prospective observational study was conducted in Microbiology department in the hospital of western U.P. North India from July 2021 to June 2022. All the clinical samples submitted to the mycology section of central microbiology laboratory were included. Statistical analysis used all the data were analysis statistically using SPSS software version 21.0. **Result:** In current study, 51.6% were C. albicans while 48.4% were NAC. Urine sample (59.4%) was the commonest clinical sample followed by respiratory specimens (13.7%) and high vaginal swab (HVS). Among NAC, C.parapsilosis (20.9%) was commonest NAC followed by C. dubliensis (9.8%), C.glabrata (8.50%), C.tropicalis (5.9%) and C.krusei (3.3%). All isolates were found sensitive to AmphotericinB, however 5.2% of the candida isolates were found resistant to Voriconazole and 18.3% to fluconazole. **Conclusion:** Such studies will enable clinicians to choose appropriate antifungal agents for the patients thereby reducing patient's mortality and morbidity.

INTRODUCTION

Hospital acquired infections (HAI) due to Candida species are rising since 1980s leading to higher morbidity and mortality rates along with increase in hospital stay thereby increasing the overall treatment cost of the patients. Candidial infections are more commonly presented in persons with immunocompromised conditions including persons undergoing cancer treatment by radiotherapy and chemotherapy, acquired immune deficiency syndrome (AIDS), major surgery and organ transplantation, patients admitted in intensive care unit (ICU) for prolonged period, or patients with medical devices.[1] Infections caused by Candida species ranges from superficial, mucosal infections to complicated systemic infections like endocarditis, peritonitis, candidemia, systemic candidiasis etc.[2] C. albicans, is the commonest fungal infection which is residing as normal flora in skin, reproductive and gastrointestinal tract of humans. However there has been an epidemiological shift towards the increase of other non-albicans candida spp. (NACs) such as C. glabrata, C. parapsilosis, C. pseudotropicalis, C.

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vishwanathi, C.kefyr, C. dubliensis etc. accounting for above fifty percent of the total Candida infections.[3] Characterization to species level at initial level is extremely important to identify strains of candida; which might be intrinsically resistant to some antifungal agents like C. krusei for fluconazole.[4] The rampant misuse of antifungal agents for various infections has increased the resistance among C.albicans and non-albicans species thereby giving serious challenge for proper treatment of candida infections.[5] The rising variation in Candida species, their susceptibility and resistance to different antifungal agents shows the importance of early diagnosis for the control and treatment of these infections. The current study was aimed to understand the isolation of various Candida spp. in different clinical specimens of patients attending a tertiary care centre in western U.P. and to analyse its association with sex and admission in the hospital. This study also extends to determine susceptibility pattern of the isolated candida spp. against different antifungal agents.

MATERIALS AND METHODS

Study design: A prospective observational study was carried out for 1 year in the Microbiology department in the hospital of western U.P. North India from July 2021 to June 2022. All the clinical samples submitted to the mycology section of central microbiology laboratory during the study period were included. These samples were collected from all areas like Outpatient Department (OPD) and inpatients (IPDs), Intensive Care Units (ICUs), neonatal ICU, medicine, gynaecology, surgical, and paediatrics wards.

Sample size: The minimum sample size was 92 candida cases for this study to be statically significant. The sample size was calculated using below mentioned formula:

$$\text{Sample Size} = \frac{Z^2\,P\,Q}{E^2}$$

Z^2: standard normal variate.

P: Prevalence (33.96)

Q: 100-P = (66.04)

E= Absolute error (5% -10%)

Selection and description of participants: Different clinical specimens like high vaginal swab (HVS), respiratory sample, urine, blood, pus, catheter tips, ear swabs etc. received in the laboratory with isolation of candida were included in the study. After taking written informed consent from the recruited subjects the sample was processed. Wet mount was prepared for appearance of yeast cells showing budding with or without pseudohyphae and cultured on Sabouraud Dextrose agar slants. Isolated colonies were identified based on cultural characteristics, gram stain, Reynold Braude phenomena, growth on chromogenic medium, biochemical reactions in sugar fermentation and sugar assimilation test [6]. All other isolates except candida were excluded from our study. Antifungal drug testing of candida was done according to CLSI M 44A standards. Antifungals tested were fluconazole (25µg), voriconazole (1µg), and Amphotericin B (100 µg) procured from HiMedia laboratories, Mumbai. Confirmation of results were done by Minimum Inhibitory Concentration (MIC) by using E- test strips according to manufacturer instructions.

Statistical analysis: The incidence of various candida spp. in different clinical samples was identified in frequency and percentage. Categorical variables association were tested using Chi square test with $P< 0.05$ as statistically significant. The statistical analysis was performed using recent SPSS software version 21.0 (IBMCorp., Armonk, NY, United States of America)

RESULTS

Total 153 Candida spp. were isolated from different clinical samples as per the study criteria and the sample size was more that the minimum sample size calculated. Out of which, 51.6% were Candida albicans while 48.4% were non-albicans candida. Candida infection was more common in females (62.09%) compared to males (37.91%). Female (62.09%) is significantly associated with the development of candida infections than males (37.91%) with a ratio of 1.6:1 with P = 0.003. 21-30 yrs (25.5%) were the most commonly affected age group followed by 41-50 yrs (17%) and 51-60 yrs (16.3%) with a mean age of 42 yrs as shown in [Table 1]. Of 153 isolates, 71 (46.40%) isolates were isolated from cases admitted in wards, while 66(43.14%) and 16 (10.45%) isolates from ICU and OPD patients respectively. Patients admitted in the hospital in wards or ICUs are significantly associated with the development of various candida infections compared to OPD patients with a ratio of 8.56:1 with P = 0.000. Urine sample (59.4%) was the common clinical sample followed by respiratory specimens (13.7%) and HVS (high vaginal swab) with positivity for candida infection as shown in [Table 2]. Candiduria has significant contribution in candida infections with P=0.000.

In the present study, 48.4% cases accounted for non-albicans candida (NAC). Among NAC, the most common species were C. parapsilosis (20.9%) followed by C. dubliensis (9.8%), C. glabrata (8.5%), C. tropicalis (5.9%) and C. krusei (3.3%) as shown in [Table 3]. Among urine sample, C. albicans (50.5%) was most commonly isolated followed by C. parapsilosis (20.9%), C. glabrata (8.8%) and C. dubliensis (8.8%). C. albicans was seen in 71.4% of isolates from respiratory samples while C. dubliensis accounted for 14.3%, followed by C. glabrata (9.5%) and C. parapsilosis (4.8%). From vaginal swabs (HVS) predominantly C. albicans (56.2%) and C.parapsilosis (37.5%) were recovered. However in blood culture, NAC contributed 73.3% of the total

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candida species while C. albicans accounted for 26.7% of the isolates as shown in [Table 3]. However the P value was 0.063 which is more than 0.05, thereby accepting the null hypothesis that any candida species can be the etiological agent in different clinical samples.

In the current study, 100% isolates were sensitive to Amphotericin B. However 5.2% were found resistant to Voriconazole and 18.3% were resistance to fluconazole. Resistance to Voriconazole and fluconazole was more common among NAC especially C. dubliensis, C. krusei and C. glabrata compared to C. albicans as shown in [Table 4].

Table 1: Age and sex-wise distribution of various Candida spp. isolated from various samples

Age group	No. of isolates	Males	Females
0-10 yrs	10 (6.5%)	7	3
11- 20 yrs	9 (5.9%)	3	6
21- 30 yrs	39 (25.5%)	5	34
31-40 yrs	18 (11.7%)	7	11
41-50 yrs	26 (17.0%)	7	19
51-60 yrs	25 (16.3%)	16	9
61-70 yrs	14 (9.1%)	9	5
>70 yrs	12 (7.8%)	4	8
Total	153	58 (37.91%)*	95 (62.09%)*

P value-0.003.*

Table 2: Distribution of Candida isolates in various clinical samples

Clinical samples	No. of isolates	Percentage
Urine	91	59.4%
Respiratory specimens (ET secretions and Broncho-alveolar lavage)	21	13.7%
HVS	16	10.4%
Blood	15	9.8%
Pus sample	08	5.2%
Peritoneal fluid	02	1.3%

P value-0.000. P is significant at the 0.001 level

Table 3: Distribution of various Candida spp. isolated from different clinical samples

Etiological agents	No. of isolates (%)	Urine	Respiratory samples	HVS	Blood	Pus	Peritoneal fluid
C.albicans	79 (51.6%)	46 (50.5%)	15 (71.4%)	09 (56.2%)	04 (26.7%)	05 (62.5%)	-
C.parapsilosis	32 (20.9%)	19 (20.9%)	01 (4.8%)	06 (37.5%)	02 (13.3%)	02 (25%)	2 (100%)
C.dubliensis	15 (9.8%)	08 (8.8%)	03 (14.3%)	-	03 (20%)	01 (12.5%)	-
C.glabrata	13 (8.5%)	08 (8.8%)	02 (9.5%)		03 (20%)		
C.tropicalis	09 (5.9%)	07 (7.7%)			02 (13.3%)		
C. krusei	05 (3.3%)	03 (3.3%)		01 (6.2%)	01 (6.7%)		
Total	153	91	21	16	15	08	02

P value-0.063, which is not significant at P value of 0.05.

Table 4: Antifungal Susceptibility pattern of various Candida Isolates

Clinical Isolates	Fluconazole (Flu)		Voriconazole (Vor)		Amphotericin B (Amp B)	
	S	R	S	R	S	R
C.albicans (79)	71 (89.9%)	08 (10.1%)	76 (96.2%)	03 (3.8%)	79 (100%)	0 (0%)
C.parapsilosis (32)	29 (90.6%)	03 (9.4%)	32 (100%)	0 (0%)	32 (100%)	0 (0%)
C.dubliensis (15)	08 (53.3%)	07 (46.7%)	12 (80%)	03 (20%)	15 (100%)	0 (0%)
C.glabrata (13)	09 (69.2%)	04 (30.8%)	12 (92.3%)	01 (7.7%)	13 (100%)	0 (0%)
C.tropicalis (09)	08 (88.9%)	01 (11.1%)	09 (100%)	0 (0%)	09 (100%)	0 (0%)
C. krusei (05)	0 (0%)	05 (100%)	04 (80%)	01 (20%)	05 (100%)	0 (0%)
Total (153)	125 (81.7%)	28 (18.3%)	145 (94.8%)	08 (5.2%)	153 (100%)	0%

DISCUSSION

A major increase in the incidence and prevalence of opportunistic fungal infections, specially caused by endogenous human commensal flora Candida species, has gained importance in the medical fieldworldwide. In our study, total 153 Candida isolates were identified from various clinical samples. Candida infection was common in females (62.09%) compared to males (37.91%) with a ratio of 1.6:1 with P = 0.003, which is comparable with findings in other studies.[7,8] The reason of high prevalence and virulence of Candida in females is that it has a receptor for female hormones and also due to poor personal hygienic conditions. However many studies suggest male dominance in candidiasis.[9] In our study, most commonly affected age group was 21-30 yrs (25.5%) followed by 41-50 yrs (17%) with a mean age of 42 yrs which was in concordance with other studies.[10] Maximum isolation of candida spp. was from admitted patients (Ward and ICUs) as compared to OPD with a ratio of 8.56:1 with P = 0.000. This could be due to various factors like immunocompromised patients, antibiotics usage, indwelling devices etc.

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In the present study, the highest number of isolates (59.4%) were from urine, followed by respiratory specimens (13.7%), HVS (10.4%) and blood (9.8%). Our study is in accordance with a study conducted by Patel et al,[11] where maximum Candida isolation is from urine and sputum. Candiduria has significant contribution in candida infections in our study with P=0.000. This could be justified as candida is one of the common commensal in gut flora and it can reach to the urinary tract via ascending and hematogenous route especially in immunocompromised conditions.

Candida albicans was the leading pathogen accounting for 51.6% of yeast infection compared to NACs which was similar to earlier reports.[11-13] Various studies have shown the importance of NAC and their increasing incidence in the past decade. In the present study, 48.4% cases accounted for non-albicans candida (NAC). Among NAC spp., C. parapsilosis (20.9%) was most commonly isolated followed by C.dubliensis (9.8%), C.glabrata (8.5%), C.tropicalis (5.9%) and C.krusei (3.3%). In a study conducted by V. Manchanda, C.tropicalis (55.03%) was the most common NAC isolated.[14] However, Nucci et al. reported C. albicans (37.6%) as the most common agent causing Candida infection followed by C. parapsilosis and C. tropicalis.[15] In the current study, no association (p value 0.063) was found between the sample received and the species isolated in it, thereby suggesting that any candida spp. can cause infection of any site. High level of resistance to commonly used antifungals in these species poses a serious threat for the society as well as clinicians in treating such patients.

Many new and advanced antifungal agents have been introduced in recent decades and their efficacy, effectiveness and sensitivity to treat fungal infections is required. Hence antifungal sensitivity testing is important in isolated strain of candida for better management of patients. In our study, all isolates were found sensitive to AmphotericinB. This finding correlates with another study where C.albicans & NAC were 100% sensitive to Amphotericin B.[2] C. krusei and C. glabrata are considered as intrinsically resistant species against azole group of antifungals. Fluconazole resistance was found in 18.3% of the clinical isolates in the present study. Fluconazole was more commonly found resistant among NAC especially C. dubliensis, C.krusei and C.glabrata compared to C.albicans. Fluconazole resistance was 18.8% in Tasneem et al,[16] and 13% in Sojakova et al.[17] However, Kaya et al,[18] reported dangerously increased fluconazole resistance in C. albicans (68.7%) and NACs (63.2%). In the current study, 5.2% isolates were resistant to Voriconazole and was higher in NAC compared to C. albicans. Voriconazole was more effective compared to itraconazole and fluconazole, but voriconazole resistance in isolates is increasing due to cross resistance, because of similar chemical structure. Oberoi et al observed cross-resistance and reduced susceptibility to fluconazole as well as voriconazole in 11.3% isolates.[19]

CONCLUSION

Candidiasis incidence has been increased throughout the world especially among hospitalised patients and in patients with immunocompromised status. C.albicans is the common isolate; however the incidence of NAC has also been increased considerably. The NAC species has changes in their sensitivity pattern to various antifungal agents used commonly in clinical practice which is a common cause of concern. Hence it is necessary to monitor closely the incidence of various species along with their antifungal susceptibility.

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