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ORIGINAL RESEARCH

Virulence and antifungal susceptibility pattern of Candida isolates in ICU patients: a prospective observational study from kumaon region, Uttarakhand, India

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ABSTRACT

Background: Candidiasis is emerging as a significant health problem in hospitalized patients especially in ICU setup. There is relative rise in the proportion of Non-albicanscandida (NAC) and antifungal resistance. Virulence factors of fungi also contribute to pathogenesis of disease process and its resistance pattern. **Aim and Objective:** Virulence and antifungal susceptibility pattern of Candida isolates in ICU patientsof Dr. Susheela Tiwari Government Hospital,Haldwani, Uttarakhand. **Materials and Methods:**A prospective observational study was conducted for two years from Nov 2015 to Oct 2017 and 2323 samples were collected from suspected cases of Candida infection, admitted in critical care units of Dr. Susheela Tiwari Government Hospital, Haldwani. Identification of Candida species was done by standard laboratory approaches. Virulence factors like Biofilm and Coagulase production were assessed by tube method whereas Haemolysin activitywas assessed on blood enriched SDA agar. Antifungal susceptibility testing was done by Disc diffusion method (M 44-A).**Results:**Out of 2323 clinical specimens, 104 (4.47%) were positive for Candida species, of which34(32.69%) were C. albicans, while 70(67.30%) were Non-albicansCandida (NAC).Most frequently isolated Non-albicans Candida was C. tropicalis (41.34%). Biofilm production was found to occur more frequently among Non-albicans Candida (72.0%). Coagulase and haemolysin production was found to be more in C. albicans than NAC. Antifungal susceptibility testing showed resistance to fluconazole was highest in both C.albicans and NAC species. The drug resistance to itraconazole was more in NAC as compared to C. albicans and this difference in the resistance pattern was statistically significant. ($p<0.005$)**Conclusion:** Fungi have emerged as an important public health problem. Identification and antifungal susceptibility testing of Candida isolates from ICU patients will help in building a data center of the prevalent Candida species and will go a long way in the management of patients suffering from candidiasis.

Keywords: Virulence, Antifungal susceptibility, Observational study, Uttarakhand

INTRODUCTION

Fungi have emerged as an important public health problem, especially among the immunocompromised and those hospitalized with serious underlying diseases. They are a cause of increased morbidity and mortality in them.[1]Intensive Care Units (ICU) are highly susceptible for opportunistic fungal infections.[2] Candida cause systemic diseases which are the fourth leading cause of nosocomial bloodstream infections in modern hospitals.[3] Nowadays, many countries are experiencing a relative rise in the proportion of Non-albicanscandida (NAC) like Candidatropicalis, Candidaparapsilosis, Candidaglabrata and Candida krusei. Moreover, though C.albicans is generally susceptible to

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fluconazole, NAC species are showing decreased susceptibility to it.[4,5]

Virulence factors of Candida like adherence, Biofilm formation, secreted enzymes, toxins, surface structures and phenotype switching contribute to pathogenesis of disease process and its resistance pattern.[6,7]Therefore, early detection of Candida species, their virulence factors and antifungal susceptibility will greatly help in making clinical decisions.[8] This will result in judicious use of antifungal therapy at a time when there is a rising trend in detection of NAC species and antifungal resistance. Since limited studies are present addressing above situation, present study was conceptualized to characterize clinical samples from various ICU's for candida species, determine their virulence factors and antifungal susceptibility pattern.

AIM AND OBJECTIVES

Virulence and antifungal susceptibility pattern of Candida isolates in ICU patientsof Dr. Susheela Tiwari Government Hospital, Haldwani, Uttarakhand.

MATERIALS AND METHODS

Study Design

A prospective observational study was conducted for two years from Nov 2015 to Oct 2017

Study setting and population

Samples were collected from suspected cases of Candida infection admitted in ICU of Dr. Susheela Tiwari Government Hospital, Haldwani.Candida isolated from the samples collected from ICU were subjected to speciation using the following techniques according to standard protocols[3]- KOH mount, Gram's stain, India Ink preparation, culture on Sabouraud's Dextrose Agar, Germ Tube test, Urea hydrolysis, Cornmeal Agar morphology (Dalmau plate technique), morphology in CHROM agar, Carbohydrate fermentation test, Carbohydrate assimilation test. Virulence factors like Biofilm and Coagulase production were assessed by tube method whereas Haemolysin activity was assessed on blood enriched SDA agar. [8] Antifungal susceptibility testing was done by Disc diffusion method (M 44-A).

Sample size

Assuming an average rate of occurrence of Candidia isolates in ICU to be 50% and a margin of error of 2.3%, the expected sample size for this study was calculated to be 1872. Considering sample spoilage rate of 10%, minimum sample size came to be 2059 but we planned to include 2323 samples in our study to add more precision to the study.

Inclusion criteria

Clinical samples from suspected Candidiasis, who were critically ill and admitted in the Medical (MICU), Surgical (SICU), Pediatric (PICU), and Anaesthesia (AICU) ICU's.

Exclusion criteria

Patients previously treated with or were on antifungal drug therapy.

Ethical Clearance

Study protocol was approved by Institutional Ethical Committee (IEC) of Government Medical College, Haldwani.

Statistical Analysis

Collected data was coded appropriately, entered in Microsoft Excel (MS Excel) spreadsheet and later cleaned for any possible errors in SPSS Statistics for Windows v. 16.0 (IBM Corp., Armonk, NY). Categorical data are presented as percentage. Descriptive analysis of categorical data is presented as frequencies and percentages. The Chi-square test is used to assess the association between two categorical variables and $p<0.05$ is considered as statistically significant.

RESULTS

A total of 2323 clinical specimens were collected during the study period, of which majority of the samples from ICU were urine 64.3% followed by blood 13.4% (Table 1). Total samples positive for Candida species were 104, of which 34 (32.69%) and 70 (67.30%) were positive for C. albicans and Non-albicans Candida (NAC) respectively. The most frequently isolated Non-albicans Candida was C.tropicalis 61.4% followed by C.krusei, C.dubliniensis (11.4%) (Table 2). Maximum no. of C.albicansand NAC were isolated from urine sample (81.73%)(Table 3). Among the virulence factors (Biofilm, Coagulase, Haemolysin) studied, out of 104 Candida isolates tested, 65.38% were found to be biofilm producers. Biofilm production was found to occur more frequently among non-albicans Candida species (70%) than C. albicans (55.9%). Coagulase and haemolysin production were found to be more in C. Albicans (67.6%, 70.6%) than NAC isolates(42.9%, 44.3%) and the difference was statistially significant. The difference in the number of isolates producing more than one virulence factors among C.albicans and NAC was found to be significant statistically ($p<0.05$) (Table 4). Among the non-albicans Candida species, Biofilm formation capacity was higher in C. krusei, C. parapsilosis (75.0%) and C. tropicalis (74.41%) (Table 5). The results of antifungal susceptibility testing showed all the Candida isolates (C. albicans and NAC) to be sensitive (100%) for amphotericin B and nystatin. The resistance to fluconazole was highest in both C.albicans and NAC species (out of VC, FLC, IT, KT). The drug resistance to itraconazole was more in NAC as compared to C. albicans and this difference in the resistance pattern was statistically significant ($p<0.005$) (Table 6).

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Table 1: Distribution of different samples in ICU's

Samples	Number (%)
Urine	1494 (64.31)
Blood	312 (13.43)
Pus	274 (11.79)
Csf	117 (5.03)
Peritoneal fluid	68 (2.92)
Pleural fluid	58 (2.49)
Total	**2323**

Table 2: Different Candida spp. isolated from ICU patients

Species	NO. (%)
C.albicans	34 (32.7)
NAC species	70 (67.3)
C.tropicalis	43 (61.4)
C.krusei	8 (11.4)
C.dubliniensis	8 (11.4)
C.glabrata	7 (10)
C.parapsilosis	4 (5.7)
Total	**104**

Table 3: Sample-wise distribution of Candida species

Candida Isolates	Urine N (%)	Pus N (%)	Blood N (%)	Peritoneal Fluid N (%)	Pleural fluid N (%)	Total n (%)
C.albicans	25 (73.52)	2 (5.88)	5 (14.70)	2 (5.88)	0 (00.00)	34 (32.70)
NAC	60 (85.71)	2 (2.85)	2 (2.85)	3 (4.28)	3 (4.28)	70 (67.30)
Total N (%)	85 (81.73%)	4 (3.84%)	7 (6.73%)	5 (4.80%)	3 (2.88%)	104 (100)

Table 4: Virulence factors of C.albicans and Non-albicansCandidaIsolates

	C.albicans **(N=34) (%)**	***NAC*** **(N=70) (%)**	**TOTAL (N=104) (%)**	***P*** **value**
Biofilmproductionn (%)	19(55.9)	49(70)	68(65.4)	0.15
Coagulase productionn (%)	23(67.6)	30(42.9)	53(51)	**0.01**
Haemolysin productionn (%)	24(70.6)	31(44.3)	55(52.9)	**0.01**
No. Of isolates producing >1 virulence factors	27(79.4)	36(51.4)	63(60.6)	**0.006**

Table 5: Virulence factors produced by Candida species

Candida isolates	No. Of isolates	Biofilm production n (%)	Coagulase production N (%)	Haemolysin production n (%)
C.albicans	34	19(55.88)	23(67.64)	24(70.58)
C.tropicalis	43	32(74.41)	27(62.79)	29(67.44)
C.glabrata	7	3(43.85)	2(28.57)	0(0)
C.krusei	8	6(75.00)	1(12.5)	1(12.5)
C.dubliniensis	8	5(62.50)	0 (0)	0(0)
C.parapsilosis	4	3(75.00)	0 (0)	0(0)
Total	**104**	**68(65.38)**	**53 (50.96)**	**55(52.88)**

Table 6: Comparison of Resistance pattern of C.albicans and Non-albicansCandidaisolates

Antifungal drug	Resistance		
	C.albicans **34 N (%)**	***NAC*** **70 N (%)**	***p-value***
Ketoconazole	9 (26.47)	27 (38.57)	0.360
Fluconazole	13 (38.23)	39 (55.71)	0.094
Itraconazole	6 (17.64)	32 (45.71)	**0.005**
Voriconazole	7 (20.58)	29 (41.42)	0.073

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DISCUSSION

Candidaspecies causes wide spectrum of infections depending on its virulence factors, susceptibility to antifungals and host defenses.[9]Patients in ICUs are more prone for acquiring nosocomial infections due to the compromised immune status of the patients and high frequency of invasive procedures needed for their monitoring and treatment.[10]In the present study, out of 2323 clinical specimens received from different ICU's, total104 samples were found positive for Candidaspecies.We observed that NAC species were more frequently (67.3%) encountered than C.albicans (32.7%). Among NACspecies, C.tropicalis was most common isolate (61.4%). Majority of the patients were in the age group of > 60 years (24.04%). Male to female ratio was 1.8:1. In our study, Candida species were mainly isolated from urine (81.73%). In the present study, biofilm production was noted in 65.38% Candida isolates. It was found to occur more frequentlyin Non-albicans Candida species (70%) than C. albicans (55.9%). The findings were in concordance with Sharma et al.[10]Among the Non-albicans Candida species, biofilm formation capacity was highest in C. krusei and C. parapsilosis (75.0%) folllowed byC. tropicalis (74.41%). These findings were similar toSharma P etal[10],Mukhia R. Ket al[11], Arora S et al[12] and Bizerra F C etal.[13]In our study, Coagulase production and haemolysin production washigher in C. albicans(67.6%, 70.6%)than NAC isolates (42.9%, 44.3%) and the difference was statistially significant.Similar results were observed in a study by Sharma et al [10]and Rodrigues AG et al. [14]The difference in the number of isolates producing more than one virulence factors among C.albicans and NAC was found to be significant statistically ($p<0.05$). Candida pathogenicity is facilitated by a number of virulence factors, the most important of which are biofilm formation and secretion of hydrolytic enzymes (proteases, haemolysins). Candida biofilms carries an important risk of increased resistance to antifungal therapy. Biomedical devices support colonization and biofilm formation by Candida.[15] Catheter related infections are the major cause of morbidity and mortality among hospitalised patients and catheter microbial biofilms are associated with 90% of these infections.[16]In current study,all the isolates (C. albicans and NAC) showed no resistance to amphotericin B and nystatin which was in concordance with Guptaet al[17], Arora S et al[12], Shivanand et al.,[18]BineshLal Y et al[19] and Dutta V et al.[20] The findings are in contrast to study conducted by AlmeidaAA et al[21] where 3.1% of NAC isolates were resistant to amphotericin B.[22]The resistance to itraconazole was lowest in Candida albicans(17.6%) whereas it was second highest in NAC species (45.7%). This difference was found to be statistically significant ($p<0.05$). The drug resistanceof NAC species toitraconazolewas also reported by Arora S et al[12], Shivanand et al.[18] In our study, C. albicans and NAC showed maximum resistance to fluconazole (38.23% & 55.71% respectively) which was similar with the finding of Sharma etal[10], Guptaet al[17] and Binesh et al.[19]The cause for this is widespread use of fluconazole at community care facilities which has facilitated development of resistance to fluconazole.[23]Possible mechanisms involved is upregulation of multidrug efflux transporter genes due to exposure to increasing concentrations of the fluconazole.[24]There are various reports from our country and world showing increasing trend of NAC species isolates in clinical samples and development of resistance towards antifungal therapy. This warrants judicious use ofantifungal therapy in hospitalswhich will greatly improve the clinical outcome in patients suffering from candidiasis.

CONCLUSION

Candidiasis is emerging as a significant health problem in hospitalized patients especially in ICU setup.The present study highlights the predominance of NAC species colonization in ICU patients and their resistance to commonly used antifungal drugs. Identification and antifungal susceptibility testing of Candida isolates from ICU patients will help in building a data center of the prevalent Candida species and will go a long way in the management of these serious patients

LIMITATIONS

Multicentric studies are required so that our findings can be generalized.

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