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Research Article

Identification of *Candida* species in neonatal septicaemia

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ABSTRACT

Background: Candidemia refers to presence of candida species in blood. It is significant cause of mortality and morbidity in neonatal intensive care unit. Candidiasis is a primary or secondary fungal infection involving a member of genus candida. The aim and objectives of the study was to identify and separate candida isolates obtained from cases of neonatal candidemia and clinical manifestations associated with it and to identify the species other than *candida albicans* from cases of neonatal candidemia and clinical manifestations associated with them.
Methods: The present study was conducted in the Department of pediatrics, Subharti Medical College from August 2014 to August 2015. Neonates with signs of septicemia were screened and blood culture was done for candida. Candida species were isolated from blood and urine. Blood was inoculated and candida species were identified by Bact/alert method of sampling. Urine was examined by direct microscopy in 10% KOH mount stain.
Results: Out of 527 clinically suspected cases of septicemia screened, 69.3% cases were blood culture positive. Non-Albicans Candida (NAC) were isolated from blood in 86.4% cases of neonatal candidemia compared to candida albicans in 13.6%
Conclusions: The Present study has given us a clear insight into the changing trend of candida species with emergence of non-albicans candida (NAC) species as an important cause of neonatal candidemia from Meerut city: definitely a changing trend.

Keywords: Brain heart infusion, Potassium hydroxide, Low birth weight, Neonatal intensive care units

INTRODUCTION

Candidemia refers to presence of candida species in neonatal septicemia. It is a significant cause of mortality and morbidity in neonatal intensive care unit. Candidiasis may be a primary or secondary fungal infection by a member of genus candida. Candiduria refers to presence of candida in urine. Candidiasis may be localised to oral cavity, throat, skin, scalp, nails, fingers, vagina, bronchi, lungs or the GI track, or become systemic as in septicaemia in endocarditis and meningitis.[1-3] Invasive candidiasis is a common complication of neonates of low birth weight requiring long intensive care.[3] Candida species are third most common organism (coagulase negative staphylococcus and staphylococcus aureus) insolated in late onset sepsis and low birth weight infants.[5] Preterm infants are predisposed to candida infections because of immaturity of their immune system and invasive interventions.[4-6] Transmission of candida may be vertical or nosocomal.[4,5] The risk Factors for candidemia include low birth weight, use of broad spectrum and/ or multiple antibiotics, central venous catheters, prolonged urinary catherization, parenteral alimentation and intervenous fat emulsion, colinization with Candida and/or previous episode of mucocutaneous candidiasis and immunosuppressive therapy.[3-5,7-11] Systemic Candidiasis in neonates is increasing in frequency especially since the survival of babies with low birth weight has increased.[8] This prompted us to carry out a study to isolate and identify the various species of candida causing neonatal candidemia in NICU of Subharti Medical College, assess the risk factors and clinical manifestations associated with neonatal candidemia and also to evaluate if candiduria, can be consider as a reliable indicator of candidemia.

The aim and objectives of the study was to identify and separate candida isolates obtained from cases of neonatal candidemia and clinical manifestations associated with it and to identify the species other than *candida albicans* from cases of neonatal candidemia and clinical manifestations associated with them.

METHODS

Inclusion criteria

Neonates with clinical presentations suggestive of septicemia such as feed intolerance, abdominal distension, failure to thrive, respiratory distress, bleeding tendency, lethargy, convulsions; with or without associated risk factors.

Study period

The present study was conducted in the Department of paediatrics, Subharti Medical College from August 2014 to 2015.

Study population

527 clinical suspected cases of neonatal septicaemia admitted in NICU over a period of one year were studied. Informed consent was taken from the parents of the neonates before collection of clinical samples.

Specimen collection and transport

Blood culture

One or two ml venous blood collected with aseptic precautions and transferred to either conventional biphasic culture bottle containing Brain heart infusion agar slant overlaid with BHI broth or to an automated Bact/alert 3D.

Urine culture

Urine sample was collected by freshly inserted "in and out" catheter with aseptic precautions and was transferred to a sterile universal container.

Candida albicans ATCC 2091 was used as a control for all the methods of identification employed in the study.

Statistical analysis

The statistical analysis for the present study was carried out using SPSS15.0, SOFA, MedCalc 9.0.1 and Systat 12.0 softwares.

The statistical formulae applied in our study were used as follows:

1. Two proportion Z-test.
2. Pearson product moment correlation coefficient.

Significance levels

The 5% level of significance has been adopted for the present study:

P-value >0.05 - statistically not significant

0.01 < P-value <0.05 - statistically moderately significant

P-value <0.01 - statistically highly significant

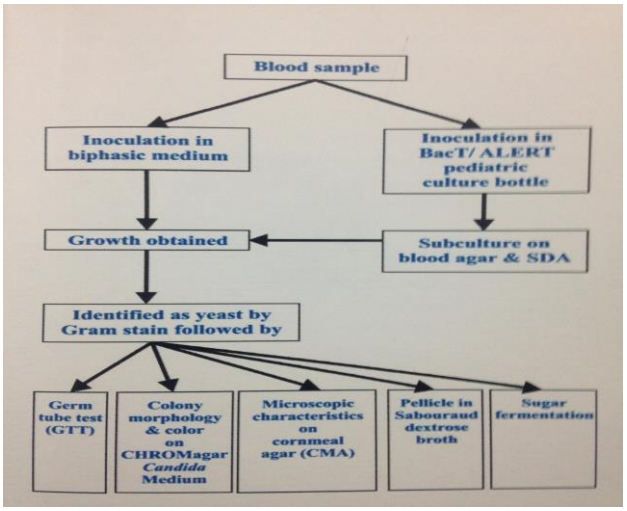

Figure 1: Various methods in the isolation and identification of candida species from blood sample.

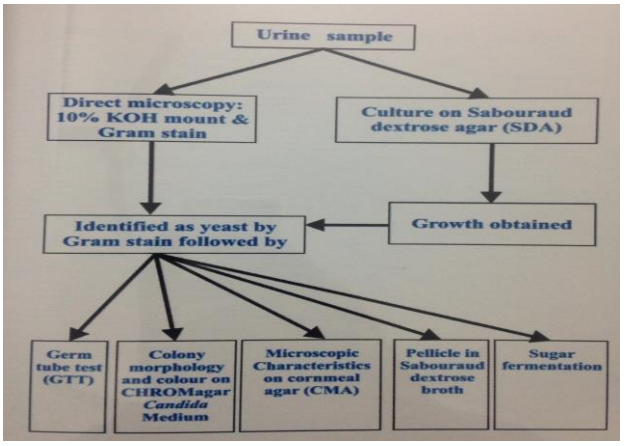

Figure 2: Various methods in the isolation and identification of candida species from urine sample.

Identification

Identification of yeast isolate on sabouraud dextrose agar (SDA) was done by following methods:

1. Colony morphology on SDA
2. Germ tube test (STT)
3. Colony morphology and colour on Chromagar candida Medium
4. Microscopic characteristics on cornmeal agar (CMA)
5. Pellicle on Sabrouraud dextrose broth
6. Sugar fermentation

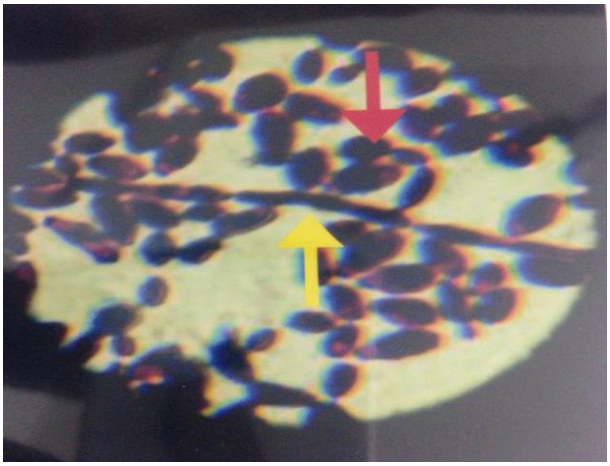

Figure 3: Gram stained smear showing gram-positive oval budding yeast cell (red arrow) with pseudo hyphae (yellow arrow) (X 1000).

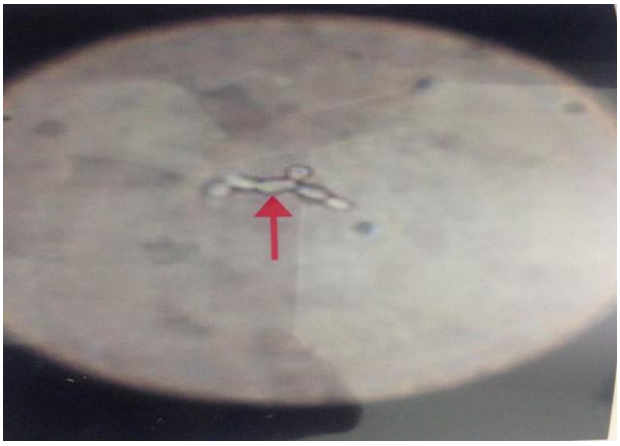

Figure 4: 10% KOH mount of urine showing budding yeast cells (arrow) (X 400).

Figure 5: Various isolates of candida on SDA (from left to right): C. albicans, C. glabrata, C. tropicalis, C. parapsilosis, C. guilliermondii, C. krusei, C. lusitaniae, C. dubliniensis.

Figure 6: Isolation of candida in oral cavity.

RESULTS

Candida isolates from cases of neonatal septicaemia

- Out of 527 clinically suspected cases of septicemia screened, 69.3% cases were blood culture positive. Non-Albicans Candida (NAC) was isolated from blood in 86.4% cases of neonatal candidemia compared to candida albicans in 13.6%.

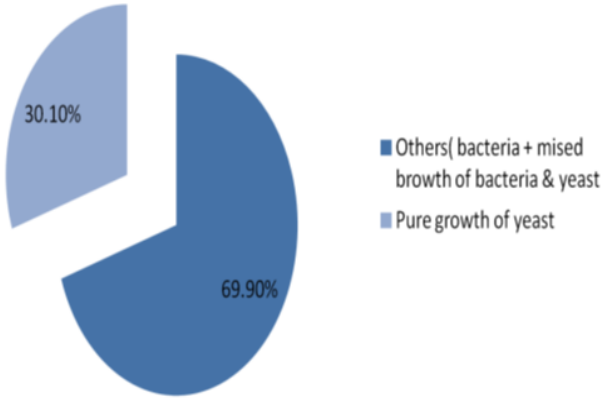

Figure 1: Distribution of micro-organisms isolated from culture proven cases of neonatal septicemia.

The most common clinical presentation in cases of neonatal candidemia was respiratory distress (74.55%) , followed by failure to thrive (70%) , lethargy (64.55%) , feed intolerance (60.09%) , bleeding tendency (57.27%) and convulsions (43.64%).

- Low birth weight (79.09%) was the commonest risk factor seen in our babies, followed by prematurity (67.27%) and prolonged intravenous antibiotic therapy (49.09%).
- Non – albicans candida (NAC) species were isolated from blood in 86.4% cases of neonatal candidemia compared to candida albicans in 13.6%.
- Among the various isolates, *Candida glabrata* (39%) was predominant species causing neonatal

candidemia followed by *Candida tropicalis* (26%), *Candida parapsilosis* (14.5%), *Candida albicans* (13.6%), *Candida guilliermondii* (2.7%), *Candida* krusei (1.8%), *Candida dubliniensis* (0.9%) and *Candida lusitaniae* (0.9%).

- Candiduria was found in 54.5% cases of neonatal candidemia.
- Among the cases of candiduria, non-albicans candida (NAC) species were isolated from urine in 81.7% compared to c. albicans in 18.3%
- However there was no statistically significant difference in the isolation rates of *Candida tropicalis* and *Candida albicans* from urine and blood at 5% level of significance.
- Positive correlation between candiduria and candidemia in our neonates was statistically significant.

DISCUSSION

Non-albicans Candida (NAC) species are assuming an increasing role in nosocomial infections in neonates. The incidence of candidemia in neonates has increased substantially, due to multiple risk factors.[3-5,7-11] The clinical manifestations are respiratory insufficiency, apnoea, bradycardia, temperature instability, feeding intolerance and abdominal distension.[12]

Agarwal J et al suggested that up to three-fourth of Candida species isolated from a single blood culture specimen from neonates with clinically suspected septicaemia, may be significant.[7] This was an important finding as candida isolated from single blood culture is sometimes ignored as a mere skin contaminant. In our study, among the various clinical manifestations observed in the cases of neonatal candidemia, respiratory distress (74.55%, $p<0.001$) was the commonest, followed by failure to thrive (70%, $p<0.01$), lethargy (64.55%, $P<0.01$), lethargy (64.55%, $P<0.01$), feed intolerance (60.91%, $P<0.05$). Studies by Narang et al, Baley et al and Roy A et al have found that symptoms related to respiratory system like persistent/recurrent pneumonia, apnoea and asphyxia neonatorum were the frequent clinical presentations of septicaemia.[13-15] In the present study, low birth weight (79.09%, $P<0.01$) was the commonest risk factor associated with candidemia in our babies, followed by prematurity (67.27%, $P<0.01$).

The Present study has given us a clear insight into the changing trend of candida species with emergence of non-albicans candida (NAC) species as an important cause of neonatal candidemia from Meerut city: definitely a changing trend. Further our study also highlights that candiduria may be considered as a reliable indicator of candidemia in neonates, however to pinpoint if candiduria is a reliable marker of candidemia, more number of cases need to be studied by extending the duration of the study.

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Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

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