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Original Research Paper

Neonatology

RISK FACTORS, CLINICAL PROFILE AND ANTIBIOTIC SENSITIVITY PATTERN OF NEONATAL SEPSIS IN A TERTIARY CARE HOSPITAL IN NORTH INDIA

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ABSTRACT

Background: Neonatal septicaemia, an immune systemic dysregulation due to bloodstream infections, causes 99% of global neonatal mortality in the low and middle income countries (LMICs) making it a key global concern. It is a major obstacle in achieving the Sustainable Development Goal 3, which aims to reduce neonatal mortality to at least 12 per 1000 live births by 2030.Gaining insights into the complex associations of neonatal sepsis and mortality will be beneficial in guiding interventions to reduce neonatal sepsis. **Aims and Objectives:** To study the association of risk factors, profile of pathogens, their antibiotic susceptibility pattern and outcome of neonates in early onset and late onset neonatal septicaemia. **Materials and Methods:** This is a prospective observational study conducted in a tertiary care hospital from October 2022 to September 2023for a period of one year. All neonates admitted to the neonatal intensive care unit with a clinical diagnosis of suspected neonatal sepsis were assessed. After obtaining written consent from their guardians, a detailed history and relevant investigations were recorded. Blood samples (1-2ml) were collected under aseptic precautions and processed according to standard microbiological procedures for identification of the pathogen based on colony morphology and biochemical reactions. The antibiotic susceptibility was performed by modified Kirby- Bauer's disk diffusion method. **Results:** In the present study 221 Clinically Suspected Cases of Neonatal Septicaemia (CSNS) were enrolled. The primary presenting features were respiratory distress in 38.46%, n=85), lethargy in 33.03% (n=73), along with any symptom like refusal of feeds, weak cry, abdominal distension, fever, diarrhoea, vomiting, and/or jaundice. Among these CSNS, males accounted for 64.7% (n=143) and 35.3% (n=78) were females. CSNS in full-term was seen in 57.5%(n=127) while42.5% (n=94) were preterm and 52%(n=114) were Low Birth Weight (LBW) babies. Growth on blood culture was observed in 39.4% (n=87) and out of these blood culture positive cases,72% (n=63)were delivered vaginally while 28% (n=24) had caesarean section. Significant association of blood culture positivity was seen between male gender and vaginal delivery ($p < 0.05$). Gram-positive bacteria were isolated in 82.75% and Gram-negative isolates were obtained in 13.85%of culture positive cases. Most of the Gram-positive organisms were susceptible to linezolid and cotrimoxazole. Gram negative bacilli were found to be 100% sensitive to gentamicin and imipenem.

KEYWORDS : Neonatal sepsis, blood culture, bacteriological profile, antibiotic susceptibility pattern, neonatal mortality

INTRODUCTION

Neonatal mortality accounts for 2.3 million neonatal deaths annually.[1] The Sustainable Development Goal (SDG) number 3, aims to reduce neonatal mortality rate to 12 or fewer deaths per 1,000 live births by 2030which would mean saving almost 1 million newborn lives every year.[2,3]In India, the present prevalence of neonatal sepsis is about 17,000/1,00,000 live birth[4]while the case fatality rate ranges from 25% to 60%[5]and bacterial causes being mainly responsible for neonatal septicemia.[6]Hence, understanding the complex associations of risk factors, bacterial profile and antibiotic sensitivity pattern of neonatal sepsis is of critical importance to formulate steps towards achieving the SDG goal 3.

Neonatal sepsis represents a clinical syndrome characterized by signs and symptoms of infection which includes temperature instability, lethargy, poor cry, and poor feeding with or without bacteraemia in the first 28 days of life.[1,5] It encompasses various systemic infections of the new born such as septicaemia, meningitis, pneumonia, arthritis, osteomyelitis, and urinary tract infections.[6]However, for LMICs an acceptable case definition is still lacking due to differences in laboratory setups and epidemiology.[7]

Neonatal sepsis is categorised into two groups based on the time of presentation after birth. In Early Onset Neonatal Sepsis (EONS) the neonates typically present with symptoms during their first 3 days while Late Onset Neonatal sepsis (LONS), refers to sepsis presenting on the 3rd day or after 3 days.[8]EONS results from vertical transmission from mother to infant occurring in the perinatal period (during or soon after delivery). The infection occurs in-utero by transplacental transmission or more commonly ascending from the vaginal environment after rupture of membranes or examination during labour. Various studies suggest that EONS also correlates to gut microbiome colonisation.[9]The new-borns become infected when exposed to pathogenic bacteria, viruses or fungi as they pass through the birth canal.[8]LONS infection occurs after delivery following interactions with the hospital or community environment and usually starts after 72 hours of age. The source of infection is either nosocomial or from the community which includes the health care workers and/or care givers.

The estimates of laboratory-confirmed neonatal sepsis in high-income countries are documented, the overall incidence being 0.5-0.9/1000 live births.[10]However, in India and LMICs the data is scarce. The exact incidence and associations are not robust enough to depict the true burden of neonatal sepsis. Apart from the variability in clinical and microbiological parameters the emergence of regional multi-drug resistant bacteria also influences the true incidence of neonatal septicaemia.

Objectives:
To study the association of risk factors, profile of pathogens, their antibiotic susceptibility pattern, outcome of neonates in early onset and late onset neonatal sepsis.

MATERIAL AND METHOD

This is a prospective study conducted in a tertiary care hospital in the Department of Microbiology in Northern India for a period of one year from October 2022 to September 2023. All the neonates admitted to the NICU based on inclusion and exclusion criteria were enrolled for the study and categorised according to standard definition of EONS and LONS.[11]

Inclusion Criteria:
All neonates more than 28 weeks of gestation clinically suspected of septicaemia were included in the study. The clinical features included i) temperature instability of 99 C ii) respiratory rate more than 60 per minute iii)grunting, flaring or use of accessory muscles and apnoea iv) abnormal cry and not accepting feed v) drowsiness, irritability or seizures vi) diarrhoea and vii) convulsions.

Exclusion Criteria
Neonates who had i) received previous antibiotic treatment ii) severe birth asphyxia iii) prolonged resuscitation at birth or iv) been given intrapartum antibiotic prophylaxis were excluded from the study.

After obtaining written consent from their guardians, detailed history and relevant investigations were recorded and Blood sample (1–2 ml) was collected under all aseptic precautions from these CSNS neonates. The blood was inoculated directly into brain heart infusion broth containing blood in a ratio of 1:5. The inoculated bottles were incubated at 37 C and examined after 24 hours. The first subculture was performed on Blood agar and MacConkey Agar. A preliminary gram staining was done after the first subculture. A final subculture on blood agar media was carried on seventh day along with the gram Staining. The blood culture was considered negative if no growth was observed on the final subculture. The organism was identified based on the colony morphology, gram staining and biochemical reactions.

Antibiotic susceptibility testing of bacterialisolates was performed by modified Kirby-Bauer disk diffusion method. A predetermined battery of organism specific antimicrobial discs was put up and zone of inhibition interpreted according to according to Clinical and Laboratory StandardsInstitute (CLSI) guidelines 2023.[12]

Statistical Analysis
All the data were collected,analysed on SPSS software 16.0. A p-value of ≤ 0.05 wasconsidered to be statistically significant.

RESULTS

A total of 5018delivieswere conducted in the hospital during the study period, out of which424neonateswere admitted to NICU. Among these 221 neonates suspected of neonatal sepsis,the presenting features were respiratory distressin 38.46%, n=85), lethargy(33.03%, n=73), poor cry and fever. Other symptoms noted were refusal of feeds, abdominal distension, diarrhoea, vomiting, and jaundice. Out of all CSNS,64.7% (n=143) were males and 35.3% (n=78) were females. CSNS with vaginal deliveries were recorded in 68% (n=151) including 6 home deliveries, while in 30% (n=67) LSCS was performed. In our study, EONSwas documented in 70.1% (n=155)as compared to LONS 29.9% (n=66)which was found to be statistically significant ($p < 0.05$). Amongst these CSNS cases, 42.5% (n=94) were preterm and 57.5% (n=127) were full-term. According to weight distribution, 51.58% (n=114) neonates were low birth weight (< 2.5 Kg)and 94 neonates were preterm (< 37 weeks of age) as depicted in Table 1.

Association with positive blood culture was seen in 39.4% (n=87) of CSNS cases of which 64% (n=56) were male neonates and 72% (n=63) neonates were delivered vaginally showing significant association between male gender, vaginal delivery with blood culture positivity ($p< 0.05$) as shown in Table2.

Gram-positive organisms were isolated commonly 82.75% (n= 72) as compared to Gram-negative organisms, accounting for only 13.80% (n=12) of cases. Candida species were isolated in 3.45%of cases. Among the Gram-positive isolates, Staphylococcus aureus was the most predominant bacteria, representing 69% of the isolates, followed by Coagulase-negative Staphylococci(CoNS) in 12.66%and is represented in Table 3.

Out of 221 clinically suspected cases of neonatal septicaemia,87.79% neonates recovered while 5.43% (n=12) neonates expired while 6.78% required referral to higher centres. Out of the 12expired neonates, three were blood culture positive, showing growth ofS. aureus, Klebsiella sp. and E. Coli while in others congenital anomaly and other conditions were associated.

Table1: Socio Demographic Profile Of The Neonates With Septicaemia (n=221)

S.No	Parameters	Number & percentage (%)
1.	Onset of sepsis	
	i. Early onset neonatal sepsis	155 (70.1%)
	ii. Late onset neonatal sepsis	66 (29.9%)
2.	Mode of delivery	
	i. Normal vaginal delivery	151 (68.33%)
	ii. LSCS	67 (30.32%)
	iii. Assisted deliveries	3 (1.35%)
3.	Clinical presentation	
	i. Poor cry	43 (19.45%)
	ii. Fever	43 (19.45%)
	iii. Respiratory distress	85 (38.46%)
	iv. Lethargy	73 (33.03%)
4.	Birth weight	
	i. 1000-1499 gm	22 (9.95%)
	ii. 1500-1999 gm	36 (16.28%)
	iii. 2000-2499 gm	56 (25.3%)
	iv. > 2500 gm	107(48.4%)

Table 2: Association Of Gender With Positive Blood Culture (n=221)

S.No	Gender	Blood culture Positive	Blood culture Negative	Total
1	Male	56	87	143
2	Female	31	47	78
3	Total	87	134	221

Table 3: Pathogens Isolated From Positive Blood Cultures (n=87)

S.No	Pathogensisolated	Number & percentage(%)
1.	i. Bacterial	
	a. Gram positive	72 (82.75%)
	b. Gram negative	12(13.80%)
2.	ii. Fungal	
	a. Candida species	3 (3.45%)

In both groups, EONS and LONS, Gram positive organisms were isolated more often. Among the EONS, 58.62% (n=51) were associated with positive blood culture out of which gram-positive organisms were isolated in 78.43% (n=40)while in LONS gram positive isolates were found in 88.88 %(n=32) cases. The gram positive bacteria mainly isolated were S.aureus, CoNS and Enterococcusspp while the Gram negative bacteria includedE. coli, Klebsiella spp., Achromobacters were isolated. The detailed distribution is shown in Table 4.

Table 4: Pathogen Isolated In EONS And LONS

Pathogen isolates	EONS	LONS	Total (n)
Staphylococcus aureus	32	28	60
CoNS	7	4	11
Klebsiella sp	4	0	4
Achromobacter	1	0	1
E. coli	1	1	2
Enterococcus	1	0	1
SphingomonasPaucimarbilis	0	1	1
Others (Non fermenter group of organisms)	3	1	4
Candida	2	1	3

Most of the Gram-positive organisms were susceptible to linezolid and cotrimoxazole. Gram negative bacilli were found to be 100% sensitive to gentamicin and imipenem.[12,13] (Table 5 & 6)

Table 5:antibiotic Sensitivity Pattern For Gram Positive Organism

Antibiotic	Sensitivity Pattern	Percentage (%)
Co-trimoxazole	21	52.5
Clindamycin	8	20
Linezolid	34	85
Rifampicin	3	100
Teicoplanin	3	100
Tetracycline	4	100
Vancomycin	6	100

Table 6: Sensitivity Pattern For Gram Negative Organism

Antibiotic	Sensitivity of isolates	Percentage (%)
Ampicillin	5	100
Gentamicin	4	100
Imipenem	3	42.85
Tobramycin	2	28

DISCUSSION

Neonatal septicaemia is a major cause of death in LMICs and with the emergence of antibiotic resistance the treatment has become more complicated. This is the first study from our tertiary care hospital that depicts the bacterial profile along with the antimicrobial sensitivity pattern, risk factors and clinical characteristics of neonatal sepsis. Our study has shown that, out of 221 neonates, 70.1% had EONS and 29.9% developed late onset neonatal sepsis and among these 87 neonates were diagnosed with bacteriologically confirmed neonatal sepsis. Culture positive EONS and LONS was documented in 51 and 36 cases respectively. Similar findings have been noted in a study from Bangladesh by Hafsa et al. (2011), where out of 104 culture positive neonates, EONS accounted for 65.38% and LONS accounted for 34.62% cases.[19,20] Comparable findings have been shown in a study conducted in Babylon where 58% neonates were presented with EONS and 42 % neonates were presented with LONS.[21]

Our study also shows an increased incidence of CSNS in male neonates (65%),consistent with studies showing male preponderance ranging from 65% to 74% positive blood cultures.[14]The mechanisms attributed to male preponderance may be linked to inherent biological difference in the regulation of gamma globulin synthesis, which is likely to be situated on the X chromosome. As males possess only one X chromosome, immunological protection may be diminished as compared to females who have two X chromosomes.[22]

A total of 67 neonates were delivered by LSCS. Among these blood cultures were positive in 35.82%(n=24) neonates, and in these 22.38%(n=15) were preterm neonates and 13.43%(n=9) were low birth weight babies indicating that both preterm and low birth weight could be the risk factors for development of neonatal sepsis[15].Similar results have been reported by Thakur S et al. stating challenging deliveries encompassing caesarean sections, forceps deliveries, or vacuum-assisted deliveries, are significant risk factors to develop CSNS, constituting 32% of cases in their study[16]. Macdorman et al. has also reported a heightened incidence of sepsis among neonates born via Caesarean Section in comparison to those delivered vaginally. [17]

In our study neonates born vaginally have been at risk to develop CSNS. Multidrug resistant bacteria have emerged and imposed challenges in treatment of neonatal sepsis. Knowledge of prevalence of local isolates and their antimicrobial sensitivity pattern is of utmost importance for appropriate antimicrobial therapy and management of neonatal sepsis. In the present study, out of 221 cases studied, 87 were culture positive, resulting in a positivity rate of 39.17%.[18]The most common organism isolated was Staphylococcus aureus (37.22%) followed by Klebsiella pneumoniae (27.01%) and Escherichia coli (19.70%)These findings were comparable with studies conducted by other authors, highlighting a culture positivity rate ranging from 25% to 42% in their study.[22]In the present study, Gram-positive organisms constituted the majority of the isolates, with a prevalence of 82.75% as compared to Gram-negative organisms, which accounted for 13.80% of cases. Fungi were isolated in 3.45% of cases. Among the Gram-positive isolates, Staphylococcus aureus was the most predominant, representing 69% of the isolates, followed by Coagulase-negative Staphylococci (CoNS) in 12.66%. These findings align with observations reported in other studies, indicating a consistent prevalence of Gram-positive organisms, particularly Staphylococcus aureus.[16,23,24]The gram-positive organisms produce more inflammation-causing cell wall constituents, as well as unbound exotoxins resulting in septicaemia.[26]Our study depicted that Klebsiella pneumoniae emerged as the predominant Gram-negative isolate, followed closely by Escherichia coli and consistent with other studies.[25]

Staphylococcus aureus exhibited varying degrees of sensitivity to different antibiotics. The maximum sensitivity was observed with linezolid in 54 (100%), followed by cotrimoxazole in 34 (65.38%), minocycline in 36 (100%), penicillin in 6 (13.63%), quinupristin in 1(100%), erythromycin in 8(14.28%), vancomycin in 7(100%), clindamycin in 18(39.13%), doxycycline in 2(100%), whereas one isolateshowed 100% sensitivity to ciprofloxacin, mupirocin, rifampicin, teicoplanin and tobramycin.

CONCLUSION

This is first study from our institution which provides valuable insights specifying important risk factors and bacteriological profile that affect outcomes of neonatal sepsis.The study has been of immense help to provideadvice to formulate the antibiotic policy in our institution.However,multicentric researchis mandatedto enable policy makers to draft preventive strategies and the clinicians to treat neonates with septicaemia well in time.

Ethical Approval Statement

Ethical approval (No HFW11/SLBSGMC/IEC 2018/19, Protocol -NO 21/2022) was obtained from the Institutional Ethics Committee notified by Government of India, Ministry of Health and Family Welfare, Department of Health Research (National Ethics Committee Registry for Biomedical and Health Research). All data were collected and kept confidential.

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