Study of bacteriological spectrum of neonatal septicemia and associated risk factors of septicemia

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Abstract:
The aim of this study was to identify the organisms causing neonatal septicemia and their antimicrobial susceptibility at Neonatal Intensive Care Unit in a tertiary care hospital; to evaluate the maternal and neonatal risk factors and to establish a correlation between the hematological markers and septicemia. This cross sectional study period extends from 1st May to 30th June 2011. 40 neonates suspected to have septicemia or had maternal history of risk factors were enrolled after ethical clearances. A septic work up was carried out in all these infants that included Complete blood counts, C-reactive protein, and blood cultures with antimicrobial sensitivity pattern. The analysis of the peripheral smear was done. Statistical analysis was done by 'Z' test. Out of total 40 suspected cases; male: female ratio was found to be 1.6:1.The blood culture was positive in 26(65%) cases, whereas negative in 14(35%). Early onset septicemia was in 27(67.5%) and late onset septicemia was in 13(32.5%). Out of these, 26 positive blood cultures, non-fermenting gram-negative bacilli (65.3%) were found to be the commonest; followed by Pseudomonas species (11.5%); Serratia species (7.69%); Enterococci (3.84%).The organisms showed highest sensitivity to Imipenem and Cefotaxime. The significant risk factors were perinatal maternal fever, prolonged rupture of membranes (>12 hours), Neonatal Resuscitation, Low Birth Weight. Hematological markers TLC<5000 cu.mm and increased CRP were highly indicative of septicemia. As of now, non-fermenters have not been reported to be isolated from the maternal genital tract. This strongly suggests that early onset hospital – acquired sepsis due NFGNB should be an area of concern and future research. But for this, a study on larger sample size is required.

Keywords: Neonatal septicemia, Antimicrobial susceptibility pattern, Neonate.

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Introduction:

Neonatal Septicemia is a significant cause of neonatal morbidity and mortality. Neonatal sepsis or sepsis neonatorum refers to systemic infection of the newborn. It is characterized by a constellation of a nonspecific symptomatology in association with bacteremia. The term "neonatal sepsis" used broadly in the clinical context encompasses diagnoses of septicemia, meningitis, pneumonia, arthritis, osteomyelitis and urinary tract infection in the newborn. In developing countries, sepsis is the commonest cause of mortality responsible for 30-50 per cent of 5 million total neonatal deaths each year (1). Wide spectrum of organisms has been described as the cause of neonatal septicemia. Moreover, the organisms isolated are often resistant to multiple antimicrobials which make treatment difficult (2). Prompt recognition; appropriate antimicrobial therapy and judicious supportive care are the key in determining the positive outcome in this serious pediatric emergency. The detection of microorganisms in patient's blood has a great diagnostic and prognostic significance. An awareness of the many risk factors associated with neonatal sepsis prepares the clinician for early detection and effective treatment, thereby reducing mortality and morbidity.

Materials and methods:

This study was conducted at NICU and Department of Microbiology at a rural tertiary care hospital from 1st May to 30th June 2011 after obtaining essential institutional ethical clearance. Clinical data was collected and recorded on a pretested proforma. This included the age, weight, mode and place of

delivery and risk factors of infection in mother like premature rupture of membrane (PROM), perinatal fever, recent or chronic maternal illnesses and neonatal risk factors like neonatal resuscitation, respiratory distress syndrome, Birth Weight, etc.

Sample collection: Total 40 samples of either sex clinically suspected of septicemia were included in the study with maternal history suggestive of possible risk factors leading to neonatal septicemia in two months period starting from 1st May to 30th June 2011. 2 ml of blood was collected from neonates for culture (by venupuncture) and hematological investigations under all aseptic precautions. Repeat samples were also collected from the patients when indicated.

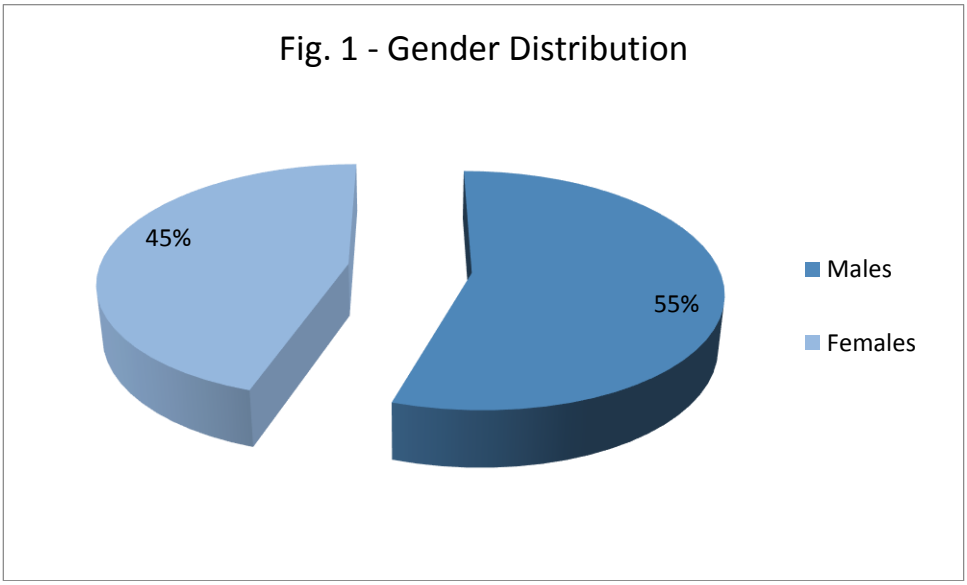
Bacterial culture: One ml of blood was inoculated in a bottle containing 10 ml 1% Glucose Broth. Blind subcultures were done on Blood agar and MacConkey's agar after 24 hours, 48 hours, 72 hours, 5th day and final on 7th day as per standard protocol (3-6). A provisional report was issued after every subculture and if after 7^{th} day, no growth was obtained; the sample was reported as negative. Antimicrobial susceptibility testing was performed by Kirby-Bauer disc diffusion method (7) performed in accordance to Clinical and Laboratory Standards Institute guideline (CLSI) guidelines(8) for Gram positive and Gram negative isolates.

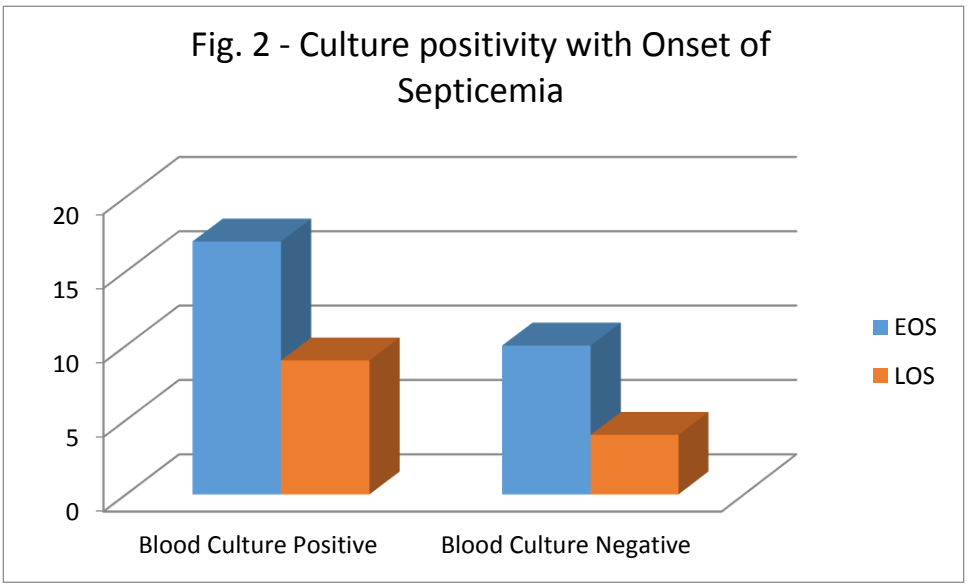
Biochemical investigations: The various organisms were identified on the basis of colony morphology, gram staining and standard biochemical tests like catalase, coagulase, oxidase, urease, sugar fermentation test, citrate utilization test, methyl red reaction, Voges-Proskauer test (6). Hematological investigations for septicemia- Hemoglobin, Platelets count, Differential Leucocyte Count (DLC), Total Leucocyte Count (TLC), and CRP were carried out. The CRP estimation was done using CRP Latex Kit (Beacon Diagnostics Pvt. Ltd). Values >0.6mg/dL were considered to be positive and indicator of septicemia (9).

Statistical analysis: Done using 'Z score' (EPI info software version 3.4.3). A difference with P value <0.05 was considered statistically significant.

Results:

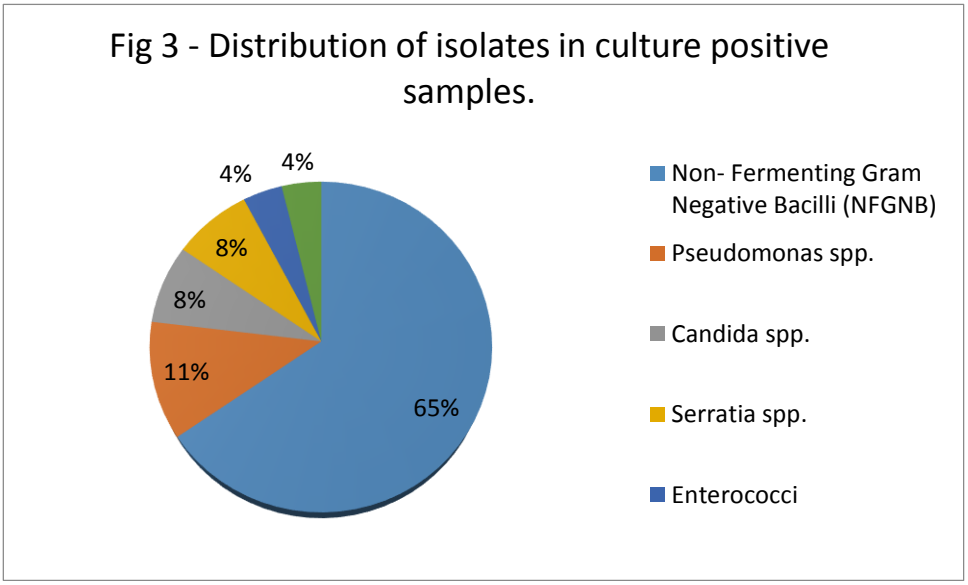
Out of 40 blood samples sent for culture, bacterial growth was obtained in 26 with the positivity rate of 65%. Male to female ratio was 1.6:1 (Fig. 1). Out of 40 cases, Early Onset Septicemia (EOS) were 27(67.5%) and Late Onset Septicemia (LOS) were 13(32.5%) (Fig. 2). Out of 27 EOS, blood culture was positive in 17(62.9%) whereas out of 13 LOS, blood culture was positive in 09(69.2%). Both in EOS and LOS, Non–Fermenting Gram Negative Bacilli (NF-GNB) was the most common isolate.

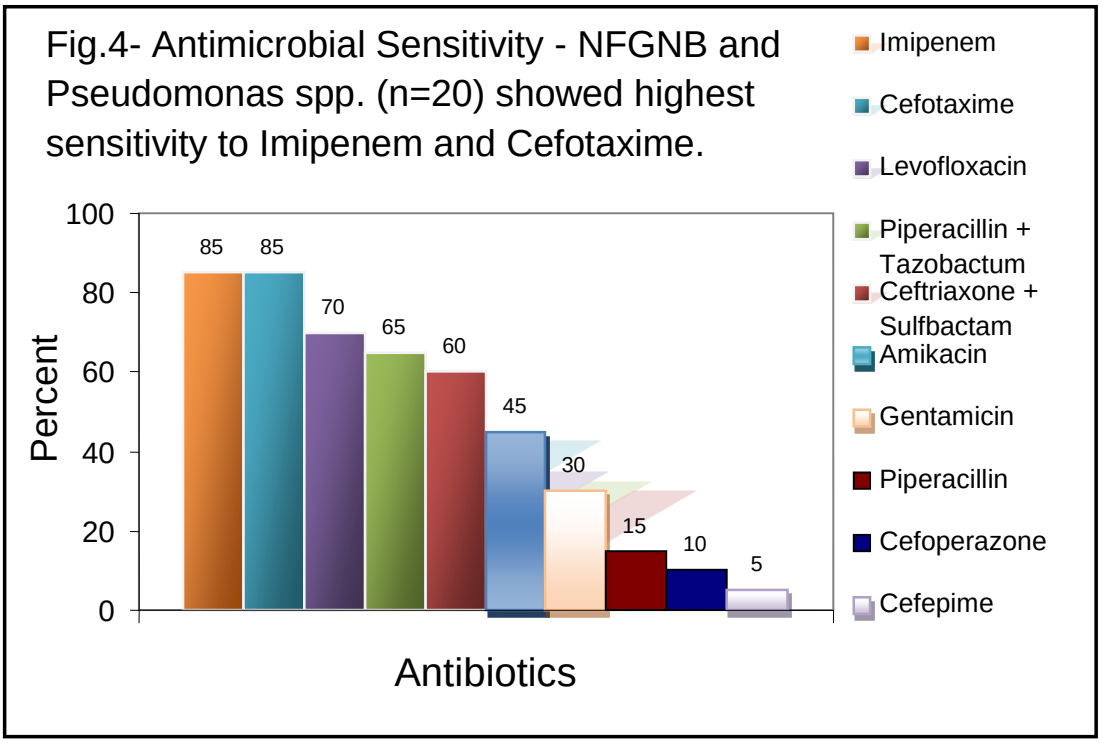




Pseudomonas species was the second most commonly isolated organisms in EOS. As Candida grows readily on bacterial culture media, isolation of 2 Candida species was a chance finding.

Out of the 26 culture positive samples, Gram negative bacteria formed the major part of isolates. NF-GNB were isolated in 65.3% which formed the most prevalent group. The other organisms were Pseudomonas species 03(11.5%), Candida species 02(7.69%), Serratia species 02(7.69%), Enterococci 01(3.84%), Acinetobacter 01(3.84%) (Fig. 3).





NF-GNB and Pseudomonas species organisms were most sensitive to Imipenem (85%) and Cefotaxime (85%). The other antibiotics to which these organisms were found sensitive are Levofloxacin, Piperacillin + Tazobactam and Ceftriaxone + Sulfbactam (Fig. 1). The correlation between maternal and neonatal risk factors with haematological investigation associated in neonatal septicemia is shown in Table 1. Maternal risk factors (Maternal fever and Prolonged rupture of membranes > 12 hours) for neonatal septicemia were found to be highly significant as $p<0.05$. Neonatal risk factors like Neonatal Resuscitation, Low Birth Weight, TLC <5000 and positive CRP showed $p<0.05$ which suggests that these factors have significant association with Neonatal Septicemia.

Table 1: Maternal & Neonatal risk factors

Sr.No	Factors	Total	Blood Culture		P value
			Positive	Percentage	
1.	Maternal Fever	12	08	66.6%	0.005
2.	PROM(>12 hours)	11	07	63.6%	0.002
3.	Caesarean Section	20	13	65%	0.50
4.	Institutional Delivery	25	17	68%	0.38

Neonatal Risk Factors					
Sr. No	Factors	Total	Blood Culture		P value
			Positive	Percentage	
1.	RDS	21	15	71.4%	0.7518
2.	IUGR	17	11	64.7%	0.1714
3.	Neonatal Jaundice	30	22	73.3%	0.1288
4.	LBW	29	19	65.5%	0.057
5.	Neonatal Resuscitation	09	05	55.5%	0.0005
Hematological Investigations					
1.	TLC<5000/cu.mm	05	05	100%	0.000001
2.	CRP	33	24	72.7%	0.0000019
3.	Platelet Count (<1.5 lac/cu.mm)	14	12	85.7%	0.1166

Discussion:

Neonatal sepsis is a life-threatening emergency and any delay in the treatment may become fatal. Appropriate treatment with antibiotics requires knowledge of common bacterial pathogens involved and their antibiotic sensitivity pattern, therefore, area based knowledge about bacteriological spectrum of antibiotics is essential as the clinicians would not wait for the culture result, so right choice for such empiric therapy is of utmost importance. New treatment regimens are discovered throughout the world due to changing bacterial profile and high mortality rate. Gram-negative organisms are on top to cause the sickness to fragile and debilitated newborns. Gram negative septicemia is a challenge to the neonatologists, microbiologist, and hospital administrators (10).

In the present study, blood culture positivity was seen in 65%. Some studies have also indicated low culture positivity. The probable reason quoted by them for lower prevalence of documented neonatal septicemia with positive blood culture was antibiotic administration in mother or neonate, difficulty in sampling, blood culture technique, and sepsis due to anaerobic, viral or fungal pathogens (11). In the present study, NF-GNB has been isolated as a leading cause of septicemia followed by Pseudomonas species 11.5%(n=03),Candida species 7.69%(n=02), Serratia species 7.69%(n=02), Enterococci 3.84%(n=01), Acinetobacter 3.84%(n=01). In our study, there were 17 isolates of NF-GNB; sensitive to Imipenem (88.2%) which is similar to other authors (12). Cefotaxime (82.3%) followed by Levofloxacin, Piperacillin+Tazobactam and Ceftriaxone+Sulfbactam (64.7%each) were also sensitive. NF-GNB was resistant to Cefepime (100%) followed by Ampicillin (94.1%), Cefoperazone (88.3 %). Resistance to Amikacin was less frequent than resistance to Gentamicin (2). Total 03(11.5%) cases of Pseudomonas species were isolated in the present study. These were sensitive to Levofloxacin and Cefotaxime (100% each) followed by Imipenem, Amikacin, Gentamicin, Piperacillin+Tazobactam (66.6 % each). 02(7.69%) of Serratia species were isolated in our study. It showed sensitivity to Levofloxacin, Ceftriaxone+Sulbactum, Imipenem, Piperacillin and was resistant to Ampicillin, Amikacin and Gentamicin. Enterococci isolates were sensitive to Vancomycin and resistant to Gentamicin, Ceftazidime and Levofloxacin.

Early onset bacterial infections occurs either due to ascending infection following rupture of membranes or during the passage of baby through infected birth canal or at the time of resuscitation in the labor room. Maternal pyrexia was noted in 12 out of 40 cases (30%). Out of these 12, 8(30.7%) had positive blood culture growth. Maternal fever is a risk factor for development of neonatal septicemia (13). Some authors (12) in their study stated a good correlation between neonatal infections and mothers on intrapartum antibiotics for pyrexia and prolonged rupture of membranes (PROM) (13.33% and 10% respectively).

In our study, 27.5% of total number of cases had history of prolonged rupture of membranes of more than 12 hours. An association of PROM and neonatal septicemia is also reported by many authors (12-14). Hence our findings are consistent with the findings of other authors.

We found that 11 out of 27 cases of EOS had PROM of >12 hours. 9 out of 11 (81.8%) cases of EOS with PROM >12 hours showed positive blood culture which signifies the role of PROM in the development of neonatal septicemia.

In the absence of maternal risk factors, the infant is at high risk for the development of sepsis due to various neonatal parameters like prematurity, low birth weight, neonatal asphyxia (14). In our study, we observed highly significant association of blood culture positivity in 55.5% (5/9) with Neonatal Resuscitation, 71.4% (15/21) with Respiratory Distress Syndrome(RDS), 64.7 %(11/17) with Intra Uterine Growth Restriction(IUGR) and 65.5 %(19/29) with Low Birth Weight (LBW). LBW is a well-accepted risk factor for neonatal septicemia (2, 11-12, 15). Jain NK et al (2003) reported that RDS was significantly associated with EOS. Authors (12) also accepted RDS to be a significant factor for neonatal septicemia. No difference in EOS or LOS was seen in term neonates. However, in preterm (<37 weeks of gestation) neonates, EOS (n=8) was predominant than LOS (n=2).

CRP has revealed a high sensitivity of 82.5 %(33/40) and specificity of 72.7 %(24/33 cases) in our study. Some investigators have also reported a high CRP sensitivity of 82.4% in their study on neonatal septicemia (13). Some studies have stated that CRP did not have a good correlation with neonatal sepsis (16). The sensitivity was only 23 % with specificity of 84 %. The authors have quoted that there were conflicting results on the CRP levels in the literature.

In our study, we found that Leucopenia and thrombocytopenia was reliable indicator. In our study, all the cases of suspected septicemia which showed blood culture positive, also showed the TLC count <5000 /cu.mm. In our study, out of 26 proven cases with septicemia, 11 neonates had thrombocytopenia

(platelet count <150,000/ cu.mm).The sensitivity in neonates with thrombocytopenia was 42.3% (11/ 26) but specificity was as high as 81.8 %(9/11) with positive blood culture. Similar findings were reported with sensitivity of 61% and specificity of 82%[16].They also found a good co-relation between low platelet count and neonatal systemic infections.

Conclusion:

As of now, non-fermenters have not been reported to be isolated from the maternal genital tract. This strongly suggests that early onset hospital – acquired sepsis due NF-GNB should be an area of concern and future research. With early diagnosis, prompt and effective therapy and with the help of good intensive care management, mortality due to neonatal infections can be reduced. Local microbiological databases suggesting the best choice of antibiotics are important for the local physicians when treatment of septic infant has to be initiated before the result of blood culture is known. But for this, a study on larger sample size is required.

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