Bacteriological profile and antibiotic susceptibility pattern of neonatal septicemia in a tertiary care hospital of Tripura

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ABSTRACT

Background: *Sepsis is the commonest cause of neonatal mortality and is influenced by the quality of intrauterine life, host and environmental factors. The present study was undertaken to identify the aetiological agents causing neonatal septicemia and analyze the antibiotic susceptibility pattern, so as to guide the Pediatrician in formulating an empirical antibiotic policy against the predominant pathogens in this geographical area of North East India.*

Methods: *On clinical suspicion of septicemia, atleast two sets of test for blood culture were performed and such neonates included in the study. Antimicrobial Susceptibility Testing was subsequently performed following Kirby Bauer Disc Diffusion method. The data obtained from culture proven septicemia was analyzed.*

Results: *Blood culture was positive in 41.1% clinically suspected cases of septicemia. Early Onset Septicemia was confirmed in 73(57.5%) and Late Onset Septicemia in 54(42.5%) neonates. The Gram Positive organisms (51.1%) predominated over Gram negative organisms (48.9%). Staphylococcus aureus (34.6%) was the most predominant isolate followed by Klebsiellapneumoniae (24.4%). The antibiotic sensitivity pattern of the gram positive blood culture isolates indicates that all isolates were sensitive to Vancomycin. The Cephalosporins, Clindamycin and Amikacin were found significantly sensitive to Staphylococcus aureus(95.5%). All Gram negative isolates were sensitive to Imipenem followed by Piperacillin-tazobactum and Amikacin.*

Conclusion: *Amikacin along with a third generation Cephalosporin like Cefotaxime were chosen to be the first line regimen for treatment of cases of septicemia in our setting. The predominant isolate of Staphylococcus aureus along with significant association of maternal risk factors indicates the possibility of cross infection in NICU from index case, for which emphasis need to be laid on Hospital infection control strategies.*

Keywords: *Neonatal septicemia, Aetiology, Risk factors, Antibiotic sensitivity, Emperical treatment.*

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INTRODUCTION

Sepsis is the commonest cause of neonatal mortality and is responsible for about 30-50% of total neonatal deaths in developing countries.[1,2] The incidence of neonatal sepsis according to National Neonatal Perinatal Database (NNPD, 2002-03) is 30 per 1000 live births. The NNPD network comprising of 18 tertiary care neonatal units across India found sepsis to be one of the common causes of neonatal mortality contributing to 19% of all neonatal deaths.[3]

Sepsis in neonates is influenced by the quality of intrauterine life, host and environmental factors. Prematurity, improper prenatal care, conduct of labour, environmental conditions in nurseries, low birth weight, presence of perinatal risk factors like premature membrane rupture, maternal sepsis, meconium stained liquor and associated chorioamnionitis have been established to be important predisposing factors.[4] The morbid neonates being deficient in their inherent protective mechanisms of humoral and cellular immunity, acquire infection rapidly. Intensive life support measures commonly used for survival of preterm and other sick babies may predispose to septicemia.[5]

The profile of causative agents of neonatal septicemia varies in different geographical areas and from time to time. In India, *Klebsiellapneumoniae* is the most frequently isolated pathogen followed by *Staphylococcusaureus*.[6] In developed nations, Group *B Streptococcus* is implicated as the leading pathogen causing neonatal septicemia.[3,7]

The recent challenge is in management of the morbid babies suffering from septicemia due to emergence of resistant strains to various classes of antibiotics and the changing pattern of susceptibility from time to time. It is essential to know the changing pattern of antibiotic sensitivity of predominant organisms to plan a strategy for treatment at a particular geographical area.[8]

Therefore, the present study was undertaken to identify the aetiological agents causing neonatal septicemia and analyze the antibiotic susceptibility pattern, so as to

guide the paediatrician in formulating an empirical antibiotic policy against the predominant pathogens in this geographical area of North East India, while waiting for the blood culture report.

MATERIAL AND METHODS

The neonates admitted in the Neonatal Intensive Care Unit (NICU) of Dr. BR Ambedkar Memorial Teaching Hospital, Agartala were recruited for a period of two years from January 2012 to December 2014. On clinical suspicion of septicemia, atleast two sets of test for blood culture were performed and such neonates included in the study.

The clinical diagnosis of sepsis was established on the basis of sign and symptoms like refusal of feed, diarrhoea, vomiting, irritability, jaundice, lethargy and respiratory distress. Neonates showing two or more risk factors like Maternal pyrexia, Urinary tract infection (UTI), Premature Rupture of Membrane (PROM), Meconium Stained Liquor (MSL), Perinatal Asphyxia, Low Birth Weight (LBW), etc were selected for the study. Onset of septicemia was considered as Early Onset Septicemia (EOS) and Late Onset Septicemia (LOS) by less than and more than 72 hours of birth respectively.

About 1-2 ml of blood was collected from the neonates and immediately transferred to 20 ml Brain Heart Infusion Broth for blood culture, maintaining all aseptic precautions. The Culture and Antimicrobial Susceptibility Testing (AST) was done in the Microbiology Laboratory of the Institute following standard CLSI guidelines.[9] Subculture was attempted in MacConkeys agar, Sheep Blood agar and Chocolate agar every alternate day till the 7th day, for possibility of any growth. The media plates showing growth were subjected to identification by Gram staining, Colonial characteristics, Biochemical tests and other applicable special tests. AST was subsequently performed following Kirby Bauer Disc Diffusion method. The data obtained from culture proven septicemia was analyzed.

RESULTS

During the study period, sepsis was suspected in 309 cases and blood culture was positive in 127 cases, thereby giving a positivity rate of 41.1%. EOS was confirmed in 73(57.5%) and LOS in 54(42.5%)neonates.

The most important maternal risk factor found associated with the proven cases of septicemia was maternal pyrexia (18.1%). In EOS, the major risk factors included maternal pyrexia (21.9%), PROM (17.8%) and MSL (15%). In LOS the observation was maternal pyrexia (12.9%), chorioamnionitis (7.4%) and maternal UTI (9.2%).

Table 1: Maternal risk factors

Risk Factor	EOS	LOS	Total
Pyrexia	16 (21.9%)	7 (12.9%)	23 (18.1%)
PROM	13 (17.8%)	2 (3.7%)	15 (11.8%)
MSL	11 (15%)	2 (3.7%)	13 (10.2)
Chorioamnionitis	2 (2.7%)	4 (7.4%)	6 (4.7%)
UTI	2 (2.7%)	5 (9.2%)	7 (5.5%)

It was observed that 59.8% cases of Caesarean section group had EOS whereas in case of Vaginal delivery 38.5% neonates had LOS. There were only two cases of Outlet Forceps delivery, of which each had EOS and LOS. It is clearly evident that operative delivery predispose in the causation of neonatal septicemia.

Table 2: Mode of delivery Versus onset of septicemia

Mode of delivery	EOS (n=73)	LOS (n=54)	Total (n=127)
LSCS	49 (64.4%)	27 (35.5%)	76 (59.8%)
NVD	19 (38.8%)	30 (61.2%)	49 (38.5%)
Assisted	1 (50%)	1 (50%)	2 (1.7%)

The male neonates (63.8%) had preponderance over female (36.2%) in both types of onset of septicemia. LBW was found as an important predisposing factor for septicemia. A total of 71.7% babies with LBW had septicemia. Also 69.9% and 74.1% neonates had LBW with EOS and LOS respectively. On analysis of the gestational age, it was observed that 62.2% Preterm neonates had septicemia compared to 37.8% full term neonates. EOS was more common in Preterm neonates (73.9%) whereas LOS was more found in Full term neonates (53.7%).

Table 3: Neonatal risk factors

Risk factor	Category	EOS (n=73)	LOS (n=54)	Total (n=127)
Sex	Male	49 (67.1%)	32 (59.3%)	81 (63.8%)
	Female	24 (32.9%)	22 (40.7%)	46 (36.2%)
Birth weight	< 2.5 Kg	51(69.9%)	40 (74.1%)	91 (71.7%)
	≥ 2.5 Kg	22 (30.1%)	14 (25.9%)	36 (28.3%)
Gestation	Preterm	54 (73.9%)	25 (46.3%)	79 (62.2%)
	Term	19 (26.1%)	29 (53.7%)	48 (37.8%)

The Gram Positive organisms (51.1%) predominated over Gram negative organisms (48.9%). *Staphylococcus aureus*(34.6%) was the most predominant isolate followed by *Klebsiellapneumoniae* (24.4%), *Staphylococcus epidermidis* (14.2%), *E.coli* (12.6%), *Pseudomonas aeruginosa*(8.7%), *Acinetobacter spp*. (3.1%) and *Enterococci spp*(2.4%).

Table 4: Blood culture isolates

Organism	EOS (n=73)	LOS (n=54)	Total (n=127)
S.aureus	36 (49.3%)	8 (14.8%)	44 (34.6%)
K.pneumoniae	27 (36.9%)	4 (7.4%)	31 (24.4%)
S. epidermidis	3 (4.1%)	15 (27.8%)	18 (14.2%)
E.coli	11 (15.1%)	5 (9.3%)	16 (12.6%)
P.aeruginosa	3(4.1%)	8 (14.8%)	11 (8.7%)
Acinetobacter spp.	-	4 (7.4%)	4 (3.1%)
Enterococci spp.	1 (1.4%)	2 (3.7%)	3 (2.4%)

The antibiotic sensitivity pattern of the gram positive blood culture isolates indicates that all isolates were sensitive to Vancomycin, whereas all isolates were resistant to Ampicillin, Cotrimoxazole and Penicillin. The Cephalosporins, Clindamycin and Amikacin were found significantly sensitive in *Staphylococcus aureus*(95.5%), *S.epidermidis* (100%) and *Enterococci spp*(100%).

Table 5: Antibiotic susceptibility pattern of Gram positive isolates.

Isolate	Ac n(%)	G n(%)	Ak n(%)	E n(%)	Cd n(%)	Va n(%)	Cf n(%)	Cp n(%)	Ci n(%)	Ce n(%)
S.aureus (n=44)	**18 (40.9)**	**16 (36.4)**	**42 (95.5)**	**16 (36.4)**	**42 (95.5)**	**44 (100)**	**34 (77.3)**	**42 (95.5)**	**42 (95.5)**	**42 (95.5)**
S.epidermidis (n=18)	**12 (66.7)**	**6 (33.3)**	**18 (100)**	**12 (66.7)**	**18 (100)**	**18 (100)**	**18 (100)**	**18 (100)**	**18 (100)**	**18 (100)**
Enterococci Spp.(n=3)	**1 (33.3)**	**0**	**3 (100)**	**0**	**3 (100)**	**3 (100)**	**3 (100)**	**3 (100)**	**3 (100)**	**3 (100)**

Ac – AmoxycillinClavulanicacid; G – Gentamicin; Ak – Amikacin; E - Erythromycin; Cd – Clindamycin; Va – Vancomycin; Cf – Ciprofloxacin;Cp – Cephalexin; Ci – Ceftriaxone;Ce – Cefotaxime.

The spectrum of antibiotic sensitivity for gram negative isolates showed all isolates to be sensitive to Imipenem followed by Piperacillin-tazobactum and Amikacin. The isolates were least sensitive to Cefixime, Ofloxacin, Piperacillin and Cefuroxime.

Table 6: Antibiotic susceptibility pattern of Gram negative isolates.

Isolate	As n(%)	Ak n(%)	I n(%)	Pt n(%)	Pc n(%)	Ci n(%)	Ce n(%)	Cpm n(%)	Cu n(%)	Cfx n(%)	Of n(%)
K.pneumoniae (n=31)	**17 (54.8)**	**23 (74.2)**	**31 (100)**	**23 (74.2)**	**8 (25.8)**	**14 (45.2)**	**14 (45.2)**	**14 (45.2)**	**8 (25.8)**	**2 (6.5)**	**8 (25.8)**
E.coli (n=16)	**14 (87.5)**	**14 (87.5)**	**16 (100)**	**14 (87.5)**	**2 (12.5)**	**6 (37.5)**	**6 (37.5)**	**6 (37.5)**	**6 (37.5)**	**0**	**2 (12.5)**
P.aeruginosa (n=11)	**4 (36.4)**	**7 (63.6)**	**11 (100)**	**11 (100)**	**4 (36.4)**	**2 (18.2)**	**2 (18.2)**	**7 (63.6)**	**0**	**0**	**0**
Acinetobacterspp (n=4)	**3 (75)**	**3 (75)**	**4 (100)**	**4 (100)**	**2 (50)**	**2 (50)**	**2 (50)**	**3 (75)**	**0**	**0**	**0**

As – Ampicillin sulbactum ; Ak – Amikacin ;I - Imipenem; Pt – PiperacillinTazobactum ;Pc – Piperacillin; Ci – Ceftriaxone ; Ce – Cefotaxime ; Cpm- Cefipime; Cu – Cefuroxime ;Cfx – Cefixime; Of – Ofloxacin.

There were total of 8 (6.3%) neonatal deaths in the present study. Maximum numbers of deaths were due to *Klebsiellapneumoniae*4(50%), followed by *P.aeruginosa*2(25%) and one each due to*S.aureus* and *Acinetobacter spp*.

DISCUSSION

During the present study, out of 1153 admissions in NICU, 309 cases were clinically suspected to have septicemia giving a clinical incidence of 26.8%. This varies from 4.4% to 51.5% as reported by other authors [10,11]. There were 127 blood culture confirmed cases, thereby giving a bacteriological incidence of 11.01%. This is similar to findings reported by Kuruvilla et al. [10] There occurred 57.5% cases of EOS and 42.5% cases of LOS. The data can be compared to one study from South India stating 59% EOS and 41% LOS cases [12].

Maternal pyrexia near term do indicate the presence of infection and thus responsible for maternal transmission to the non-immune baby. In present study, 18.1% neonates had their mothers near term with pyrexia, of

which 21.9% had EOS and 12.9% had LOS. In a study from South India, there were 6.6% cases of EOS and 1.1% cases of LOS associated with maternal pyrexia.[10]

The risk of neonatal septicemia increases with PROM, as following rupture of amniotic membrane, the organisms present in the vagina may gain access to uterine cavity leading to infection in foetus. Beargie et al. reported a preponderance of gram positive organisms in maternal vagina in late pregnancy [13]. In the present study 11.8% neonates with septicemia were born to mothers having PROM. We found 17.8% EOS cases and 3.7% LOS cases to be associated with PROM. A similar study reported this association as 24% in EOS and 7% in LOS.[14]

Presence of foul smelling liquor is indicative of amnionitis which results by colonization of amniotic fluid even in presence of intact membrane or following rupture of foetal membranes thereby predisposing to infection. In the present study, 10.2% babies were born to mothers associated with MSL. Few other Indian studies reported associated chorioamnionitis to be 3.1% and 6%. [10,15]

Any Genitourinary tract infection at parturition definitely predispose to neonatal sepsis. In present study, 5.5% babies were born to mother with UTI and out of them 2.7% were in cases of EOS and 9.2% in LOS. E.coli was isolated in urine culture from all mothers who had UTI and four of them had asymptomatic bacteruria. A study reported 1.5% mothers with UTI and 3.3% & 1.1% in EOS & LOS cases respectively. [10]

Kishore et al. studied the colonization rate of newborns from three superficial sites of throat, ear and perianal area and found that it was significantly higher in infants born following instrumental delivery (89.5%) compared to spontaneous vaginal delivery (52.4%). [16] In the present study, 59.8% babies with septicemia were delivered by LSCS, 1.7% babies born by outlet forceps, giving an incidence of 61.4% instrumental delivery. Other studies reported the number of instrumental delivery associated with septicaemia as 55.2%, 46.7%, 46% and respectively. [10,16,17]

In the present study, there were 63.8% male and 36.2% female babies giving a ratio of 1.8:1. The incidence of septicemia in males was 67.1% in EOS and 59.3% in LOS. This findings are consistent with another Indian study stating 70% males having EOS, 68.4% having LOS and 68.8% total cases of septicemia.[10] The male preponderance may be attributed to the hypothesis based on presence of genes responsible for production of immunoglobulin on X chromosome. Female receives two 'X' chromosomes each from two partners. So they have heterozygous locus for diversity in production of Immunoglobulin whereas male possess only one X chromosome. [18]

LBW and prematurity predispose to infection in a neonate as enhanced by low IgG levels. The transfer of IgG from mother to fetus is not sufficient in small for date babies who are often the product of placental insufficiency. In present study, 71.7% babies with LBW (<2.5 kg) had septicemia, of which 69.9% neonates with LBW had EOS and 74.1% had LOS. This observation is consistent with other studies stating incidence of such association at 79.3%, 59.4% and 54.5%.[19,20,21]

In the present study, 62.2% preterm neonates had septicemia, of which 73.9% preterm neonates had EOS whereas 53.7% full term neonates had LOS. Other studies have reported such association of preterm neonates with septicemia from 22% to 73%.[17,14] Preterm neonates are at higher risk of infection due to their low immunity and need for invasive procedures like Intravenous lines, Endotracheal intubation and mechanical ventilation.

The blood culture positivity rate in the present study was 41.1%. It is to be considered that a negative blood culture does not rule out septicemia. Various factors like administration of antibiotics to mother or baby before blood collection, possibility of infection with fastidious organisms and anaerobes, etc. pose difficulty in isolation unless advanced automated or molecular techniques are employed, which may be expensive in routine laboratory protocol.

In the present study, Staphylococcus aureus was the predominant isolate (34.6%) followed by Klebsiellapneumoniae (24.4%), *Staphylococcus epidermidis* (14.2%), *E.coli* (12.6%), *Pseudomonas aeruginosa* (8.7%), *Acinetobacterspp* (3.1%) and *Enterococci spp* (2.4%). The predominant isolate in EOS was *Staphylococcus aureus* (49.3%) and in LOS it was *Staphylococcus epidermidis* (27.8%).

Though most of the Indian studies have reported the Gram Negative bacteria like *E.coli* and *K.pneumoniae* as predominant isolate, few other studies have reported the incidence of *Staphylococcus aureus* isolate as 29.5%, 35% and 38%. [22,23,24] Along with the maternal risk factors, this indicates the possibility of cross infection in NICU from index case, which could be prevented by following standard measures like universal precautions, elimination of source of infection & hand hygiene by health care providers.

The gram positive isolates were found more sensitive to majority of antibiotics than the gram negative organisms, as also observed by Mathur et al.[8] The predominant isolate, *Staphylococcus aureus* showed 100% sensitivity to Vancomycin and resistance to Ampicillin and penicillin in all isolates, as alsoobserved by Kuruvilla et al. [10] They were significantly sensitive to Cephalosporins like Cephalexin, Ceftriaxone and Cefotaxime as also observed in other reference studies. The isolate was 95.5% sensitive to Amikacin compared to 36.4% for Gentamicin. This has also been observed by Kumar et al.[25] The sensitivity to Ciprofloxacin was 77.3%. But considering the poor CSF penetrability and adverse effects of Ciprofloxacin

in infants, Cephalosporins&Amikacin can be considered as first line of treatment followed by Fluoroquinolones. This observation can be compared with a study by Agnihotri et al. [23] Other gram positive isolates were significantly sensitive to Cephalosporins, Ciprofloxacin, Vancomycin and Amikacin, thereby not changing the chosen antibiotic policy.

The predominant gram negative isolates of *Klebsiellapneumoniae&E.coli* showed a pattern of multi drug resistance, which is consistent with observations cited by other authors.[8,10,25] This indicates the emergence of highly resistant mutants especially found with nosocomial pathogens. However, all of these isolates were sensitive to Imipenem, followed by Piperacillin-tazobactum. andAmikacin with significant resistance to Fluoroquinolones and Penicillins.

From the antibiogram, Imipenem was found to be the most effective drug against all gram negative isolates. But it is to be avoided in neonates due to reported incidence of seizures following its use. Moreover, such antibiotic should not be used empirically, and should be reserved for exceptional situations which warrant its use, considering the in–vitro susceptibility of the prevailing regimen. Therefore keeping aside the reserve drugs like Imipenem and Vancomycin, Amikacin was found to be the drug of choice for both groups of organisms. This observation is consistent with the observation stated by Diwakar et al. [12]

CONCLUSION

In the present study, the important predisposing maternal risk factors to neonatal septicemia were found to be maternal pyrexia and PROM with foul smelling liquor. Preterm and LBW babies had more incidences of septicemia and showed preponderance in cases of EOS. Neonatal mortality in this study was 13.5% and all of them were Preterm and LBW neonates. *Staphylococcus aureus* followed by *Klebsiellapneumoniae* were the common causative agents of septicemia. The overall drug of choice was found to be Amikacin. Considering the recommendation to use combination of drugs in septicemia, Amikacin along with a third generation Cephalosporin like Cefotaxime were chosen to be the first line regimen for treatment of cases of septicemia in our setting. Highly susceptible drugs like Imipenem, Piperacillin-tazobactum and Vancomycin were kept reserved for unusual situations depending upon Antibiotic sensitivity reports, thereby keeping rationality in use of antibiotics.

The predominant isolate of *Staphylococcus aureus* along with significant association of maternal risk factors indicates the possibility of cross infection in NICU from index case, for which emphasis need to be laid on Hospital infection control strategies like universal precautions, elimination of source of infection and appropriate antibiotic prophylaxis.

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