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Clinical and bacteriological profile of neonatal septicemia in a tertiary care hospital

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Abstract
Background: Neonatal sepsis is a clinical syndrome characterized by signs and symptoms suggestive of infection with or without accompanying bacteremia in the first month of life. Since the spectrum of organisms that cause neonatal sepsis changes over the time and varies from region to region and hospital to hospital even in the same city/country, it is necessary to conduct periodic surveillance to access the changing pattern of organisms causing neonatal sepsis. The present study was undertaken to know the clinical and bacteriological profile and antibiotic susceptibility pattern of neonatal septicemia.
Methods: Total 251 samples received during the study period were processed as per standard bacteriological techniques and antibiotic susceptibility is performed as per CLSI guidelines.
Results: Out of 251 samples received during the study period, 142 (56.57%) were found to be positive. Prematurity ($p < 0.05$), birth asphyxia ($p < 0.01$) and duration of labor more than 24 hours ($p < 0.05$) were statistically significantrisk factors for causation of early onset septicemia. Refusal of feed, lethargy and weak reflexes were most common clinical presentation. 86 (60.56%) and 56 (39.44%) neonates had Gram positive and Gram negative infection respectively. *Staphylococcus aureus* and *Klebsiella pneumoniae* were the commonest isolates. Gram positive organisms were found to be sensitive to linezolid, and vancomycin and gram negative isolates were sensitive to meropenem and piperacillin tazobactam.
Conclusions: The periodic surveillance of etiological agents and their susceptibility pattern should be done in order to use better choice of antibiotics, as the patterns of bacterial organisms are changing constantly with time and place.

Keywords: Neonatal septicemia, blood culture, antibiotic susceptibility pattern

1. Introduction
Neonatal sepsis is a clinical syndrome characterized by signs and symptoms suggestive of infection with or without accompanying bacteremia in the first month of life. It encompasses various systemic infections of the newborn such as septicemia, meningitis, pneumonia, arthritis, osteomyelitis and urinary tract infection [1]. Neonatal septicemia remains one of the main causes of mortality and morbidity despite the progress in hygiene, introduction of new and potent antimicrobial agents for treatment and advanced measures for diagnosis. Up to 10% infants have infections in the first month of life, the matter which results in 30- 50% of total neonatal deaths in developing countries [2]. These neonatal deaths are attributed principally to infection, birth asphyxia and consequences of premature birth and low birth weight [3, 4]. The incidence of neonatal bacterial sepsis may vary from country to country, as well as within the same country. In developing countries, neonatal mortality results from all expected causes of neonatal sepsis, consequently, it is about 30 per 1000 live births, occurring mainly in the first week of life, while it is only 5 per 1000 live births in the developed countries [5]. In most developing countries, gram-negative bacteria remain the major cause of neonatal sepsis [6, 7]. These organisms have developed increased drug resistance over the last two decades [8]. On the other hand Group B Streptococcus (GBS) has been the most frequent etiological agent of neonatal sepsis in developed countries, being responsible for high morbidity and mortality [9]. Since the spectrum of organisms that cause neonatal sepsis changes over the time and varies from region to region and hospital to hospital even in the same city/country, it is necessary to conduct periodic surveillance to access the changing pattern of organisms causing neonatal sepsis.

Therefore knowledge of the pattern of bacterial isolates and their antimicrobial susceptibility pattern is useful for prompt treatment of patient. So the present study was undertaken to know the clinical and bacteriological profile and antibiotic susceptibility pattern of neonatal septicemia.

Materials and Methods

The present observational study was conducted in the Department of Microbiology at tertiary care hospital in the Maharashtra state of central India from January 2014 to June 2015, after obtaining the necessary permission from Institutional Ethical Committee (IEC).

251 blood samples from clinically suspected cases of neonatal septicemia were included in the study. All newborn babies aged 0-28 days presenting with one or more clinical feature features suggestive of septicemia and having one or more risk factors like low birth weight, prematurity, birth asphyxia, premature rupture of membranes, prolonged labour, instrumentation, home delivery, were included in the present study. Neonates with clinical features suggestive of septicemia receiving antibiotics before collection of blood sample and with gross congenital malformations were excluded from the study. A detailed history based on gestation in weeks, mode of delivery, place of delivery, various risk factors and presenting clinical features for each case was taken and duly recorded in the Study Proforma. Consent was taken from the parents of these neonates for participation in the study after explaining the study protocol to them in the language that they understood best.

Blood culture and antimicrobial sensitivity test

Blood was collected with aseptic precautions before starting antibiotics and 1 ml of venous blood was added to each of the two bottles containing 10 ml of sterile glucose broth and bile broth thus making a dilution of 1 in 10 to nullify the natural bacteriostatic/bacteriocidal activity of blood. Both these bottles were incubated under aerobic conditions in the incubator for 7 days. The first subculture was done after 18-24 hours of incubation, the second on third day and final subculture was done on seventh day. Subcultures were done on nutrient agar, 5% blood agar and MacConkey's agar plates (Hi Media, Mumbai, India).

The inoculated plates were incubated aerobically in the incubator at 35-37 °C for 18- 24 hours, and the plates were observed for growth. The organisms were identified on the basis of colony characteristics, Gram's staining and biochemical test as per standard bacteriological techniques. [10] A provisional report was issued after every subculture and if after 7 days, no growth was obtained, the sample was reported as negative.

Antimicrobial sensitivity test

Antimicrobial sensitivity test was done for all the isolates on Muller Hinton agar using commercially available antibiotic discs, by Kirby-Bauer disk diffusion method as per Clinical Laboratory Standards Institute guidelines [11]. All the antibiotic disks were obtained from Hi Media, Mumbai, India. Every batch of Muller Hinton agar and antibiotic discs were tested by using ATCC control strains. The plates were incubated at 37 °C. After overnight incubation, the diameter of clear zone around the disc was measured and interpreted as sensitive or resistant according to the zone diameter as per Clinical Laboratory Standards Institute guidelines [11].

The statistical analysis was done using Open Epi, version 2.3 Statistical Package. A p value of <0.05 was considered significant, and p value of <0.01 was considered as highly significant.

Result and Discussion

During the study period, a total of 251 blood samples from clinically suspected cases of neonatal septicemia were tested. Out of 251 cases, 142 cases were culture positive thus prevalence of neonatal septicemia in the present study was 56.57%. 138 (54.98%) cases were of early onset septicemia and 113 (45.02%) cases were of late onset septicemia. Out of 138 suspected cases of early onset septicemia, 84 (60.87%) cases were blood culture positive and out of 113 suspected cases of late onset septicemia, 58 (51.33%) cases were blood culture positive (Table 1).

Table 1: Distribution of culture positivity in EOS and LOS

Septicemia	Blood Culture Positive	Blood Culture Negative	Total
Early onset Septicemia	84 (60.87%)	54 (39.13%)	138
Late onset septicemia	58 (51.33%)	55 (48.67%)	113
Total	142 (56.57%)	109 (43.43%)	251

Difference in culture positivity among early and late onset septicemia was not statistically significant (p> 0.05). These findings are consistent with the study done by Shaw CK *et al.* [12] and Bhatt SK *et al.* [13] who reported blood culture positivity in 54.64% and 56.67% cases of neonatal septicemia.

Culture positivity rate is highly variable from place to place and positive blood cultures reportedly range from 8% to 73%. [14] A study conducted by Sharma CM *et al.* [15] reported positive cultures in 37.63% cases which was lower than the present study. The lower blood culture positivity may be due to several reasons, viz. administration of antibiotics (mother/baby) before blood collection, infection with anaerobes, due to intermittent bacteremia or effective control in spread of nosocomial infection.

In the present study out of 142 culture positive cases, 70 (49.30%) were males and 72 (50.70%) were females. Male preponderance was not observed in the present study which is in concordance with the study done by Chacko *et al.* [16] and Shrestha RK *et al.* [17]. However, preponderance of septicemia in male babies was reported by Khinchi *et al.* [18] (males 65.1%, female 34.9%) and Jonnala *et al.* [19] (males being 68% and females 32%). The possible reason for the male preponderance is that the factors regulating synthesis of gamma globulins are probably situated on X chromosome and presence of one X chromosomes in male thus confers less immunological protection compared to female counter part. [20]

In the present study among 142 blood culture positive cases, 92 (64.78%) were preterm and 50 (35.22%) were term babies (Table 2). Preterm babies showed significantly higher culture positivity (p<0.001) as compared to term babies. This may attribute that the preterms are more susceptible to infection due to inherent compromised immunity, vulnerable skin and mucosal barriers, prolonged hospital stay and extensive interventions for other complications of prematurity. [21] In present study Culture positivity was found to be more in low birth weight babies, majority

(77.29%) of the neonates (194 out of 251) were of low birth weight (weight < 2.5 kg.). These findings are similar to the study done by Khinchi *et al.* (2010) [18] and Raha BK *et al.* (2014) [22] The rate of infection is inversely proportional to the birth weight, low IgG levels due to impaired cellular immunity in the low birth weight neonates contributes to the increased susceptibility to infections in these neonates.[23]

Table 2: Distribution of cases according to the gestational age

Gestational age	Culture positive	Culture negative
Preterm	92 (64.78%)	49 (44.95%)
Term	50 (35.22%)	60 (55.05%)
Total	142	109

p = 0.001

Neonatal septicemia is difficult to diagnose clinically as it presents with nonspecific signs and symptoms. Refusal of feed, lethargy and weak reflexes were most common clinical presentation in suspected cases of neonatal septicemia. The other clinical presentations like hypothermia, hyperthermia, tachypnea, apnea, high pitched cry, convulsion and loose stools were not so prominent and were in the range of 31.47% to 7.17% (Table 3).

Table 3: Distribution of clinical findings in neonatalsepticemia

S. No	Clinical presentation*	No. of cases	Percentage %
1.	Refusal of feed	171	68.13
2.	Lethargy and weak reflexes	156	62.15
3.	Hypothermia	79	31.47
4.	Hyperthermia	53	21.12
5.	Tachycardia	100	39.84
6.	Tachypnea	59	23.51
7.	Apnea	34	13.55
8.	High pitched cry	26	10.36
9.	Convulsion	26	10.36
10.	Loose stools	18	7.17

* multiple response

Khinchi *et al.* (2010) [18] in their study found that majority of neonates presented with refusal to feeds (74%), tachypnea or respiratory distress (75%) and fever (69%). Refusal of feed was the most common presentation in the present study and in the above referred study, other clinical features differs in both the studies. In a study done by Jajoo M *et al.* (2015) [24] reported common clinical presentations as lethargy/refusal to feed in 63 (77%), respiratory distress in 36 (44%) and hypothermia in 39 (47.5%) cases. The clinical features and further course in neonatal sepsis depends on various factors like birth weight, place of delivery, age of newborn, intervention in preventable factors for sepsis, availability, accessibility, affordability and timely referral of baby to an appropriate center. Therefore variation in different parameters may be observed in various studies.

In the present study, among 84 culture positive cases of early onset septicemia, prematurity ($p < 0.05$), birth asphyxia ($p < 0.01$) and duration of labor more than 24 hours ($p < 0.05$) were statistically significant risk factors for causation of early onset septicemia. Prematurity is the most important neonatal factor predisposing to infection, due to less developed immune systems they are more likely to have diseases such as necrotizing enterocolitis and hyaline membrane disease which are further complicated by infection.[21] Birth asphyxia, depresses the immune functions. Additional interventions, frequent suction, intubation, prolonged ventilator care to manage asphyxia may predisposed the neonates for infections.

Out of 142 cultures positive cases, 86 (60.56%) were gram positive isolates and 56 (39.44%) were gram negative isolates. In the present study Staphylococcus aureus 84 (59.15%) was the most common causative agent of neonatal septicemia followed by Klebsiella pneumoniae 43 (30.28%) Table 4.

Table 4: Distribution of bacterial isolates causing septicemia

Sr. No	Bacterial isolate	Number of isolates	Percentage
A	**Gram positive isolates**	**86**	**60.56**
1.	Staphylococcus aureus	84	59.15
2.	Staphylococcus epidermidis	02	1.41
B	**Gram negative isolates**	**56**	**39.44**
1.	Klebsiella pneumoniae	43	30.28
2.	Pseudomonas aeruginosa	07	4.93
3.	Acinetobacter baumannii	04	2.82
4.	Proteus mirabilis	01	0.70
5.	Klebsiella oxytoca	01	0.70
	Total	142	100

The finding of predominance of isolation of Gram positive isolates and Staphylococcus aureus as a predominant pathogen correlates well with the study done by Karthikeyan *et al.* (2001) [25] and Shrinivasa *et al.* (2014) [26]. Greater prevalence of Staphylococcus aureus in neonatal septicemia could be explained by the fact that it is a common hospital acquired organism which accounts for most of the infections and there are high chances of transmission of Staphylococcus aureus to neonates from health care workers and relatives. [27] For the same reason it is possible that prevalence of this less fastidious organism is over estimated, and also due to comparative ease of its isolation. However the studies done by Bhatt *et al.* [13], Vishwanathan *et al.* [28] and Zakaria *et al.*[29] have reported the overall predominance of gram negative organisms with Klebsiella pneumoniae as a predominant pathogen in 59.10%, 36.64% and 66% cases of neonatal septicemia respectively. This shows that there is a marked geographical variation in the microbiological spectrum of neonatal septicemia and it differs from nursery to nursery.

The second most common organism isolated in this study was Klebsiella pneumoniae. Klebsiella pneumoniae is commonly found in the environment of neonatal intensive care units and nursery.[29] It can also be present as colonizers on the hands of the health workers. There are also frequent reports of neonatal septicemia out breaks due to Klebsiella pneumoniae in the nursery and NICU.[29] Other gram negative organisms isolated includes the Pseudomonas aerugionosa 07 (4.93%), Acinetobacter baumannii 4 (2.82%), Proteus mirabilis and Klebsiella oxytoca in 1 (0.70%) cases each.

In the present study Gram positive isolates, Staphylococcus aureus and Staphylococcus epidermidis were highly resistant to ampicillin and penicillin. High level of resistance was also seen with ciprofloxacin and erythromycin. However they were found to 100% sensitive to vancomycin and linezolid, thus these drugs can be effectively used if methicilin resistance is suspected during treatment. About 73.81% of Staphylococcus aureus and 50% of Staphylococcus epidermidis were sensitive to Amikacin (Table 5). Mane AK *et al.* (2010)[30] and Mehta *et al.* (2014)[31] observed 100% sensitivity to vancomycin and

linezolid by Staphylococcus aureus. These findings were consistent with the present study.

Table 5: Antimicrobial sensitivity pattern of Staphylococcal species

Sr. No	Drugs	S. aureus (n=84) Sensitive (%)		S. epidermidis (n=02) Sensitive (%)	
1.	Penicillin	06	7.14	00	0
2.	Ampicillin	06	7.14	00	0
3.	Erythromycin	32	38.10	01	50
4.	Clindamycin	49	58.33	01	50
5.	Cefoxitin	35	41.67	02	100
6.	Linezolid	84	100.00	02	100
7.	Vancomycin	84	100.00	02	100
8.	Gentamycin	34	40.48	01	50
9.	Amikacin	62	73.81	01	50
10.	Ciprofloxacin	33	39.29	01	50

In the present study, about 49 (58.33%) of Staphylococcus aureus isolates were methicillin resistant Staphylococcus aureus (MRSA). Karthikeyan *et al.* (2001) [25] and Chelliah *et al.* [32] reported isolation of 66% and 56.6% of MRSA respectively. Findings of above referred study are consistent with the present study. So screening for MRSA in every staphylococcus aureus isolate will be of immense value for providing efficient patient care. The high resistance rates found may be associated with frequent use of antimicrobial drugs for both prophylactic and therapeutic treatment of hospitalized new born.

In case of Gram negative bacteria most common isolated organisms were Klebsiella pneumoniae (n=43), Pseudomonas aeruginosa (n=7) and Acinetobacter baumannii (n=04). All the Gram negative isolates were resistant to ampicillin. However they showed 100% sensitivity to meropenem, 67.44% of Klebsiella pneumoniae, 57.14% of Pseudomonas aeruginosa, and 75% of Acinetobacter baumannii showed sensitivity to piperacillin-tazobactum. Only 10 (23.26%) isolates of Klebsiella pnemoniae were sensitive to cefotaxime and ceftazidime and 09 (20.93%) were sensitive to aztreonam. Other isolated Gram negative bacteria includes Klebsiella oxytoca (n=1) and Proteus mirabilis (n=1) which also exhibited high resistance to commonly prescribed drugs like penicillin, cephalosporins and aminoglycosides (Table 6).The high resistance of all these organisms to third generation cephalosporins can be attributed to frequent production of extended spectrum beta lactamases (ESBL) by these organisms.

Table 6: Antimicrobial sensitivity pattern of gram negative isolates

Sr. No.	Drugs	K.pneumoniae (n=43) S* (%)		K. oxytoca (n= 01) S* (%)		P. aeruginosa (n=07) S* (%)		P. mirabilis (n=01) S* (%)		A. baumannii (n=04) S* (%)	
1.	Ampicillin	00	00	00	00	00	00	00	00	00	00
2.	Gentamycin	14	32.56	00	00	04	57.14	00	00	01	25
3.	Amikacin	17	39.53	00	00	05	71.43	00	00	01	25
4.	Ciprofloxacin	32	74.42	01	100	03	42.86	00	00	02	50
5.	Ampicillin-Sulbactum	04	9.30	00	00	01	14.29	00	00	01	25
6.	Amoxy-Clav	04	9.30	00	00	01	14.29	00	00	01	25
7.	Piperacillin-Tazobactum	29	67.44	01	100	04	57.14	00	00	03	75
8.	Cefotaxime	10	23.26	00	00	03	42.86	00	00	01	25
9.	Ceftazidime	10	23.26	00	00	03	42.86	00	00	01	25
10.	Aztreonam	09	20.93	00	00	03	42.86	00	00	02	50
11.	Meropenem	43	100	01	100	07	100	01	100	04	100

*S = Sensitive

Conclusion

The periodic surveillance of etiological agents and their susceptibility pattern should be done as the patterns of bacterial organisms are changing constantly with time and place in order to use better choice of antibiotics.

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