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Original Research Article

Bacterial etiology and antimicrobial susceptibility pattern of neonatal sepsis at a tertiary care hospital in Nepal

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ABSTRACT

Background: Sepsis is the second major cause of mortality among neonates. Present study was done to identify the common organisms which cause early and late onset neonatal sepsis in neonates admitted in our department and their antibiotic sensitivity patterns.
Methods: All neonates weighing more than 1500 gms and born to mothers with pre-existing infection, admitted to neonatal intensive care unit for suspected neonatal sepsis were included in the present study. They underwent blood culture and antibiotic sensitivity profiling.
Results: 210 newborns were admitted to the NICU of our department for suspected neonatal sepsis. Longer duration of rupture of membranes was found to be significantly associated with growth of organisms. Amongst the cases with gram positive organisms, most were due to Coagulase-negative staphylococci (CoNS) (n=25), followed by Staphylococcus aureus (n=14), and, Enterococcus (n=4). Gram negative organisms isolated constituted 17 organisms. Amongst the cases with gram negative organisms, mostly were due to Klebsiella (n=10), followed by Pseudomonas (n=5) and E. coli (n=2). The most common organism causing early onset sepsis was CoNS, while Staphylococcus aureus was the most common organism causing late onset sepsis. CoNS was fully sensitive to Vancomycin and Amikacin. Staphylococcus aureus was fully sensitive to Amikacin, α-hemolytic. Streptococcus were sensitive to Amikacin, Vancomycin and Piperacillin and Tazobactum and Enterococcus was sensitive to Amikacin and Vancomycin.
Conclusions: Antimicrobial surveillance of neonatal septicaemia is required to know the antibiotic sensitivity pattern and thus to formulate policies on use of antibiotics and to know the changing spectrum of antimicrobial sensitivity patterns.

Keywords: Blood culture, Sepsis, Surveillance, Neonates

INTRODUCTION

Sepsis is the second most common cause of neonatal deaths, accounting for higher than a million deaths every years.[1] Neonatal sepsis, pneumonia and meningitis add up to one fourths of all neonatal deaths.[2] Under the umbrella of neonatal sepsis, various clinical conditions are included like septicemia, pneumonia, meningitis, and urinary tract infections.[3] Despite such a high burden of cases, an expert consensus case definition has not been developed yet.[4] Depending on the age of the affected neonate, sepsis is classified as either early or late onset sepsis. When sepsis occurs within first 72 hours of birth, it is called early onset sepsis. Various epidemiological factors associated with maternal genital tract are usually responsible for early onset sepsis. When sepsis presents after 72 hours of birth,

it is termed as late onset sepsis (LOS). In these cases, there are numerous other factors are causative, which could be community acquired or nosocomial.

In the recent years, indiscriminate use of broad spectrum antibiotics has caused the development of bacterial strains which are resistant. This has two fold disadvantage. One for the treating physician, which are finding it more and more difficult to treat neonatal sepsis due to antibiotic resistance. Second, antibiotic resistance is causing longer hospital stays, need for more expensive antibiotics and progression to complications which need interventions. This has resulted in higher health related costs for the patients.[5] Thus, clinically it makes sense to study antibiotic sensitivity patterns in a hospital setting, especially when the pattern evolves with time. The information obtained from antibiotic surveillance studies can inform the clinicians in making treatment related decisions. The present study was done to identify the common organisms which cause early and late onset neonatal sepsis in neonates admitted in our department and their antibiotic sensitivity patterns.

METHODS

Study design and sample population

This cross-sectional observational study was conducted in the Neonatal Intensive Care Unit (NICU), Department of Pediatrics at Lumbini Medical College and Teaching Hospital, Nepal from June 2016 till May 2018. All neonates, up to 28 days of life, weighing more than 1500 gms and born to mothers with pre-existing infection, admitted to NICU for suspected neonatal sepsis were included in the present study. Babies who have undergone recent surgical interventions and those with congenital anomaly rendering them easily susceptible to infections such as; cystic fibrosis, down's syndrome, et were excluded from the study. The research protocol was submitted and approved by the Institutional review Committee of Lumbini Medical College and Teaching Hospital, Nepal.

Blood sampling and antibiotic susceptibility testing

Two ml of blood was drawn aseptically before starting antimicrobial therapy and directly inoculated into Brain Heart Infusion (BHI) broth in a ratio of blood : BHI of 1:5. The blood culture bottles were immediately sent to the microbiology laboratory and incubated at 37∘C for 24 hrs and sub-cultured on McConkey agar, blood agar, and chocolate agar daily for 7 days. The inoculated McConkey agar plates were incubated aerobically, whereas blood agar and chocolate agar plates were incubated in CO2 enriched humid atmosphere using candle jar, at 37°C for 24-48 hours. Blood culture bottles showing no growth on subculture done after incubation of 7 days were reported as negative. All the collected blood samples were processed for culture and isolation by standard microbiological methods.[6]

The antimicrobial susceptibility testing was done by Kirby-Bauer disc diffusion method as recommended by Clinical Laboratory Standard Institute (CLSI) guidelines.[7] Antibiotic disks used were ampicillin/sulbactam (10/10 μg), amikacin (30 μg), ceftriaxone (30 μg), cefotaxime (30 μg), cotrimoxazole (25 μg), clindamycin (2 μg), cefoxitin (30 μg), cefixime (5 μg), cloxacillin (5 μg), erythromycin (15 μg), gentamicin (10 μg), meropenem (10 μg), nalidixic acid (10 μg), ofloxacin (5 μg), piperacillin/tazobactam (100/10 μg), teicoplanin (30 μg), and vancomycin (30 μg). For quality control of biochemical tests, purity plate was used. Similarly, for quality control of antimicrobial susceptibility testing, E. coli ATCC 25922 and S. aureus ATCC 25923 were used.

Data collection and data analysis

Using a pre-designed semi-structured study proforma, a thorough and detailed history was taken including maternal history, maternal use of any drugs during the period of pregnancy, intrapartum use of any antibiotics. Physical examination with investigations by the resident/doctor and obtained findings was recorded as well. The demographic information captured included patients' gender, race and gestational age. Limited clinical data obtained included birth weight, gestational age, mode of delivery, place of birth, maternal booking status and HIV results. A list of positive blood cultures done on neonates admitted in NICU during the study period was obtained from Central Laboratory of our hospital by the resident/doctor. Later the antibiotic sensitivity and resistance for the given organism were followed.

The data were described as means and standard deviation for quantitative data and as frequency and percentages for qualitative data. Student's t test was used for comparing means and chi-square was used to compare percentages. The analysis was done in SPSS version 23 and a p value of less than 0.05 was considered as statistically significant.

RESULTS

During the study period, 210 newborns were admitted to the NICU of our department for suspected neonatal sepsis. Of these neonates, 74% were in the early neonatal period (≤7 days of age) and rest were in the late neonatal period (>7 days of age). Of our study population, 59% were females. We found that 2% of the neonates had very low birth weight (<1500 gm), 31% had low birth weight (1500 to 2500 gm) and rest had birth weight of more than 2500 gm (Table 1).

Normal vaginal delivery took place in 77% of the cases, LSCS in 17% and rest had vacuum assisted delivery. Gestational age was <37 completed weeks in 9% of the cases (preterm), and the rest of newborns were born term. Early onset sepsis was diagnosed in 59% of the neonates. Rupture of membrane information was obtained for 119 neonates (Table 2).

Table 1: Baseline characteristics of the neonates and their mothers.

Variables	N	Percent (%)
Age group of neonates		
≤7 days (early neonatal period)	156	74
>7 (late neonatal period)	54	26
Gender		
Female	124	59
Male	86	41
Birth weight (gm)		
<1500	4	2
>1500 to 2500	66	31
>2500	140	67
Maternal age (years)		
<18	10	5
>18 to 22	86	41
>22 to 26	82	39
>26	32	15
Mode of delivery		
Normal vaginal delivery	162	77
Lower section caesarean section	35	17
Vacuum	13	6
Gestational age		
<37 completed weeks	18	9
37 to 41 completed weeks	192	91
≥42 weeks	0	0
Onset of sepsis		
Early onset	124	59
Late onset	86	41

Among them 43 had growth of organism and rest did not. Among those who had growth, 60% had rupture of membrane for 8 to 24 hours and 35% for more than 24 hours. While among those without growth, 36% had less than 8 hours of rupture of membranes and 30% had it for 8 to 24 hours. Longer duration of rupture of membranes was found to be significantly associated with growth of organism ($p<0.001$). Mean I/T ratio was found to be significantly associated with the growth of organisms ($p<0.01$). Reactive CRP was found in 53% in those who had growth as compared to 22% in those who did not have growth ($p<0.001$). There were 62 microorganisms isolate on blood culture of 210 patients accounting for 29.5% of culture proven sepsis (Table 3). Amongst the cases with gram positive organisms, most were due to Coagulase-negative staphylococci (n=25), followed by Staphylococcus aureus (n=14), and, Enterococcus (n=4). Gram negative organisms isolated constituted 17 organisms. Amongst the cases with gram negative organisms, mostly were due to Klebsiella (n=10), followed by Pseudomonas (n=5) and E. coli (n=2). The most common organism causing early onset sepsis was Coagulase-negative staphylococci, while Staphylococcus aureus was the most common organism causing late onset sepsis (Table 4).

Table 2: Comparison of neonates with and without growth of organisms.

	Growth of organism	No. growth of organism	P value
Rupture of membrane			
<8 hours	2 (5%)	27 (36%)	<0.001
8 to 24 hours	26 (60%)	23 (30%)	
More than 24 hours	15 (35%)	26 (34%)	
Total	43	76	
Mean I/T ratio (n=133)	0.185	0.1	<0.01
Total	80	53	
CRP			
Reactive	33 (53%)	33 (22%)	<0.001
Non-reactive	29 (47%)	115 (78%)	
Total	62	148	

The antibiotic sensitivity pattern is described in table 5. All organisms isolated in our population were resistant to Ampicillin, Cefixime and Amoxycillin. Coagulase-negative staphylococci was fully sensitive to Vancomycin and Amikacin. Staphylococcus aureus was fully sensitive to Amikacin, α-hemolytic Streptococcus were sensitive to Amikacin, Vancomycin and Piperacillin and Tazobactum and Enterococcus isolated were sensitive to Amikacin and Vancomycin. All gram-negative organisms were resistant to Ampicillin, Cefixime and Amoxycillin.

Table 3: Bacteriological spectrum with the age of the neonates.

Age of the neonate	α-hemolytic Streptococcus	Coagulase-negative staphylococci	Enterococcus	E. coli	Klebsiella	Pseudomonas	Staph. aureus
≤7 days (n=156)	2	24	0	2	7	5	6
>7 (n=54)	0	1	4	0	3	0	8

Table 4: Bacteriological spectrum with the onset of sepsis.

	Early onset sepsis (n=124)		Late onset sepsis (n=86)	
	N	Percent (%)	N	Percent (%)
Gram positive				
Coagulase-negative staphylococci	19	15	6	7
Staphylococcus aureus	4	3	10	12
α-hemolytic Streptococcus	2	2	0	0
Enterococcus	0	0	4	5
Gram negative				
Klebsiella	7	6	3	3
Pseudomonas	5	4	0	0
E. coli	2	2	0	0

Table 5: Antimicrobial susceptibility profile of the organisms isolated in the neonates.

	HS			CoNS			SA			Ent			Ec			Kleb			PDM		
Antibiotic	**R**	**I**	**S**	**R**	**I**	**S**	**R**	**I**	**S**	**R**	**I**	**S**	**R**	**I**	**S**	**R**	**I**	**S**	**R**	**I**	**S**
Ampicillin	2	0	0	25	0	0	14	0	0	4	0	0	2	0	0	10	0	0	5	0	0
Cefotaxim	1	1	0	2	10	13	0	6	8	2	2	0	2	0	0	10	0	0	5	0	0
Gentamicin	0	0	2	2	2	21	0	5	9	1	1	2	1	0	1	3	3	4	0	3	2
Amikacin	0	0	2	0	0	25	0	0	14	0	0	4	0	0	2	0	0	10	0	0	5
Cephalexin	2	0	0	25	0	0	12	0	2	4	0	0	2	0	0	10	0	0	5	0	0
Cefixime	2	0	0	25	0	0	14	0	0	4	0	0	2	0	0	10	0	0	5	0	0
Amoxycillin	2	0	0	25	0	0	14	0	0	4	0	0	2	0	0	10	0	0	5	0	0
Ceftazidime	0	1	1	0	7	18	o	5	9	1	1	2	0	2	0	4	4	2	0	4	1
Ceftriaxone	2	0	0	18	6	1	13	1	0	4	0	0	2	0	0	10	0	0	5	0	0
Imipenem	0	2	0	0	7	18	0	3	11	0	2	2	0	0	2	0	0	10	0	0	5
Vancomycin	0	0	2	0	0	25	0	2	12	0	0	4	2	0	0	4	5	1	5	0	0
Piperacillin+tazo	0	0	2	0	7	18	0	11	3	1	1	2	0	2	0	4	5	1	0	5	0
Ciprofloxacin	0	0	2	1	16	8	3	5	6	0	3	1	0	1	1	2	3	5	0	3	2
Cefoperazone	0	0	2	7	9	9	8	3	3	0	2	2	2	0	0	8	0	2	3	1	1

HS: α-hemolytic Streptococcus; CoNS: Coagulase-negative staphylococci; SA: Staphylococcus aureus; Ent: Enterococcus; Ec: E. coli; Kleb: Klebsiella; PDM: Pseudomonas

DISCUSSION

The present study describes the risk factors which were associated with the development of neonatal sepsis. We observed that the of duration of rupture of membranes was significantly associated with the growth of the organisms. Neonates with growth of organisms had a higher mean I/T ratio and higher proportion with reactive CRP. In addition, early onset sepsis was diagnosed in 59% of the neonates. It could be because of the immature immunologic response of neonates in the first week of life, making them more susceptible to infection in this period. Lamba et al reported that 61.41% had early onset sepsis while the rest had late onset sepsis.[8] They also observed that Of the various maternal risk factors affecting neonatal septicaemia, rupture of membranes for more than 18 hours was the commonest factor with 21.85% cases.

We observed that 29.5% of the neonates had culture proven sepsis. Lamba et al reported culture positivity in 37.8% of the neonates included in their study. In our study, coagulase-negative staphylococci were the most common gram-positive organism causing early onset sepsis, while Klebsiella was the most common gram-negative bacteria.[10]

For late onset sepsis, Staphylococcus aureus was the most common gram-positive bacteria, while Klebsiella was the only gram-negative organism. CoNS is normally considered as a skin contaminant when isolated from blood. The presence of this bacterium in blood in critically ill babies, should be considered as significant and should be treated, as the clinical manifestation of CoNS sepsis can be varied. In our study, contamination with skin flora is improbable since adequate precautions were taken while collecting the sample. Jajoo et al reported that Klebsiella pneumoniae, Staphylococcus aureus, and Escherichia coli were the common causes of early onset sepsis.[9] Lamba et al observed that coagulase negative Staphylococci was the commonest bacterial isolate followed by Klebsiella spp. Thapa et al in a similar study reported the predominant isolate in early onset sepsis to be Acinetobacter species, while it was Staphylococcus aureus for late onset sepsis.[10]

We observed that all organisms were resistant to Ampicillin, Cefixime and Amoxycillin. Coagulase-negative staphylococci was fully sensitive to Vancomycin and Amikacin. Staphylococcus aureus was fully sensitive to Amikacin, α-hemolytic Streptococcus were sensitive to Amikacin, Vancomycin and Piperacillin and Tazobactum

and Enterococcus isolated were sensitive to Amikacin and Vancomycin. All gram-negative organisms were resistant to Ampicillin, Cefixime and Amoxycillin. In the study by Jajoo et al, all gram-negative bacteria isolates were found to be susceptible to meropenam (80%) and piperacillin-tazobactum (75%) followed by cefotaxime (50%), imipenem (50%) and chloramphenicol (50%) while all resistant to ofloxacin and least sensitive to amikacin (25%). In the study by Lamba et al, gram positive organisms showed highest sensitivity to linezolid (97.15%) followed by vancomycin (95.23%), and teicoplanin (88.57%) and least sensitivity to ceftazidime (14.28%). Gram negative organisms showed good sensitivity to Colistin, imipenem and Meropenam that is 89.89%, 86.43%, and 77.88% respectively. Cephalosporins showed poor sensitivity. In the study by Thapa et al, S. aureus showed highest rate of susceptibility towards vancomycin, amikacin, teicoplanin, meropenem, cotrimoxazole, clindamycin, erythromycin, and ofloxacin, while Enterobacteriaceae showed highest susceptibility towards amikacin, piperacillin/tazobactam, ampicillin/sulbactam, meropenem, ofloxacin, and gentamicin.

There are a few limitations of the study. This was a single centre study. The bacteriological profile of neonatal sepsis can vary, and so can the antibiotic sensitivity. Thus the results of the present study may not be generalizable to other hospitals. Second, detailed medical history of the mothers was not obtained, which could have affected the spectrum of organisms involved and their antibiotic sensitivity.

CONCLUSION

Based on the results of the present study, we can conclude that Coagulase negative Staphylococci is the predominant isolate among Gram positive organisms. Resistance to antibiotics is a global concern. Antimicrobial surveillance of neonatal septicaemia is required to know the antibiotic sensitivity pattern. This would enable the hospital administration to formulate policies on use of antibiotics and to know the changing spectrum of antimicrobial sensitivity patterns.

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REFERENCES

1. Abubakar I, Tillmann T, Banerjee A. Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990–2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet. 2015;385(9963):117-171.
2. Chan GJ, Lee AC, Baqui AH, Tan J, Black RE. Risk of early-onset neonatal infection with maternal infection or colonization: a global systematic review and meta-analysis. PLoS Med. 2013;10(8): e1001502.
3. Aggarwal R, Sarkar N, Deorari AK, Paul VK. Sepsis in the newborn. Indian J Pediatr. 2001;68(12):1143-1147.
4. Schlapbach LJ, Kissoon N. Defining pediatric sepsis. JAMA Pediatr. 2018;172(4):312-314.
5. Karki S, Rai GK, Manandhar R. Bacteriological Analysis and Antibiotic Sensitivity Pattern of Blood Culture Isolates in Kanti Children Hospital. J Nepal Paediatr Soc. 2010;30:94-97.
6. Winn Jr W. Konemann's color atlas and diagnostic text of microbiology. Lippencott Williams & Wilkins Publishers, Philadelphia, PA, Edition. 2006;6:945-1021.
7. CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 27th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute. 2017.
8. Lamba M, Sharma R, Sharma D, Choudhary M, Maheshwari RK. Bacteriological spectrum and antimicrobial susceptibility pattern of neonatal septicaemia in a tertiary care hospital of North India. The Journal of Maternal-Fetal & Neonatal Medicine. 2016;29(24):3993-8.
9. Jajoo M, Kapoor K, Garg LK, Manchanda V, Mittal SK. To study the incidence and risk factors of early onset neonatal sepsis in an out born neonatal intensive care unit of India. J Clin Neonatol. 2015;4:91-5.
10. Thapa S, Sapkota LB. Changing Trend of Neonatal Septicemia and Antibiotic Susceptibility Pattern of Isolates in Nepal. International journal of pediatrics. 2019;3784529.-5.

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