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Characterization and Antifungal Susceptibility Pattern of Candida Isolated From Blood Stream Infections among Patients Admitted In NICU and PICU of A Tertiary Care Centre

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ABSTRACT

Characterization of Candida species is of importance because non albicans Candida infections are significantly increasing. Also resistance to Azole group of drugs is not uncommon. This study was aimed to characterize Candida isolated from blood stream infections in NICU and PICU and their sensitivity to Fluconazole. A total of 312 samples of blood cultures were incubated at 37ºC and sub cultured on blood agar, MacConkey agar and Sabouraud dextrose agar. Susceptibility of *Candida* to Fluconazole was performed by disk diffusion method as per CLSI. Out of 312 patient samples, Candida was isolated in 44 (14.1%), of which 9 were *Candida albicans*, *14 Candida tropicalis, 7* C. parapsilosis, 5 *C. glabrata*, 5 *C. krusei* , and 4 were *C. gulermondii*. Out of 42 isolates tested, 7 were resistant to Fluconazole. Timely and accurate diagnosis and susceptibility testing is urgently required to minimize the mortality, morbidity and prolong hospital stay due to blood stream infections by Candida.

1. INTRODUCTION

Bloodstream infections are on a rise worldwide. Among the causes, *Candida* ranks fourth in the United States and seventh in Europe [1,2,3].

Candida blood stream infections (BSI) are associated with a very high mortality, morbidity and prolong hospital stay specially in the newborns and pediatric population [4,5]. Overall the prevalence of Candida causing blood stream infections range from 6-18% [6,7] .

Characterization of Candida species is of importance because non albicans Candida infections are now significant and even replacing Candida albicans at places. Also resistance to Azole group of drugs is not uncommon and is often problematic because of non judicious use antifungal agents.

Knowledge of local variability and resistance pattern becomes critical especially when no data base is available and very few studies have been carried out in central India. [8,9,10]

The aim of this study was to Characterize Candida isolated from blood stream infections in NICU and PICU and their sensitivity to Fluconazole.

2. METHODS

A hospital based prospective study was conducted in Department of Microbiology, GMC and Intensive Care Unit of the Department of Pediatrics. The duration of study was 18 months. Written term of consent was obtained from all the newborns' parents or guardians after explaining the nature and purpose of the study. The study was approved by institutional ethical committee.

A total of 312 blood samples, one from each patient, 1-3 ml volume were collected in 20 ml BHI broth and brought to the laboratory without delay. Samples were collected prior to antimicrobial treatment. The blood cultures were incubated at 37ºC and sub cultured on blood agar, MacConkey agar and Sabouraud's Dextrose Agar (SDA) on 2nd, 3rd, 5th, and 7th day.

Candida was identified by colony morphology, and Gram stain. It was further characterized by germ tube test, growth on corn meal agar for production of chlamydiospore and characteristic patterns of pseudohyphae, specific colour production on CHROMagar (Hichrome, Himedia Pvt Ltd.), and by carbohydrate assimilation.

The susceptibility of *Candida* strains to Fluconazole was performed by disk diffusion method as per CLSI M44-A2 protocol. In this method, Muller-Hinton agar supplemented with 2% glucose and 0.5µg/ml methylene blue dye (GMB) medium was used [11].

Statistical analysis - Data was collected as per the protocol. Various statistical methods were applied as per the requirement to analyze the data.

Tests applied were Z test and Fisher's exact test. P values less 0.05 was taken as statistically significant with 95% confidence interval.

3. RESULTS

A total 312 blood samples for culture were received during the study period. Of these, 198 were male and 114 were female.

Out of 312 patient samples, Candida was isolated in 44 (14.1%), bacterial growth was observed in 97 (31.1%), combined growth of fungal and bacterial in 9(2.9%), 10(3.2%) samples were contaminated, and no growth was seen in 152 (48.7%). There was no significant difference in male and female patients having Candidemia. Only samples that revealed pure growth of candida (44) were further characterized by various phenotypic techniques and antifungal susceptibility testing was performed by disc diffusion method.

Among the Candida growth observed, *Candida albicans* contributed 20.4 % (9 of 44) to Candida sepsis, and a predominance of non-albicans Candida was noted [79.6 %]. The distribution of Candida species and their anti fungal susceptibility pattern is shown in chart 1. Among non albicans, *Candida tropicalis* was predominant (14), followed by C. parapsilosis (7), *C. glabrata* (5), *C. krusei* (5), and least isolated was *C. gulermondii*(4).

Most of the Candida species were sensitive and 7of 42 isolates tested were resistant to Fluconazole by disk diffusion method (Chart 1). Maximum sensitivity was observed for *C. parapsilosis* (100%) and minimum for *C. gulermondii* (66.7%).

4. DISCUSSION

During past few years a marked increase in the incidence of candidaemia has been reported in India [12]. Further there has been a rise in infections by non albicans candida specially C. tropicalis.[13, 14]

There appears to be change in the trend of occurrence of candidal infections towards non albicans species as we found in our study also where 79.6% isolates were non albicans Candida.Among non albicans group, we found C. *tropicalis* as prominent non *albicans* Candida species (31.8%). There are various studies with similar observations.[15, 16, 17, 18] Chrome agar was very useful for rapid (with in 48 hrs) and presumptive identification of candida species specially for C. albicans, C. parapsilosis, and C. tropicalis (Fig 1). There are various studies with similar observations [19, 20].

In spite of availability of CHROMagar and corn meal agar methods, carbohydrate assimilation test remains most specific for phenotypic characterization of Candida. In our study we found that most of the Candida isolated could be easily confirmed within 48 – 72 hrs by this method.

Azoles are amongst the most useful antifungal drugs for the treatment of Candida infections. The resistance against Azole class of antifungal agents has been increasing very rapidly due to frequent use of the drug.[21]

There has been sequential increase in Fluconazole resistance among *Candida* species over past few years [22]. In our study, 7 out of 42 isolates tested (16.7%) were found resistant to Fluconazole by disc diffusion method. However, no resistance was observed against Fluconazole in *C. parapsilosis* isolates. These results are similar to a few previous studies [23, 24].

Another observation in our study was that the proportion of Fluconazole resistance observed was high in non albicans species (82.2%) than for *Candida albicans* [77.8%]. This observation is similar to several studies published in the past and favours the statement of increasing drug resistance among non albicans candida [25].

Because of drug resistance to azole group of drugs which are mainstay of therapy, at times it becomes difficult to treat these infections. Therefore timely and accurate diagnosis and susceptibility testing is urgently required to minimize the mortality, morbidity and prolong hospital stay.

However, the no. of isolates and susceptibility testing only for Fluconazole remains the shortcomings of this study, and therefore similar large scale studies must be performed in various parts of India to generate a data base for appropriate selection of antifungal agents and to control drug resistance among clinical isolates.

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Fig 1 growth of Candida on CROM agar

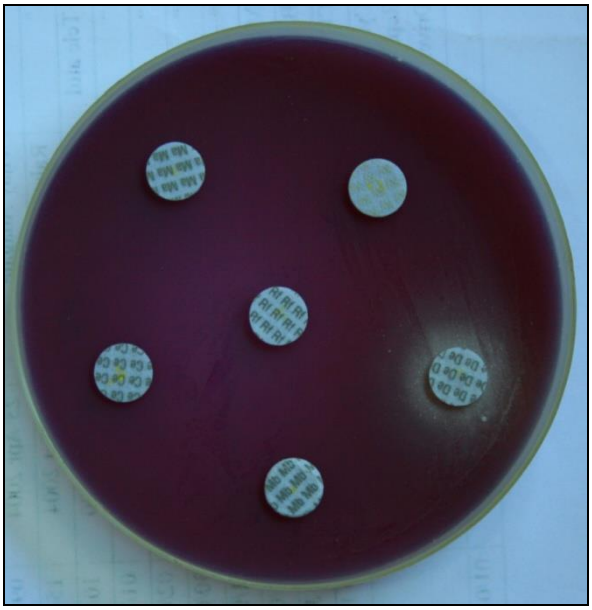

Fig 2: Carbohydrate assimilation test

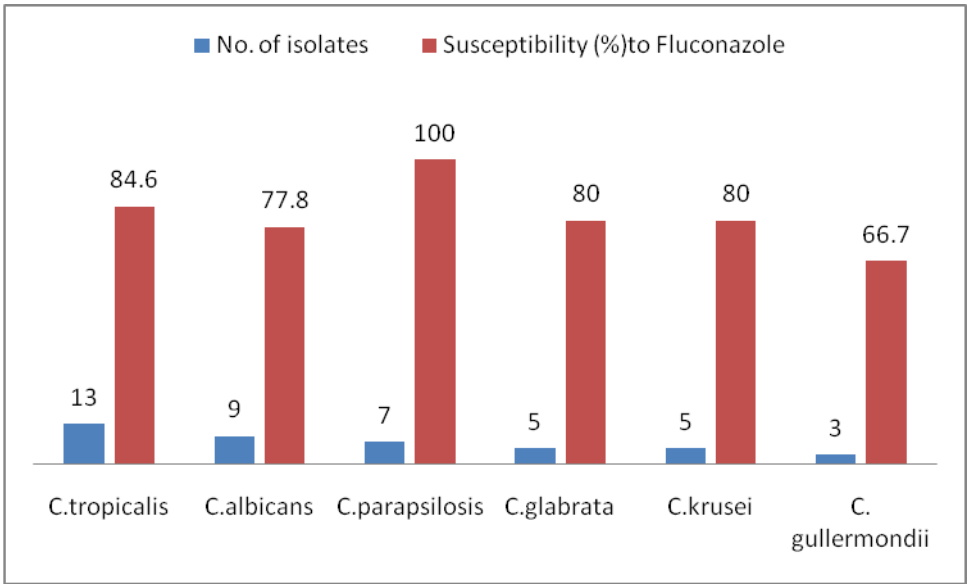

Chart 1: Distribution of Candida species and their antifungal susceptibility pattern