International Journal of Contemporary Pediatrics
Das M et al. Int J Contemp Pediatr. 2022 Nov;9(11):1056-1062
http://www.ijpediatrics.com
pISSN 2349-3283 | eISSN 2349-3291
DOI: https://dx.doi.org/10.18203/2349-3291.ijcp20222766

Original Research Article

Clinical and bacteriological profile of neonatal sepsis: a prospective hospital-based study in a tertiary care hospital in eastern zone of West Bengal

Mousumi Das[1]*, Ira Das[1], Sudip Saha[1], Saptadweepa Sanghamitra[2]

[1]Department of Pediatrics, Chittaranjan Seva Sadan and Sishu Sadan Hospital of Obstetrics, Gynaecology and Child Health, Kolkata, West Bengal, India
[2]Department of Pediatric Medicine, Shri Dadab Dev Mother and Child Hospital, Janakpuri, South West Delhi, India

Received: 07 September 2022
Revised: 04 October 2022
Accepted: 10 October 2022

***Correspondence:**
Dr. Mousumi Das,
E-mail: mousumids99@gmail.com

ABSTRACT

Background: Neonatal sepsis remains a leading cause of morbidity and mortality among neonates and clinical manifestations are non-specific. Delayed identification and inappropriate treatment remain as key factors causing high neonatal mortality. Increasing emergence of multidrug-resistant organisms reduces antibiotic options. Hence there is a need for institutional guidelines based on local microbial prevalence and their antibiotic susceptibility patterns.
Methods: Blood cultures were collected from all suspected cases prior to the initiation of antimicrobial therapy. Neonatal sepsis with positive blood culture cases is included in the study.
Results: Among 170 neonate blood culture was positive in 41 neonates (preterm - 71% and term neonate - 29%). Early onset neonatal sepsis (EONS) constituted 61% and Late onset neonatal sepsis (LONS) constituted 39%. Respiratory distress, hypoglycaemia, seizures, lethargy were more common presentations in EONS while cellulitis, sclerema, septic arthritis, loose stool, vomiting, blood in stool, hyperthermia and recurrent apnoea were more common presentations in LONS. Gram positive organisms constituted 51.2%, gram negative organisms were 46.3%, fungi were 9.8% of total pathogens. Among gram positive cons (43.9%) was most common while amongst gram negative *Klebsiella* (22%) was most common. *Candida peliculosa* was the most commonly isolated fungus. Cons, staphylococcus and enterococcus were seen to be 100% sensitive to linezolid. Enterococcus also showed 100% sensitivity to vancomycin, teicoplanin and tigecycline. Gram-positive bacteria showed the highest sensitivity to linezolid and vancomycin. *Acinetobacter baumanii* showed 80% sensitivity to gentamicin, 60% sensitivity to tigecycline and colistin. *E. coli* showed 66% sensitivity to tigecycline.
Conclusions: We were able to analyze common causative pathogens, associated risk factors, and the antibiotic susceptibility pattern of neonatal sepsis.

Keywords: Neonatal sepsis, Microorganisms, Antibiotic pattern

INTRODUCTION

Neonatal sepsis is a syndrome of clinical manifestations of inflammatory responses in neonates due to a spectrum of systemic infections, such as septicaemia, pneumonia, bone-related infections, and meningitis.[1,2] Clinical manifestations of sepsis are non-specific and resemble many non-infection-related disorders. It remains a leading cause of morbidity and mortality among neonates, especially in middle and lower-income countries.[3] Neonatal sepsis is divided into two groups based on the time of presentation after birth: early-onset sepsis (EOS)

and late-onset sepsis (LOS). EOS refers to sepsis in neonates at or before 72 hours of life (some experts use seven days), and LOS is defined as sepsis occurring at or after 72 hours of life.[4]

Despite recent advances in health care, delayed identification and inappropriate treatment remain as key factors causing high neonatal mortality; however, these problems can be averted through judicious antimicrobial selection and advanced adjuvant care.[1,2,5] Contributing factors in developing countries include the lack of people awareness of alarming signs of sepsis, lack of training of medical personnel, and limited availability of reliable laboratories, particularly blood culture, in areas far from hospitals; these factors lead to compromised care, outdated antibiotic guidelines, and emergence of resistance.

Empirical antibiotics are usually initiated once sepsis is suspected. However, increasing emergence of multidrug-resistant organisms reduces antibiotics options and defers adequate treatment implementation; hence, there is a need for institutional guidelines based on local microbial prevalence and their antibiotic susceptibility patterns.[6,7]

The objective of this article was to investigate the bacterial pathogens, to analyze the associated risk factors, and to confer the antibiotic susceptibility pattern of common causative pathogens of neonatal sepsis in this tertiary care hospital in eastern part of West Bengal, which may provide guidance on empirical antimicrobial treatment for neonatal sepsis.

METHODS

This retrospective cohort study was conducted between 01 January 2019 and 31 December 2019 at Chittaranjan Sevasadan and Sishusadan Hospital of Gynaecology, Obstetrics and Child Health after obtaining proper ethical permission.

Neonates were included in the study based on the following criteria, neonates (0–28 days) present with the risk factors and clinical symptoms of sepsis and whose blood culture came positive for any organism.

Inclusion criteria

Neonatal sepsis cases with positive bacterial blood culture were included in the study.

Exclusion criteria

Suspected sepsis with negative blood cultures were excluded from the study. Among 52 culture positive neonates, 11 cases with incomplete data were excluded.

Clinical data were collected, including name, age, gender, gestational age, associated diagnoses, and birth weight. Neonates were screened for complete blood counts, particularly total leucocyte count (immature/total ratio and absolute neutrophilic count were calculated), and C-reactive protein level, micro erythrocyte sedimentation rate (ESR).

Blood cultures were collected from all cases on admission prior to the initiation of antimicrobial therapy. Next, 1 to 2 ml of blood was drawn from a unilateral venipuncture under aseptic conditions, and then injected to a blood culture bottle for analysis with an automated BacT/ALERT 3D 60 mi-crobial system (bioMerieoux, France).

The 41 selected cases were later divided into 2 subgroups: early-onset neonatal sepsis (EONS, onset of symptoms before 72 hours of life) and late-onset neonatal sepsis (LONS, onset of symptoms beyond 72 hours after birth and before 28 days).

The antimicrobial susceptibility for isolated pathogens was determined. Antimicrobial susceptibility testing of isolated pathogens was done with ATB susceptibility system (BioMerieux La Balmes-les Grottes, France) by the Kirby Bauer disk diffusion method according to Clinical and Laboratory Standards Institution (CLSI) recommendations.[10]

Statistical analysis

Collected data was checked for consistency and completeness and entered into Microsoft excel spreadsheet for analysis. Data were organised and presented using the principles of descriptive statistics in the form of frequency and percentage and also in tables and diagrams. Diagrams were made by Microsoft excel/statistical package for the social sciences (SPSS) software. Categorical data were expressed in proportions (%) and continuous variables were presented as mean±standard deviation (SD). Depending upon the type and distribution of data, inferential statistics were applied to test significance. P value less than 0.05 was considered as statistically significant. Analysis of the data was done by IBM SPSS (version 20.0).

RESULTS

Most neonates are in the gestation group 28 to 34 weeks, both in EONS and LONS. Male neonates are more affected than females. Most presentations were before 5 days age (Table 1).

The numbers may add up to more than 41 because of more than one symptom in the patient at presentation. Respiratory distress was the most common clinical presentation as a whole (Table 2).

Gram positive organism was present in 51.2% of neonate (Table 3).

All gram-positive bacteria were 100% sensitive to Linezolid (Table 4).

Amikacin was seen to be highly effective against klebsiella, while tigecycline was found highly effective against *Acinetobacter baumanii* and *E. coli* (Table 5).

All the isolated fungi were highly sensitive to both voriconazole and amphotericin B (Table 6).

Table 1: Gestational age, sex, lab parameters, day of presentation in patients with early-onset and late-onset neonatal sepsis (n=41).

Variables and levels	Total (n=41)		EONS (n=25)		LONS (n=16)		Chi square value	P value
	Frequency	Percent	Frequency	Percent	Frequency	Percent		
Gestational age groups								
<28	4	9.8	4	16	0	0	9.322	0.025*
28-34	15	36.6	9	36	6	37.5		
34-37	10	24.4	5	20	5	31.3		
>37	12	29.3	7	28	5	31.3		
Sex								
Male	30	73.2	19	76	11	68.8	0.784	0.376
Female	11	26.8	6	24	5	31.3		
CRP								
Positive	29	70.7	17	68	12	75	0.693	0.405
Negative	12	29.3	8	32	4	25		
Sepsis screen								
Positive	25	61.0	15	60	10	62.5	0.077	0.782
Negative	16	39.0	10	40	6	37.5		
CSF								
Positive	11	26.8	8	32	3	18.8	5.067	0.079
Negative	29	70.7	16	64	13	81.3		
Not done	1	2.4	1	4	0	0		
Day of presentation								
<5 days	30	73.2	25	100	5	31.5	70.469	0.000*
5-9 days	6	14.6	0	0	6	37.5		
15-19 days	1	2.4	0	0	1	6.3		
≥20 days	4	9.8	0	0	4	25		

*Statistically significant

Table 2: Clinical symptoms of patients with early-onset and late-onset neonatal sepsis (n=41).

Clinical presentation	Total (n=41)		EONS (n=25)		LONS (n=16)		Chi square value	P value
	Frequency	Percent	Frequency	Percent	Frequency	Percent		
Respiratory distress	11	26.8	7	28	4	25.0	0.04	0.833
Hypoglycemia	8	19.5	6	24	2	12.5	0.82	0.365
Seizures	7	17.1	5	20	2	12.5	0.39	0.534
Poor suckling/ poor cry	7	17.1	4	16	3	18.8	0.05	0.819
Lethargy	6	14.6	4	16	2	12.5	0.10	0.757
Birth asphyxia	3	7.3	3	12	0	-	1.84	0.175
Others*	10	24.3	3	12	7	43.9	3.12	0.078

*Others include cellulitis, sclerema, septic arthritis, Loose stool, vomiting, blood in stool, hyperthermia and recurrent apnoea

Table 3: The distribution of causative organisms in patients with early-onset and late-onset neonatal sepsis (n=41).

Organisms	Total (n=41)	EONS (n=25)	LONS (n=16)
	Frequency (%)	Frequency (%)	Frequency (%)
Gram positive pathogens	21 (51.2)	9 (36)	12 (75)
CONS	18 (43.9)	7 (28)	11 (68.8)
Staph hemolyticus	6 (14.6)	2 (8)	4 (25)
Staph epidermidis	3 (7.3)	2 (8)	1 (6.3)
Staph hominis	1 (2.4)	0	1 (6.3

Continued.

Organisms	Total (n=41)	EONS (n=25)	LONS (n=16)
	Frequency (%)	Frequency (%)	Frequency (%)
Staph saprophyticus	1 (2.4)	1 (4)	0
Staphylococcus aureus	7 (17.1)	2 (8)	5 (31.3)
Enterobacter cloacae	1 (2.4)	0	1 (6.3)
Enterococcus fecalis	1 (2.4)	1(4)	0
Enterococcus durans	1 (2.4)	1(4)	0
Gram negative organisms	19 (46.3)	15 (60)	4 (25)
Klebsiella	9 (22)	9 (36)	0
Klebsiellapneumonae	6 (14.6)	6 (24)	0
Klebsiellaoxitoca	3 (7.3)	3 (12.5)	0
E coli	4 (9.8)	3 (12.5)	1 (6.3)
Acinetobacterbaumanii	5 (12.2)	3 (12.5)	2 (12)
Enterobacter cloacae	1 (2.4)	0	1 (6.3)
Fungi	4 (9.8)	0	4 (25)
Candida peliculosa	3 (7.3)	0	3 (18.8)
Candida glabrata	1 (2.4)	0	1 (6.3)

Table 4: Antimicrobial susceptibility patterns among gram positive bacteria of septic neonates.

Antibiotics	*Enterococcus*	CONS	*Staphylococcus aureus*
Amikacin	0/2 (0)	1/10 (10)	2/7 (28.6)
Ciprofloxacin	0/2 (0)	1/10 (10)	0/7 (0)
Coamoxyclav	0/2 (0)	1/10 (10)	0/7 (0)
Gentamicin	0/2 (0)	1/10 (10)	1/7 (14.3)
Linezolid	2/2 (100)	10/10 (100)	7/7 (100)
Vancomycin	2/2 (100)	7/10 (70)	3/7 (42.9)
Teicoplanin	2/2 (100)	7/10 (70)	3/7 (42.9)
Tigecycline	2/2 (100)	3/10 (30)	1/7 (14.3)

Table 5: Antimicrobial susceptibility patterns among gram negative bacteria of septic neonates.

Antibiotics	*Acinetobacter baumanii*	*E. coli*	*Enterobacte-riaceae*	*Klebsiella pneumoniae*	*Klebsiella oxitoca*
Amikacin	2/5 (40)	0/3 (0)	NT	8/8(100)	1/1 (100)
Gentamicin	4/5 (80)	0/3 (0)	NT	4/8 (50)	0/1 (0)
Colistin	3/5 (60)	0/3 (0)	NT	3/8 (37.5)	0/1 (0)
Tigecycline	3/5 (60)	2/3 (66.6)	NT	2/8 (25)	1/1 (100)
Pipzo	NT	NT	1/1 (100)	3/8 (37.5)	0/1 (0)
Cefoperazone	1/5 (20)	0/3 (0)	0/1 (0)	0/1 (0)	0/1 (0)
Levofloxacin	2/5 (40)	0/3 (0)	0/1 (0)	0/1 (0)	0/1 (0)
Meropenem	NT	1/3 (33.3)	1/1(100)	2/8 (25)	NT

Table 6: Antimicrobial susceptibility patterns among fungi of septic neonates.

Antifungal agent	*Candida glabrata* n (%)	*Candida peliculosa* n (%)
Voriconazole	1 (100)	3 (100)
Amphotericin B	1 (100)	3 (100)

DISCUSSION

Among 170 neonates admitted with suspicion of sepsis blood culture was positive in 41 neonates. So, the blood culture positivity rate comes as 24.1%. Neonatal blood culture positive rate have been found to range from 25-54% in some other studies.[8-11]

Of all the culture positive sepsis, preterm (71%) were seen to be more involved than term neonate (29%). In a systemic review and meta-analysis it was found that preterm babies were 3.36 more likely to develop neonatal sepsis than term babies.[12] Immaturity of the premature neonatal immune system, including low immunoglobulin levels related to decreased transplacental transfer of maternal IgG, also increases the risk of sepsis in preterm infants.[13] Barrier function of the skin and mucus

membranes is diminished in premature infants and is additionally compromised in ill premature infants by multiple invasive procedures, including intravenous (i.v.) access and intubation.

EONS constituted 61% of total culture positive sepsis while LONS constituted 39%. In a study done in Chennai it was found that the incidence of EOS was 20.7 per 1000 live births and it constituted 55.4% of overall sepsis.[14] In another study done in Indonesian tertiary neonatal care unit it was seen of all culture positive neonates 13 (25%) in early-onset neonatal sepsis (EONS) and 39 (75%) in late-onset neonatal sepsis (LONS).[15] More incidence of EONS in our study may be explained by more preterm neonate being affected than term neonate in this study. And EONS is more in preterm than in term. Infant factors associated with early-onset sepsis includes prematurity.[16]

Male neonates were seen to be more affected than female neonates in both EONS and LONS. These findings correspond to the male to female ratio of 1.3:1 reported by Eman et al.[17] In a study done at Bangladesh male neonates were affected more 42 (55.26%) than female 34 (44.74%).[18] Though the difference in male to female predominance in this study was not statistically significant ($p>0.05$), male predominance was found in almost all the studies of neonatal sepsis.[19-21] Increased male septicemic neonates in this study may be due to gender biasness for hospital care in Bangladesh like other developing countries. Moreover, males are more prone to infection as genetic loci on the X chromosome. Presence of one X chromosome in the male baby confers less immunological protection compared to the female counterpart.[22,23]

Sepsis screen was positive in 61% cases with CRP being positive in 70.7% cases of total culture positive neonatal sepsis. So, the sensitivity of sepsis screen and CRP in detecting true positive neonatal sepsis among all cases of culture positive sepsis was 61% and 70.7% respectively. In another study done in Pune it was seen CRP was positive in 90.7% of culture positive cases.[24]

In another study the sensitivity of CRP in diagnosis of acute neonatal sepsis was 76.92%.[25] C-reactive protein was first described by Tillet and Francis in 1930. They concluded that it is a protein that helps in complement binding to foreign or damaged cells in response to inflammation and rising to peak levels after fifty hours.[26]

Raised CRP levels are found in 50-90% of neonates from six hours of onset of bacteremia. Raised levels are not specific for bacterial infection.[27] Other conditions in which CRP levels are raised are asphyxia, shock, intraventricular haemorrhage, surgery and meconium aspiration.[28,29]

Maximum (73.2%) presented before 5 days of age. This can be explained by the fact that incidence of EONS was more in this study than LONS.

Respiratory distress, hypoglycaemia, seizures, lethargy were more common presentations in EONS while cellulitis, sclerema, septic arthritis, loose stool, vomiting, blood in stool, hyperthermia and recurrent apnoea were more common presentations in LONS. In a study done at Nigerian hospital, hypothermia, pallor, poor activity, poor suckling and respiratory distress were more commonly observed in EOS than LOS. Fever and irritability were more common in babies with LONS.[30]

Gram positive organisms constituted 51.2% of total pathogens while gram negative organisms constituted 46.3% of total pathogens causing neonatal sepsis. Among gram positive coagulase-negative staphylococcus (CONS) (43.9%) was most common while amongst gram negative *Klebsiella* (22%) was most common. Gram positive organisms constituted 75% of cases of LONS and 36% cases of EONS while gram negative organisms were responsible for 60% of cases in EONS and 25% cases of LONS. In a study done in a tertiary care hospital in China it was found the primary pathogenic microorganism of NS was gram-positive bacteria, which accounted for 48.33% and 65.98% of all infections in EONS and LONS group, respectively. CONS was the most common Gram-positive bacteria, accounting for 72.41% and 67.97% in EONS and LONS group, respectively. Gram-negative pathogens accounted for 36.67% and 29.90% of all infections in EONS and LONS group, respectively.[31]

In another study done at Cairo it was found that of the 75 organisms found, 49 (65.3%) were gram-negative and 26 (34.7%) were gram-positive.[32] The predominantly isolated strains were *Klebsiella* species (34/75, 45.3%), whereas the second-most prevalent organism was CONS (17/75, 22.7%), followed by *Acinetobacter* (8/75, 10.7%). Gram-positive organisms were significantly more prevalent in LOS than in EOS (19/26, 73.1%), specifically CONS (13/17, 76.5%), which is similar to finding in our study.

In our study the majority of sepsis-positive cases were LOS (51.2%); a comparable result (55.8%) and a higher result (71.2%) have also been reported.[33,34] This might reflect the higher incidence of community-acquired infections among neonates. The result of our study was contrast to that of another study where EOS predominated at 78.3%.[35]

Fungi constituted 9.8% of all sepsis, all fungus were found in cases of LONS. *Candida peliculosa* was the most commonly isolated fungus.

Enterococcus, Cons and Staphylococcus were seen to be 100% sensitive to linezolid. Enterococcus also showed 100% sensitivity to vancomycin, teicoplanin and tigecycline. CONS showed 100% sensitivity to linezolid and 70% sensitivity to vancomycin and teicoplanin. The gram-positive bacteria isolated in our study showed the highest sensitivity to linezolid and vancomycin, consistent with other findings.[35]

Acinetobacter baumanii showed 80% sensitivity to gentamicin, 60% sensitivity to tigecycline and colistin and 40% sensitivity to amikacin and levofloxacin. *E. coli* showed 66% sensitivity to tigecycline and 33% sensitivity to meropenam. In the study done by Mohammed et al done at Cairo, Egypt, *Acinetobacter* showed limited susceptibility (25%) to levofloxacin and ciprofloxacin, consistent with the report by Shrestha et al as well as to gentamycin and amikacin, consistent with the result of Ahmed et al.[32,36,37]

Higher sensitivity of *Acinetobacter* to gentamicin (66.7%) was reported by Sharma et al.[38] *Klebsiella* showed 100% sensitivity to amikacin. All the isolated fungi showed 100% sensitivity to voriconazole and amphotericin B.

Taking these findings together, short- and long-term strategies can be planned. Our understanding of this pattern would help us avoid using ineffective empirical choices of antibiotics. Changes should then be made based on further identification of isolated organisms and results of specific sensitivity assay. In the long term, such data can complement similar and consecutive studies in meta-analyses to further develop local, national, and international guidelines.

Limitations

The limitations of this study included the relatively small number of sepsis-positive blood cultures in addition to being a single centre study of over one year.

CONCLUSION

Bacterial prevalence and antibiotic sensitivity differed considerably among studies. Our cases showed higher prevalence of gram-positive bacteria in LOS cases, whereas gram-negative bacteria were common in EOS cases. Appropriate implementation of likely susceptible antibiotics would have a considerable impact on outcomes.

Recommendations

Longer periods of study are recommended to include more patients and to monitor changing patterns. Although each NICU needs to develop local protocols based on their specific microbial pattern, multicentre collection of data and analysis of antibiotic sensitivity pattern are suggested to help develop a national protocol for better outcomes.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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Cite this article as: Das M, Das I, Saha S, Sanghamitra S. Clinical and bacteriological profile of neonatal sepsis: a prospective hospital-based study in a tertiary care hospital in eastern zone of West Bengal. Int J Contemp Pediatr 2022;9:1056-62.