Original Research Article Journal of College of Medical Sciences-Nepal, Vol-15, No 1, Jan-Mar 2019

ISSN: 2091-0657 (Print); 2091-0673 (Online) **Open Access**

Bacteriological Profile of Neonatal Sepsis in a Neonatal Intensive Care Unit of a Tertiary Care Hospital of Eastern Nepal

Sunil Kumar Yadav,[1] Arun Giri[1]

[1]Department of Pediatrics and Neonatology, Nobel Medical College Teaching Hospital, Biratnagar

ABSTRACT

Background: Neonatal sepsis is a common and serious problem of neonates who are admitted for intensive care. It is a leading cause of neonatal morbidity and mortality worldwide. The objective of the study was to detect the common causative microorganisms of neonatal sepsis and their antibiotic susceptibility patterns in NICU of Nobel Medical College Teaching Hospital (NMCTH). **Methods:** This was a cross-sectional study conducted in a 17- bedded teaching and referral NICU of NMCTH from March to August, 2018. All neonates of clinical sepsis were enrolled in the study, blood cultures taken and were followed up till final outcome, which was discharge or death, irrespective of culture report. Descriptive statistics including percentages and frequencies was used.complications. **Results:** Among the 55 neonates with diagnosis of clinical sepsis, 13(23.6%) had shown bacteria in the culture. The predominant organisms were *Staphylococcus aureus and Klebsiella pneumoniae* and most of them were resistant to Ampicillin and Amikacin. **Conclusions:** The culture positivity rate among the neonates with clinical sepsis in the study was 23.6%. Pathogens isolated were resistant to the first line drugs for management of neonatal sepsis. Hence, the need for a review of first line drug for empirical treatment of neonatal sepsis.

Keywords: antibiotic susceptibility; neonatal intensive care unit; neonatal sepsis.

INTRODUCTION

Neonatal sepsis refers to a clinical syndrome that is marked by signs and symptoms of infection in the first 28 days of life, with or without isolation of a pathogen.[1] Neonatal sepsis is a leading cause of neonatal mortality in the world. [2] It is also one of the common morbidities in neonatal units in Nepal and India.[3, 4] Majority of neonatal sepsis occurs in developing countries.[5]

Neonatal sepsis can be categorized as early onset sepsis (EOS) and late onset sepsis (LOS). EOS is defined as onset of signs and symptoms of infection within 72 hr of life and may be associated with pathogen isolation or not. In the LOS, signs and symptoms present after 72 hr of life [6] and categorization of EOS and LOS is to show the varying causes and pathophysiology of common isolates related to the time of onset of the condition. The bacterial agents associated with neonatal sepsis are Group B Streptococci, Escherichia coli, Listeria monocytogenes, coagulase-negative Staphylococci (CoNS), Staphylococcus aureus, Klebsiella spp., Enterobacter spp., Pseudomonas spp., and Streptococcus pneumonia.[7-9] In developing countries, unsafe birthing practices have critical role to cause neonatal infections.

Globally, the neonatal morbidity and mortality cases have been estimated to 2.5–3 million, annually. [10] Neonatal mortality rate (NMR) distribution disparities can be seen based on socioeconomic, educational and geographical parameters. In Nepal, neonatal mortality has been found due to sepsis and emergence of drug resistant bacteria. According to Nepal Demographic and Health Survey(2011), 85% of total death is accounted to neonatal sepsis which is higher than previous surveys, 70% in 2006 and 69% in 2001. [11] NMR is higher in rural areas (34 per 1000 live births) than in urban areas (23 per 1000 live births). Currently, emergence of multidrug resistant bacteria imposes challenges in treatment of neonatal sepsis.[12, 13] Therefore, the knowledge of prevalence of local isolates and their antimicrobial sensitivity pattern is of utmost necessary for prompt antimicrobial therapy of neonatal sepsis.

Correspondence: Dr. Sunil Kumar Yadav, Department of Pediatrics and Neonatology, Nobel Medical College Teaching Hospital, Biratnagar, Nepal. **Email:** dr.sunil_yadav@yahoo.com. **Phone:** +977-9817930222 . **Orcid ID:** http://orcid.org/0000-0002-6584-3768. **DOI:** 10.3126/jcmsn.v15i1.22351. **Article received:**2018-07-17. **Article accepted:** 2019-02-18.

This study aims to determine bacteriological profile of neonatal sepsis along with antibiotic susceptibility pattern of the isolates from neonates admitted in neonatal intensive care unit of Nobel Medical College Teaching Hospital (NMCTH), Biratnagar.

METHODS

This was a hospital based cross-sectional study conducted in a 17- bedded teaching and referral NICU of NMCTH from March to August, 2018. Neonates (0–28 days) admitted to this newborn care unit from March to August, 2018, with a diagnosis of clinical sepsis were studied. Out born babies and babies with gross congenital anomalies were excluded. Written informed consent was taken.Neonates with one or more of the following risk factors: maternal fever, prolonged rupture of the membranes for more than 24 h, foul-smelling or meconium-stained liquor, or frequent (>3) unclean vaginal examinations, and/or having severe prematurity, or birth asphyxia necessitating active resuscitation were considered at risk for sepsis. All such neonates who had signs and symptoms of sepsis as per the Young Infant Study Algorithm standard definitions of sepsis, adapted from National Healthcare Safety Network which included tachypnea, respiratory distress, prolonged capillary refill time, abnormal color, abdominal distension, poor sucking, irritability, convulsions, temperature abnormalities, lethargy, or apnea were considered as clinical sepsis and included in the study. Blood culture was done for all neonates with a diagnosis of clinical sepsis.

Two milliliters of venous blood was aseptically obtained from the antecubital fossa of each neonate and dispense into a sterilized universal bottle containing 20 ml of brain heart infusion broth to make a 1:10 dilution. The samples were processed by standard bacteriological procedure[14]. Each sample was sub-cultured into commercially prepared blood agar and Mac Conkey agar. There were five samples which were sub-cultured in a week. The sub-cultured agars were incubated at 37 °C and observed for growth. Samples that did not show growth after 24 h were observed for 7 days before regarded as no growth. Pure colonies of samples that showed growth were taken for Gram staining and biochemical tests using commercially prepared reagents. These tests together with characteristic morphology of pure colonies were used for isolation and identification of pathogens.

Antibiotic susceptibility test of isolates was performed by modified Kirby-Bauer disk diffusion method according to guidelines of Clinical and Laboratory Standards Institute (CLSI). [15] The antibiotics used in this study were ampicillin, piperacillin, tazobactum, amikacin, gentamici, cefotaxime, ceftazidime, ciprofloxacin, meropenem. All the data were entered in the worksheet of SPSS software version (16.0) and descriptive statistics including percentages and frequencies was done. The level of statistical significance adopted was $p<0.05$.

RESULTS

There were 514 newborns who were admitted to neonatal intensive care unit (NICU) for various indications during the study period. The most common indications for NICU admissions were birth asphyxia, meconium aspiration syndrome, neonatal sepsis and prematurity. The study included 55 neonates who satisfied the inclusion and exclusion criteria. The details of neonatal admissions are shown as a flowchart in Figure 1.

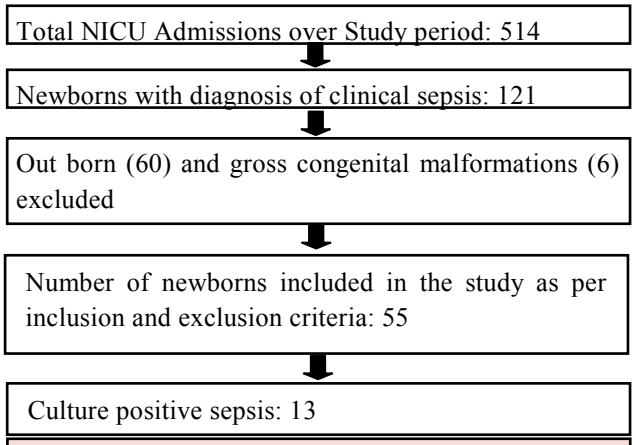

Figure 1. Details of NICU admissions.

Of the 55 babies with a diagnosis of clinical sepsis, 35 (63.63%) were males and 20 (36.36%) were females. Twenty five were preterm babies, and 45% had birth weight <2.5 kg. The basic characteristics of study neonates are shown in Table 1. Among the 55 neonates, 13 (23.6%) blood samples had shown bacteria in culture,while 76.4% of neonates showed no growth in blood culture. A total of 41 (74.54%) individuals were

Table 1. Characteristics of neonates with clinical sepsis. (n=55)

Characteristics	Categories	Frequency (%)
Gender	Male	35(63.63)
	Female	20(36.36)
Gestational age	Term	30(54.54)
	Preterm	25(45.45)
Birth weight (Kg)	<1.5	10(18.18)
	1.5-2.49	15(27.27)
	≥2.5	30(54.54)

of early-onset sepsis and 14 (25.45%) individuals were of late-onset sepsis. The predominant organisms identified were *Staphylococcus aureus* (38.46%) and *Klebsiella pneumoniae* (30.76%). The details of organisms isolated are shown in Table 2.

Table 2. Details of organisms causing culture positive neonatal sepsis. (n=13)

Microorganisms	Number	Percent
Staphylococcus aureus	5	38.47%
Klebsiella pneuminiae	4	30.76%
Pseudomonas aeruginosa	2	15.39%
Enterococcus species	1	7.69%
E. coli	1	7.69%

Eighty seven percent of Gram-negative bacilli and 80% of *S. aureus* were resistant to Ampicillin. Gentamicin resistance among Gram-negative bacilli and *S. aureus* was 50.0% and 80.0%, respectively. The prevalence of ciprofloxacin resistance was 20.0% among *S. aureus* and 12.5% among Gram-negative bacilli. None of the *S. aureus* was resistant to meropenem and 87.5% of gram-negative bacilli are resistant to ampicillin. In the study, 37.5% of Gram-negative bacilli and 20.0% of *S. aureus* were resistant to third-generation cephalosporins. The details of antimicrobial resistance are shown in Table 3.

Table 3. Resistance pattern of organisms isolated.

Antibiotics	Gram-negative bacilli (%) (n=8)	*Staphylococcus aureus* (%)(n=5)
Cefotaxime	3(37.5)	1(20.0)
Meropenem	2(25.0)	0
Amikacin	6(75.0)	4(80.0)
Ciprofloxacin	1(12.5)	1(20.0)
Piperacillin	3(37.5)	2(40.0)
Gentamicin	4(50.0)	4(80.0)
Ampicillin	7(87.5)	4(80.0)

DISCUSSION

Neonatal sepsis is defined classically as a clinical syndrome characterized by systemic signs of infection frequently accompanied by bacteremia. Positive blood culture confirms sepsis, and when the blood culture is negative, the condition is considered as clinical sepsis. The culture positivity rate among the neonates with clinical sepsis in the current study was 23.6%. In previous studies done in India, it has ranged from 16% to 54%.[4, 5] A study conducted in Kanti Children's hospital, Kathmandu in 2018 showed the culture positivity rate of 16.9%.[15] A similar study conducted in Ghana 2016, also revealed the prevalence of culture positive sepsis of 17.3%.[16]The culture positivity might be an underestimation of actual status in our study, as anaerobic organisms were not tested in our institution. Out born babies were excluded from our study due to difficulties in obtaining accurate antenatal and perinatal data. Most common isolates in this study were *S. aureus* and *Klebsiella pneumoniae*. A review of studies on neonatal sepsis in India has found that *Klebsiella* species, *Escherichia coli*, and *S. aureus* were the most common isolates.[18] Similar study from Ghana has indicated the preponderance of gram positive organisms over the gram negative organisms in neonatal sepsis.[17] Since *Staphylococcus epidermidis* and *Staphylococcus aureus* are the major normal flora located on the skin and in the nose respectively, suboptimal hand hygiene by persons who handle neonates, manipulation of peripheral intravenous lines set up on neonates could contribute to the acquisition of these bacteria. Findings from this study did not correspond to a study done in a Neonatal Intensive Care Unit (NICU) in Bangladesh, where they identified gram negative organisms (78%) to be the most common pathogen of neonatal sepsis.[19] However, in a similar study in Ghana in a tertiary hospital, gram positive organisms had a preponderance over gram negative organisms; similar to findings in this study except that their study had a larger sample size.[17] However, a similar study in a NICU in China, found that gram positive organisms were responsible for a greater proportion of early onset sepsis (83.3%) and late onset sepsis (70%) as compared to gram negative organisms [19] which corroborates the findings of this study. In a study in Nepal, results revealed that Staphylococcus epidermidis accounted for the greatest proportion (57.3%), followed by (28.1%) of Escherichia coli, (11.2%) of Staphylococcus aureus and (1.1%) of Pseudomonas aeruginosa that were isolated in EOS.[20] This is in contrast to what was observed in this study. Results of antibiotic susceptibility in the present study indicate that, Staphylococcus aureus, shows 80% resistance to *ampicillin, and amikacin. This is alarming considering that, either ampicillin or* penicillin in combination with gentamicin or amikacin is recommended as first line drugs for empirical treatment of neonatal sepsis. The study also indicates that most of the gram negative bacilli are resistant to ampicillin and amikacin. The susceptibility patterns of the study are consistent with a high degree of antibiotic resistance reported in other studies from India.[22,23] Thus, results in this study could serve as evidence of increasing

resistance to commonly used antibiotics. In this study, *Staphylococcus aureus* was 80% resistant to ampicillin, and amikacin. The overall high resistance rate exhibited could be attributed to the frequent and unrestricted use of the commonly used antibiotics. Consequently, this could limit future antibiotic choice for treating neonatal infections thereby, affecting survival of septic neonates. In this study, we observed a lower resistance to cefotaxime, and meropenem. High sensitivity to third generation cephalosporin by *Pseudomonas aeruginosa* is good for neonatal care. However, the complete resistance to ampicillin and amikacin, as exhibited by other organisms in this study is devastating.

Limitations

This study has enrolled neonates which were only admitted to Nobel Medical College Teaching Hospital, Nepal and excluded the out born neonates. Future research should covered suspected neonates from different parts of Nepal to determine overall neonatal prevalence of culture positive sepsis. We have not cultured the anaerobic organisms which are responsible for sepsis. Methicillin was not contained in the multidisc used for the culture, there sensitivity tests for *S. aureus* could not be carried out.

CONCLUSIONS

The prevalence of culture proven neonatal sepsis in this study is 23.6%. Gram positive organisms were the prevalent neonatal sepsis causing organisms in this study. Of the gram positive organisms, *Staphylococcus aureus* was the most common isolate, followed by gram negative bacilli *Klebsiella pneumoniae*. Since, both the organisms are highly resistant to ampicillin and amikacin, which are the first line drugs in sepsis, there is need to review the empirical treatment for neonatal sepsis.

ACKNOWLEDGEMENTS

We are grateful to all our nursing staffs and junior doctors who are dedicated for the care of newborn.

Funding: None

Conflict of Interest: None

REFERENCES

1. Verma P, Berwal PK, Nagaraj N, Swami S, Jivaji P, Narayan S. Neonatal sepsis: epidemiology, clinical spectrum, recent antimicrobial agents and their antibiotic susceptibility pattern. International Journal of Contemporary Pediatrics. 2015; 2 (3):176–180. DOI: http://dx.doi.org/10.18203/2349-3291.ijcp20150523
2. Lawn JE, Cousens S, Zupan J. Lancet Neonatal Survival Steering Team. 4 million neonatal deaths: When? Where? Why? Lancet. 2005; 65: 891–900. DOI:10.1016/S0140-6736(05)71048-5
3. Jain A, Awasthi AK, Kumar M. Etiological and antimicrobial susceptibility profile of nosocomial blood stream infections in neonatal Intensive Care Unit. Indian J Med Microbiol. 2007; 25: 299–300. DOI: 10.4103/0255-0857.34783
4. Kumhar GD, Ramachandran VG, Gupta P. Bacteriological analysis of blood culture isolates from neonates in a tertiary care hospital in India. J Health Popul Nutr. 2002; 20:343–347. https://www.ncbi.nlm.nih.gov/pubmed/12659415
5. Thaver D, Zaidi AK. Burden of neonatal infections in developing countries: A review of evidence from community-based studies. Pediatr Infect Dis J. 2009; 28:S3–9. DOI:10.1097/INF.0b013e3181958755
6. Shane AL, Stoll BJ. Recent developments and current issues in the epidemiology, diagnosis, and management of bacterial and fungal neonatal sepsis. Am J Perinatol. 2013; 30(2):131–141. DOI:10.1055/s-0032-1333413
7. Arora U, Devi P. Bacterial profile of blood stream infections and antibiotic resistant pattern of isolates.JKSci.2007;9:186–190. http://jkscience.org/archive/volume94/Original%20Article/Bacterial%20profile%20of%20Blood.pdf
8. Zakariya BP, Bhat V, Harish BN, Arun Babu T, Joseph NM. Neonatal sepsis in a tertiary care hospital in South India: bacteriological profle and antibiotic sensitivity pattern. Indian J Pediatr. 2011; 78:413–417. DOI: 10.1007/s12098-010-0314-8
9. Camacho-Gonzalez A, Spearman PW, Stoll BJ. Neonatal infectious diseases: evaluation of neonatal sepsis. Pediatr Clin N Am. 2013; 60:367–389. DOI: 10.1016/j.pcl.2012.12.003
10. Darmstadt GL, Zaidi AKM, Stoll BJ. Neonatal infections: a global perspective. In: Infectious diseases of the fetus and newborn infant. Philadelphia: Elsevier; 2011. p. 24–51. DOI: 10.1186/s13104-018-3394-6

11. Ministry of Health and Population (MOHP) [Nepal], New ERA, and ICF International Inc. Nepal Demographic and Health Survey. Kathmandu: Ministry of Health and Population, NewERA, and ICFInternational; 2011.p.2012https://dhsprogram.com/pubs/pdf/FR257/FR257[13April2012].pdf
12. Shrestha RK, Rai SK, Khanal LK, Mandal PK. Bacteriological study of neonatal sepsis and antibiotic susceptibility pattern in Kathmandu, Nepal. Nepal Med Coll J. 2013; 15:71–73. http://nmcth.edu/images/gallery/Original%20Articles/rE6F5RK%20Shrestha.pdf
13. Shrestha NJ, Subedi KU, Rai GK. Bacteriological profle of neonatal sepsis: a hospital based study. J Nep Paedtr Soc. 2011; 31:1-5 https://www.nepjol.info/index.php/JNPS/article/view/4158
14. Koneman EW, Allen SD, Janda WM, Schreckenberger PC, Winn WC., Jr . 6th ed Philadelphia: Lippincott Williams & Wilkins; 2006. Koneman's Color Atlas and Textbook of DiagnosticMicrobiology.http://www.scirp.org/1787397
15. Clinical Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing. In: 24th informational supplement (M100-S23) CLSI, Wayne PA, USA; 2014.https://www.researchgate.net file.PostFileLoader.html id=59202a0696b7e4d462166956&assetKey
16. Nikita Singh Yadav, Saroj Sharma, Dhiraj Kumar Chaudhary, Prabhat Panthi, Pankaj Pokhrel, Anil Shrestha, et al. Bacteriological profile of neonatal sepsis and antibiotic susceptibility pattern of isolates admitted at Kanti Children's Hospital, Kathmandu, Nepal. BMC Res Notes 2018; 11:301-306 DOI: 10.1186/s13104-018-3394-6
17. Fortress Yayra Aku, Patricia Akweongo, Kofi Nyarko, Samuel Sackey, Fredrick Wurapa, Edwin Andrew Afari, et al. Bacteriological profile and antibiotic susceptibility pattern of common isolates of neonatal sepsis, Ho Municipality, Ghana-2016. Maternal Health, Neonatology, and Perinatology 2018; 4: 2-8. https://doi.org/10.1186/s40748-017-0071-z
18. Investigators of the Delhi Neonatal Infection Study (DeNIS) collaboration. Characterization and antimicrobial resistance of sepsis pathogens in neonates born in tertiary care centres in Delhi, India: A cohort study. Lancet Glob Health. 2016; 4: e752–760. DOI: 10.1016/S2214-109X(16)30148-6.
19. Naher BS, Afroza S, Roy S, Nahar N, Kundu TN. Neonatal Sepsis in A Tertiary Care Hospital: Evaluation of Causative Agents and Antimicrobial Susceptibilities. Bangladesh J. Child Health. 2013; 37 (1):14–17. DOI: http://dx.doi.org/10.3329/bjch.v37i1.15346
20. Li Z, Xiao Z, Li Z, Zhong Q, Zhang Y, Xu F. 116 cases of neonatal early-onset or late-onset sepsis: a single center retrospective analysis on pathogenic bacteria species distribution and antimicrobial susceptibility. Int J Clin Exp Med. 2013; 6(8):693–699. https://www.ncbi.nlm.nih.gov/pubmed/24040479
21. Adhikari N, Pk S, Acharya G, Km V. Bacteriological profile and associated risk factors of neonatal sepsis in Paropakar Maternity and Women' s Hospital Thapathali, Kathmandu Neonatal Sepsis is one of the most common reasons for admission to neonatal units in developing. 2014; 16: 161–164. https://www.ncbi.nlm.nih.gov/pubmed/26930737
22. Jyothi P, Basavaraj MC, Basavaraj PV. Bacteriological profile of neonatal septicemia and antibiotic susceptibility pattern of the isolates. J Nat Sci Biol Med. 2013; 4:306–309. DOI: 10.4103/0976-9668.116981
23. Agnihotri N, Kaistha N, Gupta V. Antimicrobial susceptibility of isolates from neonatal septicemia. Jpn J Infect Dis. 2004; 57:273–275. https://www0.nih.go.jp/JJID/57/273.pdf

Citation: Yadav SK, Giri A. Bacteriological Profile of Neonatal Sepsis in a Neonatal Intensive Care Unit of a Tertiary Care Hospital of Eastern Nepal. JCMS Nepal. 2019; 15(2):93-7.