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Research Article

CANDIDEMIA PROFILE AND ANTIFUNGAL DRUGS SUSCEPTIBILITY PATTERN IN NEONATAL INTENSIVE CARE UNIT PATIENTS IN A TERTIARY CARE TEACHING INSTITUTE OF PUNJAB, INDIA

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A B S T R A C T

Introduction: Invasive candidiasis is one of the most common encountered problem in neonatal intensive care unit (NICU). During last two decades there is change in emerging species and sensitivity pattern. This study aims to find magnitude and drug susceptibility pattern of *Candida* species in NICU at tertiary level center. **Methods:** Total 426 blood cultures were tested. Identification was done by both conventional and automated VITEK-2 system. Antifungal susceptibility was done according to CLSI M27-A3 method. **Results**: From 44 isolates, all (100%) were Non *albicans Candida* (NAC). From NAC, *C. tropicalis* (40.91%) was predominant followed by *C. pelliculosa* (34.09%) and *C. krusei* (25%). From all the isolates, fluconazole, flucytosine, amphotericin B and caspofungin were sensitive in 67%, 79%, 90.9% and 92.5% respectively isolates. Voriconazole and micafungin were 100% sensitive. **Conclusion :** Since, *Candida* spp. are assuming an increasingly important role in nosocomial infections in neonates, preventive measures such as appropriate use of multiple invasive medical devices and a restrictive policy of antibiotic use to decrease *Candida* colonization rates should be implemented to decrease the emergence of NAC spp.

INTRODUCTION

Candida blood stream infections have become an increasingly important problem in the neonates. *Candida* spp. are third common cause of invasive fungal infections in neonate intensive care unit (NICU) and account for 9-13% of such infections[1].*Candida* spp. can also spread through vertical transmission from maternal flora or via horizontal transmission from hands of healthcare workers (HCW)[2]. Invasive candidiasis is associated with substantial morbidity and mortality and is difficult to diagnose due to lack of specific signs and symptoms. Although *Candida albicans* has historically been the most frequently isolated species, recently *Non-albicans Candida* (NAC) have emerged as important opportunistic pathogen. Speciation and susceptibility testing of *Candida* spp. is still not routinely being done at most of the centers. The present study was conducted to determine the *Candida* spp. causing candidemia in NICU and to find their antifungal susceptibility pattern. It helps in the selection of appropriate antifungal agents, successful treatment and to prevent the emergence of drug resistance.

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MATERIALS AND METHODS

The present study was conducted in the departments of Microbiology and Pediatrics of Guru Gobind Singh Medical College and Hospital, Faridkot, Punjab (India) on neonates who were admitted in NICU with clinical suspicion of sepsis. During study period (March 2016-August 2017), a total of 426 consecutive blood cultures were received from NICU after obtaining permission from institutional ethics committee. After taking informed written consent from parents or care takers, detailed history and clinical findings were recorded. It was a descriptive study.

Testing of Samples

1.5ml of blood sample was inoculated aspectically into BD BACTEC Peds plus/F culture vials of automated blood culture system (BACTEC 9120, Becton Dickinson,USA). It was incubated in BACTEC 9120 system till signal for growth was detected in the form of positive alarm. Vials giving no signals were reported negative after 5 days of incubation in BACTEC 9120 system. Once signal was there, the vial was taken out and further processing was done. Gram staining of smear prepared from positive Bactec bottle done. Subcultures were done on 5% sheep blood agar, MacConkey's agar and Sabouraud's

dextrose agar (SDA) with chloramphenicol (0.05%) and incubated at 37°C[3]. The *Candida* spp. was identified by conventional methods like germ tube test, corn meal agar test, carbohydrate fermentation and carbohydrate assimilation test. Identification was also done by automated VITEK-2 compact system.

Antifungal Susceptibility test Method

24 hour subcultures of these *Candida* spp. were used for antifungal susceptibility testing of amphotericin B (0.0313-16µg/mL), flucytosine (0.125-64µg/mL), fluconazole (0.125-64µg/mL), voriconazole (0.0313-16µg/mL), caspofungin and micafungin (0.015 -8µg/mL). The MICs for all isolates were determined by following the reference microdilution CLSI M27-A3 method. MIC endpoints were determined spectrophotometrically at 24h. MIC values for fluconazole, voriconazole, flucytosine, caspofungin and micafungin were defined as the lowest concentration of antifungal that resulted in a 50% reduction in the OD relative to the drug-free control. For amphotericin B, the MIC was defined as the concentration of antifungal that reduced growth by 100% compared with the control. *C. parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258 were included in CLSI BMD assays to provide on-scale MIC results and to represent fluconazole susceptible and fluconazole resistant spp., respectively.[4]

RESULTS

Out of 426 blood culture samples 44 were found to be positive for *Candida* spp. From 44 isolates, all the *Candida* spp. were *non albicans Candida* (NAC). From NAC, 40.91% (18/44) were *C. tropicalis*, 34.09% (15/44) were *C. pelliculosa* and 25% (11/44) were *C. krusei* (Fig 1). It shows that *C. tropicalis* was the most common isolated spp. Among 44 isolates of NAC, all the 18 isolates of *C. tropicalis* were 100% sensitive to fluconazole, voriconazole, flucytosine, amphotericin B and micafungin. However in case of caspofungin, 77.78% isolates were sensitive and 22.22% were observed as resistant. In case of *C. pelliculosa,* all 15 strains showed 100% sensitivity to fluconazole, voriconazole, flucytosine, amphotericin B, caspofungin and micafungin. *C. krusei* showed 100% sensitivity to voriconazole, 36.37% strains were sensitive and 63.63% intermediate were to flucytosine, 72.72% and 27.28% strains were sensitive and resistant to amphotericin B respectively, 100% isolates were observed as intermediately sensitive to caspofungin and micafungin was (Table 1).

Table 1 Antifungal susceptibility pattern of Isolated *Candida* spp. (n=44)

	C. tropicalis **(n=18)**	***C. pelliculosa*** **(n=15)**	***C. krusei*** **(n=11)**
Antifungal agent	No. (%)	No. (%)	No. (%)
Fluconazole			
Sensitive	18 (100%)	15 (100%)	
Intermediate	-	-	NA*
Resistance	-	-	
Voriconazole			
Sensitive	18 (100%)	15 (100%)	11(100%)
Intermediate	-	-	-
Resistance	-	-	-
Flucytosine			
Sensitive	18 (100%)	15 (100%)	4(36.37%)
Intermediate	-	-	7(63.63%)
Resistance	-	-	-
Amphotericin B			
Sensitive	18 (100%)	15 (100%)	8 (72.72%)
Intermediate	-	-	-
Resistance	-	-	3 (27.28%)
Caspofungin			
Sensitive	14 (77.78%)	15 (100%)	-
Intermediate	04 (22.22%)	-	11 (100%)
Resistance	-	-	-
Micafungin		15 (100%)	
Sensitive	18 (100%)	-	-
Intermediate	-	-	11 (100%)
Resistance	-		-

*NA- not applicable (*C. krusei* has innate intrinsic resistant to fluconazole)

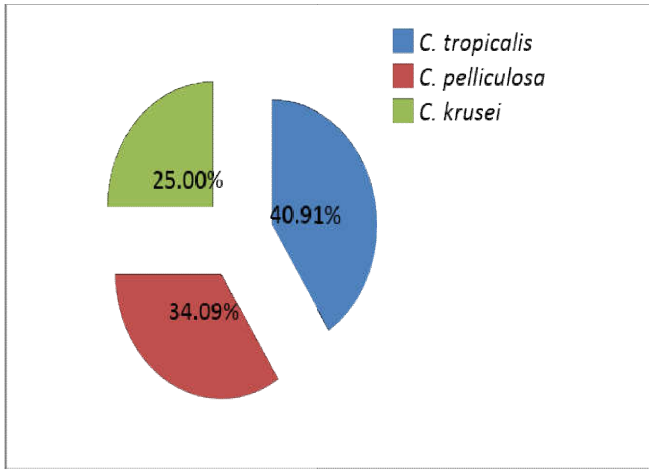

Fig 1 Distribution of non albicans Candida spp.

DISCUSSION

Candidemia is a significant cause of morbidity and mortality in neonates admitted in the NICU. Although historically *C. albicans* was the most frequently isolated *Candida spp*. from cases of neonatal septicemia, recently NAC notably *C. tropicalis, C. krusei, C. parapsilosis* and *C. glabrata, C. auris* have emerged as important opportunistic pathogens.[5] Because of lack of specific signs and symptoms and sensitive and specific laboratory tests for diagnosis of *Candida* infection, early diagnosis of candidemia remains a challenge for both pediatricians and microbiologists. The isolation rate of neonatal candidemia varies from place to place, candidemia was found to be responsible for 10.33% cases of neonatal septicemia in present study, which is consistent with the observations of Goel *et al*.[5] and Benjamin *et al*.[6]. However some authors have observed lower rates (1.5-4%) of neonatal candidemia in their study[7,8] On the other hand, there are also reports which showed frequency of isolation of neonatal candidemia 21-58%.[1,9]

In the present study NAC spp. accounted for all the 44(100%) cases of neonatal candidemia, whereas *C. albicans* was not found even in a single case. Increasing rates of NAC also have been reported by various workers from different regions of India.[9,10]. Rani *et al*. observed prevalence of NAC as 96% and *C. albicans* 4% in their study[11]Goel *et al*. also reported the

emergence of NAC as the major cause of neonatal candidemia at Rohtak, India.[5] The difference in the prevalence of various *Candida* spp. in candidemia could be because of variation in geographic regions. Some researchers have even suggested that the study should focus at the local level rather than at worldwide scale.[12] The increase in the incidence of NAC may be because of increased use of fluconazole prophylaxis in neonates, use of central venous cannulations and prior gastrointestinal surgery.

The most frequent *Candida* spp. isolated from the blood stream in the present study was *C. tropicalis*(40.91%) followed by *C. pelliculosa*(34.09%) and *C. krusei*(25.00%). Juyal *et al*. reported the following rate of NAC spp. from neonatal candidemia- *C. parapsilosis* (25%), *C.tropicalis*(21.97%), *C. albicans* (19.70%), *C. glabrata* (14.39%), *C. krusei* (10.61%).[1] In our study, all Candida isolates were found to be 75% susceptible to fluconazole (Table 1), which is similar to the findings of Nazir (2016).[13] A study done by the International Fungal Surveillance Participant Group reported higher (90%) rate of susceptibility to fluconazole.[14] However, Bansal *et al*.[15] and Shin *et al*.[16] reported only 20% of Candida isolates were susceptible to fluconazole in their studies. Amphotericin B has been reported as the drug of choice for the treatment of neonatal candidiasis. In our study amphotericin B susceptibility was 95.5% (Table 1), which is in accordance to the findings of other authors.[13,17]. Although resistance to amphotericin B in these strains in the present study was quite low (4.5%), but it is a matter of great concern as the emergence of such resistant strains poses serious therapeutic challenge to the neonates and also increases the risk of nosocomial NAC infections.[17] In the present study, 75% isolates were found to be sensitive to flucytosine, while 25% showed intermediate sensitivity (Table 1). However, other authors have reported lower rate of sensitivity to flucytosine in their studies.[13,18] Among echinocandins, 95% and 100% sensitivity was observed against caspofungin and micafungin respectively. In the present study, strains of *C. krusei* were found to be 18% resistant to caspofungin (Table 1), which is in accordance with the observation of Nazir who also reported 17% resistant strains of Candida to caspofungin in his study.[13]

CONCLUSION

Candidemia in neonates is an ominous prognostic sign and is an important entity in our hospital. Although previously *Candida albicans* accounted for majority cases of candidiasis, recently NAC spp. have been increasingly reported. Although amphotericin B is the drug of choice for treating neonatal candidiasis, it should be reserved for life threatening conditions. Azoles can be good alternative for treating these infections.

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