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Original Article

Phenotypic Characterization of *Candida* species in Tertiary Care Hospital of Eastern Nepal

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Abstract

Background

Candida albicans is one of the most frequently isolated yeast in clinical laboratories and studies have shown that it accounts for up to 80% of the yeast recovered from the site of infection. *Candida* species have emerged as significant opportunistic fungal pathogens and the conventional methods of yeast identification are reported to be cumbersome with delayed diagnosis and initiation of treatment. Thus rapid identification and speciation of *Candida* species is essential in clinical laboratories.

Materials and Methods

A hospital based cross sectional study was carried out in the department of Microbiology, Nobel Medical College from January 2020 to December 2020. Approval was acquired from the institutional review committee. Various clinical specimens were obtained and identification as per the standard microbiological procedures. Data were analyzed by SPSS, version 20.

Results

A total of 62 *Candida* species were isolated out of which 65% were from male and 35% were from female patients. *Candida albicans* was found to be the most common species with 68%. The highest incidence was seen in the age group below 20 years and 21–40 years with 32.25% cases each followed by 41-60 years with 20.96% cases. Among 62 specimens, urine samples yielded the highest number of *Candida* species 48.38% followed by sputum samples with 29.03% *Candida* species. High vaginal swab (HVS) yielded 12.9% *Candida* species and blood samples yielded 9.67% *Candida* species.

Conclusion

Candida albicans is still the most significant clinically but other non albicans are also emerging significant pathogens and warrant routine discrimination in clinical laboratories.

Keywords: *Candida albicans*, *Candida tropicalis*, *Culture media*

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Introduction

Candida albicans is one of the most frequently isolated yeast in clinical laboratories and studies have shown that it accounts for up to 80% of the yeast recovered from the site of infection [1]. The ability of *C. albicans* to produce germ tube and chlamydospores is the basis of its preliminary identification. However *Candida dubliniensis* has recently been described as very similar to *C. albicans* in many characteristics, especially germ tube formation and chlamydospore production [2].

Candida species other than *C. albicans* have also emerged as significant opportunistic fungal pathogens with an increase in number of patients with impaired immunity such as transplant recipient, cancer patient receiving chemotherapy and human immunodeficiency virus infection [1]. *Candida* species have developed resistance to antifungal agents in recent time particularly with the triazole compounds and emergence of drug-resistance has largely attributed to the use of prolonged and inappropriate empirical therapy which has further complicated the patient management and outcome. The conventional methods of yeast identification, which mainly consist of assimilation and fermentation characteristics are reported to be cumbersome and beyond the range available in local laboratories especially in resource-limited settings with delayed diagnosis and initiation of treatment [3]. But the diversity and spectrum of *Candida* species of clinical significance means there is a need to develop fast and cost effective methods of identification [1].

CHROM agar *Candida* technique has been used and has been useful in discriminating different *Candida* species as well as mixed infestations. It is a reliable and sensitive method for presumptive identification of more commonly isolated yeast species of the genus *Candida*. However, no single phenotypic test is highly effective in identifying *Candida* species and combination of tests is sometimes necessary for identification. Molecular technique has been employed to characterise *Candida* species. Although sensitive and specific it is not cost effective for routine clinical mycology laboratories in resource constrained setup [4].

Materials and Methods

A hospital based cross sectional study was conducted in the Department of Microbiology, Nobel Medical College and Teaching Hospital, Biratnagar over a period of one year from January 2020 to December 2020. This study was started after acquiring approval from the institutional review board of Nobel Medical College. The yeast that grew in the culture media used for the isolation of bacterial pathogens from the urine, sputum, high vaginal swab (HVS) and blood specimen were included[5]. The sample size was estimated to be 62 by using the formula, $n= Z^2 P (1-P)/e^2$, where Z is confidence level at 95% (1.96); e is margin of error taken as 10% and p is expected prevalence from literature [1].

All suspected yeast colonies were confirmed by Gram staining [5]. A total of 62 isolates of *Candida* species were recovered among them. The isolates were inoculated into sabouraud's dextrose agar containing chloramphenicol incubated at 37°C for 24-72 hours and subsequent tests were carried out for species identification [1].

Germ tube test: This test is used as a presumptive test for identification of *Candida albicans*. A small inoculums of the yeast cells from a pure culture were suspended in 0.5 ml human serum and incubated at 37°C for three hours. A drop of incubated serum was placed on a microscopic slide and covered with a cover slip to examine the presence of germ tubes under microscope. A yeast cell having about half the width and 3 to 4 times the length of the mother cell and no constriction at the neck of the parent cell was considered as a true germ tube [6]. Temperature tolerance: The isolates were cultured into SDA and incubated at 45°C for 72 hours. Growth observed was identified as *Candida albicans* [7]. CHROM agar: It was used for presumptive identification of different *Candida* species and to detect any mixed colonies. This is based on the differential release of chromogenic breakdown products from various substrates following differential exoenzyme activity. A single colony from pure culture was inoculated into CHROM agar and incubated at 37°C for 72 hours after which colour change was noted [8].

Chlamydospore production: Chlamydospore production on Corn Meal Agar (CMA) was used as a presumptive confirmatory test for the identification of *Candida albicans*. To see the isolate's ability to produce chlamydospore, test strains were inoculated into CMA plates by slide culture technique. This test was done by streaking and stabbing the media with isolates and covered with sterile cover slip and incubated at 25° C for 72 hours. The growth was stained with lactophenol cotton blue and examined for the chlamydospore production [9]. The collected data were entered

in Microsoft Excel 2007 and analysed using SPSS version 20.

Results

A total of 62 *Candida* species were isolated out of which 40 (65%) were from male patients and 22 (35%) were from female patients which is depicted in figure 1. *Candida albicans* was found to be the most common species with 42 (68%) cases followed by *Candida tropicalis* 11 (18%), *Candida parapsilosis* 5 (8%) and *Candida glabrata* 4 (6%) as shown in figure 2. The highest incidence was seen in the age group below 20 years and 21 – 40 years with 20 (32.25%) cases each followed by 41-60 years with 13 (20.96%) cases and the least number of cases were seen in age above 60 years with 9 (14.51%). Among 20 isolates from the age group below 20 years 16 were *Candida albicans*, 4 were *Candida tropicalis*. Out of 20 isolates between the age group of 21- 40 years, 11 were *Candida albicans*, 6 were *Candida tropicalis*, 2 were *Candida parapsilosis* and 1 was *Candida glabrata*. Among 13 isolates between the age group of 41-60 years showed 9 *Candida albicans*, 3 *Candida parapsilosis* and 1 *Candida glabrata*. Out of 9 isolates above 60 years of age, 6 were *Candida albicans*, 2 were *Candida glabrata* and 1 was *Candida tropicalis* as shown in figure 3. Among 62 specimens, urine sample yielded the most number of *Candida* species 30 (48.38%) where *Candida albicans* 24, *Candida tropicalis* 3, *Candida parapsilosis* 2 and *Candida glabrata* 1 followed by sputum sample with 18 (29.03%) Candida species where *Candida albicans* 11, *Candida tropicalis* 4, *Candida parapsilosis* 2 and *Candida. glabrata* 1. High vaginal swab (HVS) yielded 8 (12.9%) *Candida* species with *Candida albicans* 4, *Candida tropicalis* 2, *Candida parapsilosis* 1, *Candida glabrata* 1. Blood samples yielded 6 (9.67%) *Candida* species with *Candida albicans* 3, *Candida tropicalis* 2 and *Candida glabrata* 1 as shown in table 4. Among *Candida albicans* all the 42 isolates showed light green colour, chlamydospore production and growth at 42°C, 11 isolates of *Candida tropicalis* showed blue colour, no chlamydospore production and no growth at 42°C, 5 isolates of *Candida parapsilosis* showed pale pink colour, no Chlamydospores production and no growth at 42°C, 4 isolates of *Candida glabrata* showed cream to pink smooth colonies and no chlamydospore production and no growth at 42°C. Growth and colonial characteristics were shown in table 5.

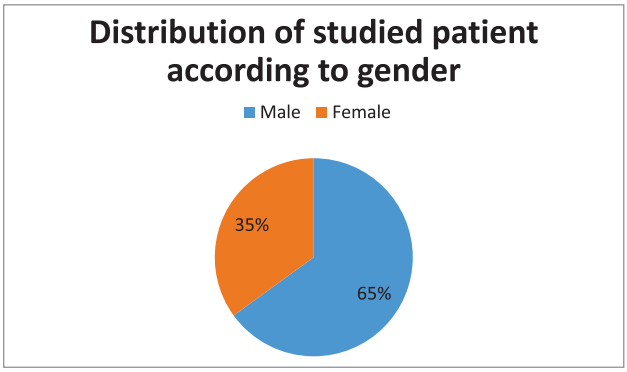

Figure 1: Distribution of studied patient according to gender

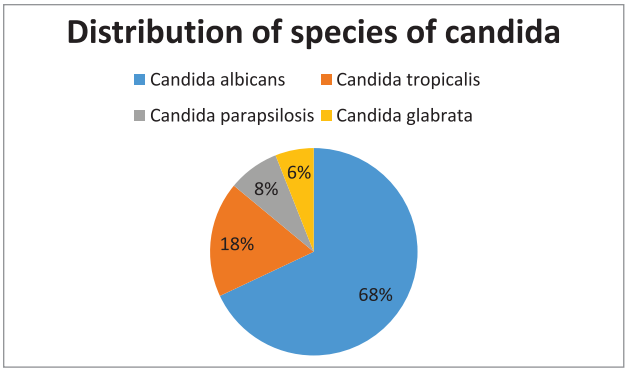

Figure 2: Pie diagram showing distribution of *Candida* species

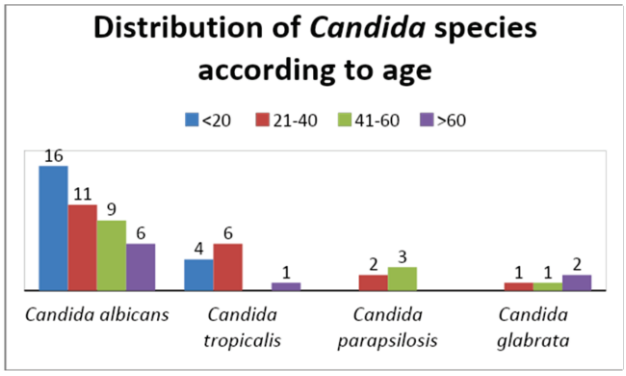

Figure 3: Distribution of *Candida* species according to age

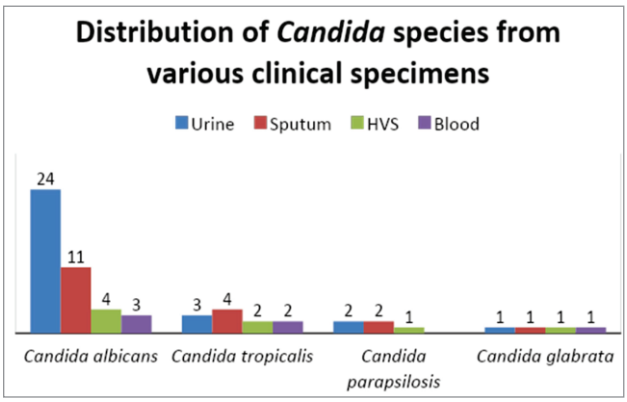

Figure 4: Distribution of *Candida* species from various clinical specimens

Species	Total number of isolates	Colour on Chrome agar	Germ tube test		Chlamydospores on CMA	Growth at 45°C
			Positive	Negative		
C. albicans	42	Light green	42		+	+
C. tropicalis	11	Blue		11	-	-
C. parapsilosis	5	Pale pink		5	-	-
C.glabrata	4	Pink smooth		4	-	-

Figure 5: Growth and colonial characteristics of Candida species

Discussion

Over the past decades the infection caused by fungal agents has increased dramatically and *Candida* species have become an important pathogen causing severe rapidly progressive disease which has become difficult to manage especially in immunocompromised individuals [10]. In our study most of the isolates were obtained from male 40 (65%) than female 22 (35%) patients which represents male preponderance, a finding similar to that of S. Swathi *et al*. in which 62.3% were male and 37.73% were female [5]. *Candida albicans* was the commonest species isolated in our study, which accounts for 68% of the total isolates and non albicans *Candida* species contribute 32% of the Candidal infections which was similar to the study done by various studies [5, 11]. but the study found that non albicans isolates contribute more of the candidal infections i.e. more than that of *Candida albicans* [12]. However *Candida albicans* was the commonest among *Candida* species.

In the present study, the majority of the isolates were from urine samples 30 (48.38%) followed by sputum 18 (29.03%) and HVS 8 (12.9) and blood 6 (9.67%) which was similar to the study done by Swathi S. *et al*. Our findings of a higher number of isolation of *Candida* species from urine samples is in agreement with reports which have shown the increased incidence of *Candida* infection in the genitourinary tract in all fields of medical and surgical practice [5]. But the study done by Sahal G *et al* found that the majority of the isolates were from HVS followed by urine samples [12]. In our study, the highest numbers of isolates were seen in the age group of below 20 and 21 to 40 whereas in most of the studies highest numbers of isolates were seen in the age group between 21-40 years [5]. SDA is the medium most widely used for the isolation of *Candida* species and other yeast from the clinical specimens. But SDA is not a differential medium as CHROM exhibits their properties [13]. The chromogenic medium, CHROM agar is a simple and rapid method for identification of common *Candida* species [1]. In terms of colony growth CHROM AGAR showed sensitivity and specificity with 95% confidence level in the range of 95.3%-100%. CHROM agar correctly identified 99% of *Candida albicans*, 98% of *Candida tropicalis* and 94% of *Candida glabrata* but for other *Candida* species other tools of identification should be used for the correct identification [1]. In our study, no *C. dubliniensis* was isolated, all the *C. albicans* isolates grew at both 37°C and 45°C and chlamydospores were not typical of C. dubliniensis. In the present study 100% of *Candida* isolates were identified accurately to the species level using CHROM agar. CHROM agar appears to be quite accurate in identifying the common *Candida* species so in resource-limited settings, availability of this type of media not only facilitates the provision of rapid patient care but may also assist to control the rise in antifungal agent resistance by reducing the time taken for presumptive identification of the organism at species level to start the therapeutic regimen [1].

Limitation of our study was to have a better understanding of the role of *Candida dubliniensis* in clinical infections, it is essential to identify this species accurately in clinical specimens as *C. dubliniensis* lack the ability to utilise xylose (XYL) and α-methyl-d-glucoside (MDG), whereas *Candida albicans* isolates utilise XYL and MDG [14].

Conclusion

Candida albicans is still the most significant clinically but other non albicans are also emerging significant pathogens and warrant routine discrimination in clinical laboratories. *Candida dubliniensis* was not isolated in our study however it does not rule out its presence and may warrant further investigations.

Acknowledgement: None

Conflict of interest: None

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