INCIDENCE OF NEONATAL FUNGAEMIA IN A TERTIARY CARE HOSPITAL IN NORTH INDIA

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ABSTRACT

In the neonatal intensive care units, infection with uncommon organisms is an increasing problem. Due to advances in medical and surgical management, an increase in nosocomial fungal infection rate has been observed, with Candida species being the most common nosocomial fungal pathogen.

MATERIALS AND METHODS

A total of 462 clinically suspected cases of septicaemia from neonatal ICU in Kamla Raja Paediatrics Hospital, Gwalior were studied for one year from November 2014 to October 2015. Blood samples were aseptically collected in duplicate into Brain Heart Infusion broth and incubated at 37°C up to 05 days and further another 05 days. Subcultures were made on Blood agar and MacConkey agar on regular interval. Both bacteria and yeast were isolated. The yeasts were studied further for identification and susceptibility as per the standard procedures.

RESULTS

Out of 103 culture positive cases, bacteria were isolated from 69 cases (66.7 %) and yeast from 34 cases (33.3 %). Among yeasts, C. tropicalis was isolated from 21 cases (61.76 %), C. albicans from 6 (17.6 %), C. glabrata from 3 (8.8 %), C. parapsilosis and C. kefyr were isolated from 2 cases (5.9 %) each.

CONCLUSION

Neonatal candidaemia is associated with significant morbidity and mortality. C. tropicalis has been reported as the predominant species involved in the cases of fungaemia.

KEYWORDS

Neonatal fungaemia, Nosocomial, Candida albicans, Non-albicans Candida (NAC).

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INTRODUCTION: With the advancement of diagnostic techniques and judicious use of medical and surgical treatment, invasive fungal infections are on rise.¹ With increasing survival of small for gestational age, more immunocompromised, preterm infants, the incidence of invasive fungal infection is increasing among NICU patients, with high associated morbidity and mortality. Systemic fungal infections, previously considered to be a rare clinical condition, occur now in as many as 5% of low birth-weight babies.²

The vast majority of fungal infections in preterm neonates are due to Candida species, with a small number being due to Malassezia and other rare fungi.³ Candida is the third most common cause of late onset sepsis in NICU patients and accounts for 9-13% of blood stream infections (BSI) in neonates.⁴ Although C. albicans has long been considered the predominant aetiologic agent, there has been an increase in the incidence of candidiasis caused by other Candida species, such as C. glabrata, C. krusei, C. tropicalis and C. parapsilosis.⁵

Malassezia furfur, M. pachydermatis, and Trichosporon species are not highly virulent but have been associated with nosocomial infections in preterm infants. M. furfur is a lipid-dependent fungus which can colonise the skin, gastrointestinal tract, and intralipid solutions of NICU patients and can be spread from patient to patient via the hands of health care workers. bloodstream infection with M. furfur is more common in infants with lower birth weight, younger gestational age and longer NICU stays. Aspergillus spp. and zygomycetes are extremely rare filamentous fungi but can cause severe infections in preterm infants.⁶

Various risk factors for the development of fungaemia in neonates include prematurity, use of broad spectrum antibiotics, prolonged duration of endotracheal intubation, receipt of parenteral hyper alimentation, presence of central venous catheters, surgical procedures, use of theophylline, administration of corticosteroids, presence of mucocutaneous candidiasis, perineal dermatitis or colonization with the organism.⁶,⁷,⁸,⁹

Candida yeast cells adhere preferentially to intermediate layers of the vaginal tract that are increased during pregnancy, increasing maternal fungal colonisation and exposure of vaginally delivered infants. Candida

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colonalisation may also be acquired horizontally, primarily from the hands of health care workers. Virulence factors like tissue adhesion, phenotypic switching, biofilm formation and production of extracellular hydrolytic enzymes play an important role in colonisation and invasion of host tissues.

Clinical presentation of fungaemia resembles sepsis syndrome and to establish a clinical diagnosis is difficult. Respiratory insufficiency, feeding intolerance, abdominal distension, temperature instability, lethargy, and decreased perfusion are the various clinical manifestations associated.

In laboratory findings other than blood culture, these neonates show decrease in leukocytes count, increase in level of micro ESR and CRP. Presence of band cells in blood in raised number also indicates toward neonatal septicaemia. The majority of preterm infants with fungal sepsis develop thrombocytopenia, but this is a common laboratory finding in patients with sepsis due to other organisms as well.

At the time fungal infection is clinically apparent, the organisms have often disseminated from the blood, urine, or CSF to adhere to and proliferate in body fluids, tissues, and organs. Candida species can cause endocarditis, endophthalmitis, dermatitis, peritonitis, osteomyelitis, and septic arthritis, and fungal abscesses may form in the CNS, kidneys, liver, spleen, skin, bowel, and peritoneum. Widespread infection despite negative cultures is common.

Prompt treatment with antifungals is required in these babies. The mainstay of therapy for neonates with disseminated fungal infection remains amphotericin B deoxycholate along with azole group of drugs. Regarding the antifungal susceptibility pattern, data is limited in this part of the country.

AIMS AND OBJECTIVES
1. To study the incidence of neonatal fungaemia in a tertiary care hospital of North India.
2. To characterise various fungus species isolated from the blood.
3. To find the antifungal susceptibility pattern for various fungus with special reference to Candida isolates.

MATERIAL AND METHODS
A prospective observational study was conducted in Department of Microbiology, G R Medical College, Gwalior between November 2014 to October 2015, to determine the incidence of fungal blood infections among neonates admitted in neonatal intensive care unit (NICU).

Inclusion Criteria:
- All neonates admitted in neonatal Intensive Care Unit with clinical presentation of septicaemia.
- Growth of fungal isolates on blood culture.

Exclusion Criteria:
- All culture negative neonates.
- Neonates with blood culture positive for bacterial isolates.

Any neonate with any other associated clinical condition which is not a part of our study.

Fully informed and voluntary consents were obtained from the parents or attendants and approval was obtained from institutional ethical committee. Detailed history and complete physical examinations of each neonate was carried out and recorded.

For this study, blood samples were collected from 462 clinically suspected cases of septicaemia from neonatal ICU in Kamla Raja Hospital, Gwalior. Approximately 1-3 mL of blood was collected from the neonates with proper antisepctic precautions and inoculated in Brain Heart infusion broth (HiMedia Pvt. Ltd, Mumbai, India) in duplicate bottles. Broth was incubated at 37°C for 10 days. Blind subculture was done on 1st, 3rd, 5th and 10th day from the broth on the solid media. Blood agar and MacConkey agar (HiMedia Pvt. Ltd, Mumbai, India) were used as primary plating media for subculture. If no growth was observed on plates after 10th day, the sample was reported as negative. On the basis of colony morphology and Gram staining, suspected yeasts were subcultured on Sabouraud dextrose agar (SDA) (HiMedia Pvt. Ltd, Mumbai, India). On SDA, Candida produces creamy, smooth, pasty and convex colonies which may become wrinkled on further incubation.

Identification of yeast was done with germ tube test, chlamydospore formation on corn meal agar and plating on HiCrome Candida Differential Agar medium. Confirmation was done by sugar assimilation test and sugar fermentation test.

Germ tube test: Yeast cells were inoculated into 0.5 mL of serum and incubated at 37°C for 2.5 h. After this period, aliquots were removed for microscopic examination. Germ tube was considered as a slender tube with straight walls, without septum and without constriction at the junction between the cells. Germ-tube was indicative of C. albicans, C. dubliniensis or C. stellatoidea.

Chlamydospore production test: Chlamydocanidia production test was performed using corn meal medium (HiMedia Pvt. Ltd, Mumbai, India). The samples previously grown in SDA were seeded as 3 parallel streaks on the agar plate and covered with a sterile cover slip. Plates were incubated under the condition of a higher humidity at 25°C for 72 hrs. and visualised in an optical microscope (10 X and 40 X magnifications). The formation of rounded spores with double-wall isolates was observed as chlamydospore, and was indicative of C. albicans, C. tropicalis or C. dubliniensis.

HiCrome Candida Differential Agar: HiCrome Candida Differential Agar medium. Confirmation was done by sugar assimilation test and sugar fermentation test.
inoculated and incubation was done at 37°C. The appearance of colonies, including colour, size, and textures on HiCrome Candida Differential Agar was analysed.\(^{(13,14)}\) The strains were identified according to the manufacturer’s instructions, which define C. albicans or C. dubliniensis as green colonies, C. tropicalis as steel blue colonies, C. krusei colonies as showing rose colour and rough aspect, and the other species as developing colonies from white to rose.

**Carbohydrate assimilation test:** Carbohydrate assimilation test was performed as follows. Yeast cells were suspended in Phosphate buffer saline. A yeast nitrogen base agar (HiMedia Pvt. Ltd, Mumbai, India) plate with no carbohydrate was taken and holes were cut in it using a hot sterile glass test tube of size 10 x 75 mm. A loopful of yeast suspension equivalent to McFarland Standard no. 0.5 was spread on agar plates. 1% (w/v) solutions of various carbohydrates were placed in the holes in plate. The results were read after incubating the plate at 25 °C for 2–3 days.\(^{(13)}\)

**Antifungal Susceptibility testing** was performed for Fluconazole (FLK, 25 mg), itraconazole (ITR, 10 mg), and amphotericin B (AMB, 100 units) (HiMedia Pvt. Ltd, Mumbai, India) using disc diffusion method (16.17) on Muller-Hinton agar supplemented with 2% glucose and methylene blue (5 mg/mL). Zone diameters were interpreted as per the approved National Committee for Clinical Laboratory Standards (NCCLS) guidelines. Quality control for AFS was performed using C. albicans-ATCC 90028 and C. parapsilosis-ATCC 22019.\(^{(13,15)}\)

**RESULTS:** Total 462 blood samples from septicaemic neonates were tested, among which 103(22.3%) samples were culture positive. Out of these, 103 culture positive samples, 69 (66.7%) were bacterial isolates and 34 (33.3%) were yeasts.

More fungal isolates were isolated from male neonates 64.7% (22/34) as compared to females 35.29% (12/34).

In 34 yeast isolates, 06 (17.6%) were Candida albicans, 26 (82.4%) were non-albicans Candida (NAC). Candida tropicalis was the most isolated fungal species, with 21/34 (61.76%) isolates, among NAC as well as in general. Other species isolated were C. glabrata 3/34 (8.8%), C. parapsilosis 2/34 (5.9%) and C. kefyr 2/34 (5.9%).

Among the risk factors observed for candidaemia, prematurity (85.29%) and LBW (73.53%) were the commonest followed by indwelling catheters (58.82%) and broad spectrum antibiotic use (52.94%).

Antifungal susceptibility testing was done for fluconazole, itraconazole and amphotericin B. Among these antifungals, amphotericin B was found to be most sensitive with 97% (30/34) susceptibility, itraconazole 67.6% (23/34) and fluconazole 70.6% (24/34) respectively. The results for individual species though calculated were not much significant in case of NAC as they are less in number.

### Table 1: Result of Blood Cultures

<table>
<thead>
<tr>
<th>Blood Samples</th>
<th>Culture Positive</th>
<th>Culture Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>462</td>
<td>103(22.3 %)</td>
<td>359(77.7 %)</td>
</tr>
</tbody>
</table>

### Table 2: Various Bacterial and Fungal Isolates

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Number (Percentage)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial Isolates</td>
<td>69(66.7 %)</td>
</tr>
<tr>
<td>Fungal Isolates</td>
<td>34(33.3 %)</td>
</tr>
<tr>
<td>Total Isolates</td>
<td>103</td>
</tr>
</tbody>
</table>

### Table 3: Sex wise Distribution Fungal Isolates

<table>
<thead>
<tr>
<th>Total Fungal Isolates</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>34</td>
<td>22(64.7 %)</td>
<td>12(35.29%)</td>
</tr>
</tbody>
</table>

### Table 4: Characterisation of Various Fungal Species Isolated from Neonates (Total 34 Isolates)

<table>
<thead>
<tr>
<th>Fungal Isolates</th>
<th>Percentage (%)</th>
<th>Germ Tube Test</th>
<th>Chlamydospore Formation</th>
<th>Carbohydrate Assimilation Test</th>
<th>Carbohydrate Fermentation Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n)</td>
<td></td>
<td></td>
<td>Glu</td>
<td>Suc</td>
</tr>
<tr>
<td>C. tropicalis</td>
<td>61.76%(21)</td>
<td>Negative</td>
<td>Absent</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>C. albicans</td>
<td>17.6%(6)</td>
<td>Positive</td>
<td>Present</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>C. glabrata</td>
<td>8.8%(3)</td>
<td>Negative</td>
<td>Absent</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>C. parapsilosis</td>
<td>5.9%(2)</td>
<td>Negative</td>
<td>Absent</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>C. kefyr</td>
<td>5.9%(2)</td>
<td>Negative</td>
<td>Absent</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

Note: Glu = Glucose, Suc = Sucrose, Mal = Maltose, Lac = Lactose, A = Acid production, G = gas production.
### Colour of Various Fungal spp. on HiCrome Candida Differential agar for Identification

<table>
<thead>
<tr>
<th>Name</th>
<th>Colour on HiCrome Candida Differential Agar</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. albicans</td>
<td>Light green</td>
</tr>
<tr>
<td>C. tropicalis</td>
<td>Metallic blue</td>
</tr>
<tr>
<td>C. glabrata</td>
<td>White</td>
</tr>
<tr>
<td>C. parapsilosis</td>
<td>White</td>
</tr>
<tr>
<td>C. kefyr</td>
<td>Pink</td>
</tr>
</tbody>
</table>

**Table 5:** Colour of Various Fungal spp. on HiCrome Candida Differential agar for Identification

### Antifungal Susceptibility Profile of Fungal Isolates

<table>
<thead>
<tr>
<th>Organism</th>
<th>Antifungals tested</th>
<th>Total No. of Isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FLK n (%)</td>
<td>ITR n (%)</td>
</tr>
<tr>
<td>C. tropicalis</td>
<td>17(80.9)</td>
<td>18(95.7)</td>
</tr>
<tr>
<td>C. albicans</td>
<td>5(83.3)</td>
<td>5(83.3)</td>
</tr>
<tr>
<td>C. glabrata</td>
<td>1(33.3)</td>
<td>1(33.3)</td>
</tr>
<tr>
<td>C. parapsilosis</td>
<td>0(0)</td>
<td>0(0)</td>
</tr>
<tr>
<td>C. kefyr</td>
<td>0(0)</td>
<td>0(0)</td>
</tr>
<tr>
<td>Total</td>
<td>23(67.6)</td>
<td>24(70.6)</td>
</tr>
</tbody>
</table>

**Table 6:** Antifungal Susceptibility Profile of Fungal Isolates

### Potential Risk Factors for Fungaemia among Neonates

<table>
<thead>
<tr>
<th>Risk Factors</th>
<th>Number of Cases/Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prematurity</td>
<td>29(85.29)</td>
</tr>
<tr>
<td>Low Birth Weight</td>
<td>25(73.53)</td>
</tr>
<tr>
<td>Indwelling Catheters</td>
<td>20(58.82)</td>
</tr>
<tr>
<td>Broad Spectrum Antibiotic Use</td>
<td>18(52.94)</td>
</tr>
<tr>
<td>Total Parenteral Nutrition</td>
<td>5(14.70)</td>
</tr>
<tr>
<td>Ventilator Support</td>
<td>3(8.82)</td>
</tr>
<tr>
<td>Prolonged hyperalimentation</td>
<td>2(5.88)</td>
</tr>
</tbody>
</table>

**Table 7:** Potential Risk Factors for Fungaemia among Neonates

### Discussion:

In the present study, isolation rate for Candida spp. was 34% from blood samples, which is comparable to some studies and significantly higher than other studies. In Juyal et al. study, 34.65% Candida spp. were isolated from blood samples. In another study by Sardana et al., 30.1% Candida spp. were isolated. About 7.48% Candida spp. were isolated during study of Shrivastav et al. (17)

Of the total 34 neonates included in study, 22 (64.7%) were male and 12 (35.29%) were female. This was in contrast to the study of Juyal et al. in which out of 132 neonates included in the study, 73 (55.30%) were females and 59 (44.70%) were males. In this study, non-albicans Candida (NAC) account for 82.4% of total fungal cases in contrast to 17.6% of Candida albicans. Though during the last few decades, Candida albicans was the predominant species causing blood stream infections, our results are comparable with the present day trends where increasing rates of NAC have been reported by various workers from different regions of India. Our results correlate with the study of Juyal et al. 2013, in which NAC species were responsible for 80.30% of the cases of neonatal candidaemia, whereas C. albicans was for 19.70% of cases. In another study, conducted by Sardana et al. 2012 in Meerut the percentage of NAC associated neonatal candidaemia was found to be 86.4 %. Similar results were seen by Agarwal et al., where NAC was isolated in 84.4% cases from Lucknow. (18) In our study, C. tropicalis was isolated in highest amount (61.76%). In a similar study conducted by Shrivastav et al. (2015) in Indore, Candida tropicalis was found to be the commonest agent of Candida bloodstream infection. Candida tropicalis is the second or third leading...
cause of candidaemia. Candida tropicalis as a cause of fungaemia in neonatal intensive care units have been linked to the presence of the fungus on the hands of the hospital personnel. Once introduced into a host with an impaired immune system, Candida tropicalis may be more virulent than Candida albicans and proceed from colonisation to invasion more easily. C. tropicalis has been considered to exhibit increased virulence, especially in those individuals with disrupted mucosal integrity.(19) In another study by VP Baradkar in 2008, C. glabrata was isolated in highest number (61.22 %).(20)

Increased prevalence of Candida krusei and emergence of Candida glabrata can be attributed to their innate resistance or indirectly due to the selective pressure resulting from its resistance to azole drugs. Candida glabrata that is found to be the predominant species in many countries, including United States but less common in Asia-Pacific regions and Latin America, has started emerging in the Indian subcontinents.(21,22)

Combinations of various risk factors are known to be strongly associated with development of fungaemia, and our results also suggest the same. The major risk factors identified in our study were prematurity, LBW, indwelling catheters, and broad spectrum antibiotic therapy.

In this study, amphotericin B was found to be most susceptible against Candida spp. with 97% sensitivity. Susceptibility for other two drugs fluconazole and itraconazole were 67.6% and 70.6 %, respectively. In the study of Juyal et al. (2013), antifungal susceptibility results were, 65.91% isolates sensitive to fluconazole, 73.49% to itraconazole, and 96.21% to amphotericin B.(4) In the study of Agrawal et al. (18) NAC species, especially C. tropicalis, C. krusei, C. glabrata and C. parapsilosis, tend to be less-susceptible to azoles, particularly fluconazole, than C. albicans. C. krusei was innately resistant to fluconazole.

CONCLUSION: Candida remains the most common causes of invasive fungal infections, but non-albicans Candida species are emerging more frequently. The present study emphasises the clinical importance and mycological shift of Candida species in neonatal candidaemia with predominance of NAC species in the Central India region. In our study, Candida tropicalis (61.76 %) was found to be most isolated fungal species from cases of neonatal sepsicaemia, exceeding the Candida albicans infection rate. This signifies the changing trend seen in the epidemiology of Candida infections.

Blood culture, although not a sensitive test, remains the only reliable method for diagnosis. It is important to diagnose the fungal infections meticulously as they create the hidden burden and increases the rate of morbidity and mortality.

Preventive measures such as use of filters for parenteral nutrition, prophylactic antifungal use, and a restrictive policy of antibiotic use to decrease Candida colonisation/infection rates should be implemented to reduce the morbidity and mortality associated with these infections.

REFERENCES


