# INCIDENCE AND RISK FACTORS ASSOCIATED WITH BLOOD CULTURE PROVEN NEONATAL SEPSIS

Nikita Singh Yadav,[1] Pranav Kumar Yadav,[3] Rajeshwar Reddy Kasarla,[1] Pramila Parajuli[2]

## ABSTRACT

### INTRODUCTION

Neonatal sepsis (sepsis neonatorum) is a clinical syndrome resulting from the pathophysiologic effects of local or systemic infection. This is a major cause of morbidity and mortality around the world affecting newborns up to one month of age with clinical symptoms and positive blood cultures. This study aimed at examining the risk factors of neonatal sepsis at pediatric tertiary care hospital.

### MATERIAL AND METHODS

This was a hospital based cross-sectional case control study conducted among 350 neonates admitted within April to September 2015 at the Kanti Children's Hospital, Kathmandu Nepal. Cases were neonates who had sepsis and controls were neonates who did not have sepsis with their index mothers. CRP screening tests and blood culture was performed. Data were entered using the SPSS (Version 22). Bivariate and multivariate logistic regression was used to determine the risk of neonatal sepsis.

### RESULTS

A total of 59 (17%) neonates who had sepsis (cases) with their index mothers' and 291 (83%) neonates who had no sepsis (controls) with their index mothers were enrolled. Maternal factors that predicted the occurrence of sepsis among neonates were parity ($p$<0.027), mode of delivery ($p$<0.001) and PROM ($p$<0.001). Neonatal risk factors which predicted the occurrence of sepsis were duration of stay in the facility ($p$<0.001) and neonatal age on admission ($p$<0.001).

### CONCLUSION

The study found both maternal and neonatal factors to have a strong association with the risk of developing neonatal sepsis. Encouraging maternal antenatal care utilization would help identify the risk factors during prenatal and postnatal care and appropriate interventions implemented to reduce the likelihood of the neonate developing sepsis.

### KEYWORDS

Blood culture, Neonatal Sepsis, Risk factors.

1. Department of Microbiology, Universal College of Medical Sciences, Bhairahawa
2. Department of Microbiology, St. Xavier's College, Maitighar, Kathmandu
3. Department of Anatomy, Universal College of Medical Sciences, Bhairahawa

DOI: http//doi.org/10.3126/jucms.v9i01.37963

**For Correspondence**
Ms. Nikita Singh Yadav
Department of Microbiology,
Universal College of Medical Sciences
Bhairahawa, Nepal
Email:yadaavnikita00@gmail.com

## INTRODUCTION

Neonatal sepsis is a clinical syndrome resulting from the pathophysiologic effects of local or systemic infection: an important cause of morbidity and mortality affecting newborns up to one month of age with clinical symptoms and positive blood cultures. Sepsis falls mainly into two categories: early onset of sepsis (EOS) occurring within 72 hours of age and late-onset of sepsis (LOS) occurring after 72 hours of age. EOS is acquired through the placenta or as an ascending infection of the cervix or during the passage of the baby through colonized birth canal whereas LOS is associated with the colonization of microorganisms from the environment and caretakers.[1] The normal fetus is sterile until shortly before birth as the placenta and amniotic sac are highly effective barriers to infections. At birth, the newborn loses the protection afforded to it in the uterus and gets exposed to the microbial world.[2] Birth asphyxia, prematurity, low birth weight, delivery settings, type of delivery, antenatal care received, newborn mixed feeding, and some cultural practices for cord care contribute to the incidence of neonatal sepsis causing morbidity and mortality among neonates. Several maternal and neonatal risk factors have been related to neonatal sepsis.[2]

Previous studies have looked at the common causative agents of neonatal sepsis with their sensitivity patterns; there are limited studies on the risk factors of neonatal sepsis in Nepal, particularly in the study setting. Research shows that neonatal sepsis receives less substantial international investment as a public health priority compared with other major conditions.[3] Early identification of risk factors of neonatal sepsis and early institutional interventions can reduce neonatal mortality and morbidity rates in the country and the world at large. With this background, this study was undertaken to know the risk factors (maternal and neonatal) associated with neonatal sepsis in a pediatric tertiary care hospital.

## MATERIAL AND METHODS

### Study design and source of population

The hospital-based cross-sectional case control study was conducted among 350 neonates admitted within April to September 2015 at the Kanti Children's Hospital, Kathmandu Nepal. All symptomatic neonates aged 1-28 days of either sex (with a history of convulsions, history of difficulty feeding, movement only when stimulated, respiratory rate ≥60 breaths per minute, severe chest in drawing axillary temperature ≥37.5$^0$C and ≤35.5$^0$C) admitted to NICU, Kanti Children's Hospital during the six-month study period was included in the study. Whereas neonates with early discharge, incomplete patient chart information and expired without taking any treatment was excluded from the study. The detailed history of selected cases: neonate age, sex, gestational age, birth weight, total white blood cells (TWBC), duration of stay, maternal age, mode of delivery, parity and premature rupture of membrane (PROM) were included in the study.

### Sample size and distribution

Sample size was calculated using formula N= $(Z)^2pq/ d^2$ with 95% of confidence interval, 5% of margin error and 30.8% was estimated proportion in population.[3] The initial sample size was 327 but considering a 5% non-response rate, the final sample size was 350. The sampling technique adopted was non-probability purposive sampling where clinically suspected neonates of sepsis aged between 1-28 days were accounted in the study.

### Sample collection, screening tests and blood culture

About 1-2 ml blood was drawn from neonates. On one hand serum were extracted from blood samples for performing CRP screening tests and on the other hand blood samples were dispensed into brain heart infusion (BHI) broth media with 1:5 blood broth dilutions. Firstly, CRP test was performed where one drop of a serum sample, one drop of positive control and one drop of negative control were placed in three different circles in cards respectively. Then one drop of CRP reagent was placed in each of the circles and the card was rotated for 2-5 min for agglutination. Marked agglutination signified CRP level ≥5 mg/dl and slight agglutination signified CRP level ≤5 mg/dl i.e. CRP positive and negative respectively. Simultaneously culture bottles were incubated immediately at 37$^0$C for 5-7 days unless the visible growth was obtained. The culture bottles were examined daily for any visual growth and turbidity, hemolysis of red cells, gas bubbles and clot formation of discrete colonies. This helped in the presumptive diagnosis of positive broth culture. The broth cultures were sub-cultured on blood agar and MacConkey agar. Repeated subcultures of culture bottles were made at different times during their aerobic incubation from 24 hours to 7 days. The MacConkey agar plates were incubated aerobically at 37$^0$C for 24 hours and blood agar plates were incubated at $CO_2$ enriched humid atmosphere by using candle jar at 37$^0$C for 24 hrs.

Blood culture plates were examined for distinguishing the growth and non-hemolytic colony. MacConkey agar plates were observed for lactose and non-lactose fermenting organisms. The standard microbiological techniques were followed for the identification of bacteria from positive subculture plates.

### Data analysis

Data were entered into the statistical package for the social sciences (SPSS) version 22 for analysis. Pearson's chi-squared

test as well binary and multivariate logistic regression analysis were employed for analysis. The magnitude of association was measured by using an odds ratio at a 95% confidence interval. Statistical significance was declared at $p<0.05$.

### Ethical Consideration

Ethical approval was taken from the institutional review committee (IRC No:1048) of Kanti Children's Hospital, Maharajgunj, Kathmandu. Also, written informed consent (in Nepali language) was taken from the participant (child's parent/guardian) and observer with their respective address and signature during the study period.

## RESULTS

A total 350 neonates (age in between 0-28 days) accounted for this study, 204 were male (58.3%), 298 (85%) were the age 3 days (EOS) or above 3 days (LOS), 260 (74%) were in Good Birth Weight (GBW) and 280 (80%) were full term. Three hundred and twenty-four neonates (93%) stayed for a week in the hospital and 162 (46%) had a total WBC count between 5000-12000/mm$^3$. Among 350 mothers, 205 (59%) were aged between 20-29 years and 164 (47%) were primiparous. 290 (80%) were delivered per vaginal and PROM was seen in 79 (23%) cases (Table 1).

**Table 1. Socio-demographic and clinical data distribution of neonates (n=350)**

| S.N | Variables | | Number (n=350) | Percentage |
|---|---|---|---|---|
| 1 | Neonate sex | Male | 204 | 58.3 |
| | | Female | 146 | 41.7 |
| 2 | Gestational age (weeks) | (Preterm) <37 | 70 | 20 |
| | | (Term) 37-42 | 280 | 80 |
| 3 | Birth weight (kg) | VLBW (1.5 kg) | 3 | 1 |
| | | LBW (1.5-2 kg) | 87 | 25 |
| | | GBW (>2kg) | 260 | 74 |
| 4 | Neonate age | <3days | 52 | 15 |
| | | 3 or above 3 days | 298 | 85 |
| 5 | Duration of stay | <1 week | 324 | 93 |
| | | 2-3 weeks | 26 | 7 |
| 6 | TWBC | >12000/mm$^3$ | 170 | 49 |
| | | 5000-12000/mm$^3$ | 162 | 46 |
| | | <5000/mm$^3$ | 18 | 5 |
| 7 | Maternal age(years) | <20 | 24 | 7 |
| | | 20-29 | 205 | 59 |
| | | 30-39 | 121 | 34 |
| 8 | Parity | 1 | 164 | 47 |
| | | 2 | 65 | 19 |
| | | 3+ | 62 | 34 |
| 9 | Mode of delivery | Vaginal | 290 | 83 |
| | | Cesarean | 60 | 17 |
| 10 | PROM | Yes | 79 | 23 |
| | | No | 271 | 77 |

VLBW=Very low birth weight, LBW=Low Birth weight, GBW=Good birth weight, TWBC=total white blood cells, PROM=premature rupture of membrane

### Socio-demographic and obstetrical characteristics of neonate's index mothers

In the current study, a total of 59 neonates who had sepsis (cases) with their index mothers and 291 neonates who had no sepsis (controls) with their index mothers were enrolled. The majority of mothers were within the age range of 20-29 years, constituting 36 (18%) of cases and 169 (82%) of controls (Table 2).

### Pregnancy and obstetric history of neonatal mothers

Twenty-one (7%) of cases and 269 (93%) of controls had a spontaneous vaginal delivery with the majority of the cases 38 (63%) delivered through cesarean section. Similarly, PROM was higher in the controls, 77 (97%) compared to cases, 2 (3%) (Table 2).

### Maternal risk factors of neonatal sepsis

Using both bivariate and multivariable logistic regression, only three variables had shown an overall significant effect on the risk of neonatal sepsis at the 5% level of significance. Maternal parity was strongly related to the risk of neonatal sepsis ($p<0.027$). Primiparous women had 1.54 times higher odds of having neonates with sepsis as compared to multiparous women (Crude odd ratio (COR=1.89; 95% CI (1.050–4.498)). Mode of delivery appeared statistically associated with neonatal sepsis ($p<0.001$). The study also showed that CS deliveries were the majority among the cases, 38 (63%). PROM had a significant association with the risk of neonatal sepsis ($p<0.001$) (Table 2).

**Table 2. Bivariate and multivariate logistic regression analysis of maternal risk factors of neonatal sepsis**

| S.N | Variables | Cases n=59 (17%) | Control n=291(83%) | Total n=350 (100%) | Chi square | p-value | COR (95%CI) | AOR (95%CI) |
|---|---|---|---|---|---|---|---|---|
| 1 | **Maternal** | | | | | | | |
| | <20 | 4 | 18 | 22 | | | | |
| | 20-29 | 35 | 168 | 203 | 0.517 | 0.915 | 1.06 (0.46-2.44) | 0.54 (0.16-1.79) |
| | 30-39 | 19 | 101 | 120 | | | 1.19 (0.50-2.85) | 0.59 (0.15-2.24) |
| | 40+ | 1 | 4 | 5 | | | 1.71 (0.19-2.85) | 0.94 (0.07-12.50) |
| 2 | **Mode of delivery** | | | | | | | |
| | Vaginal | 21 | 269 | 290 | 14.364 | 0.04 | 2.07 (1.24-3.46) | 1.21 (0.61-3.41) |
| | Caesarean | 38 | 22 | 60 | | | | |
| 3 | **PROM** | | | | | | | |
| | Yes | 2 | 77 | 79 | 10.774 | <0.001 | 5.68 (0.06-0.57) | 0.34 (0.08-1.39) |
| | No | 57 | 214 | 271 | | | | |
| 4 | **Parity** | | | | | | | |
| | 1 | 29 | 164 | 193 | | | | |
| | 2 | 17 | 65 | 82 | 9.184 | <0.027 | 1.89 (1.05-4.49) | 0.16 (0.07-0.39) |
| | 3+ | 13 | 62 | 75 | | | 0.89 (0.51- 1.55) | 0.05 (0.02-0.17) |

PROM=premature rupture of membrane, COR= Crude odd ratio, AOR= Adjusted odd ratio, *Significant at $p<0.05$, Reference category=1

### Neonatal characteristics

Thirty-seven (13%) of cases and 243 (87%) of controls were delivered between gestational ages of 37-42 weeks. Good

birth weight (above 2.5 kg) neonates were higher in controls 240 (93%) than cases 20 (7%). The majority of the neonates had <1-week duration of stay in the facility with 45 (14%) of cases and 279 (86%) controls (Table 3).

**Neonatal risk factors of neonatal sepsis**

Duration of stay at the health facility ($p$<0.05) and neonatal age ($p$<0.05) showed a significant effect on the risk of neonatal sepsis. The probability of developing neonatal sepsis increased with increasing neonatal age in both the crude logistic regression analysis and the adjusted one. There was no discernible pattern in the probability of developing neonatal sepsis based on birth weight. In the crude odds analysis, females were less likely (COR=0.90; 95% CI (0.595–1.360)) to develop neonatal sepsis than males. Also TWBC was less likely (COR=0.81; 95% CI (0.2-3.51) determinant of neonatal sepsis (Table 3).

**Table 3. Bivariate and multivariate logistic regression analysis of neonatal risk factors of neonatal sepsis**

| S.N | Variables | Cases n=59 (17%) | Control n=291 (83%) | Total n=350 (100%) | Chi square | p-value | COR (95%CI) | AOR (95%CI) |
|---|---|---|---|---|---|---|---|---|
| 1 | Neonate sex | | | | | | | |
| | Male | 31 | 173 | 204 | 1.4404 | 0.3 | 0.90 (0.59-1.36) | 1.33 (0.82-2.19) |
| | Female | 28 | 118 | 146 | | | | |
| 2 | Gestational age | | | | | | | |
| | Preterm (<37 weeks) | 22 | 48 | 70 | 0.072 | 0.072 | 0.78(0.47-1.30) | 0.46 (0.23-0.92) |
| | Term (37-42 weeks) | 37 | 243 | 280 | | | | |
| 3 | Birth weight (kg) | | | | | | | |
| | VLBW (1.5 kg) | 2 | 1 | 3 | | | | |
| | LBW (1.5-2 kg) | 37 | 50 | 87 | 6.135 | 0.05 | 2.51 (1.02-6.14) | 1.38 (0.45-4.28) |
| | GBW (>2 kg) | 20 | 240 | 260 | | | 1.48 (0.73-3.03) | 0.53 (0.19-1.44) |
| 4 | Neonatal age (days) | | | | | | | |
| | <3 | 17 | 35 | 52 | | | | |
| | 4-7 | 22 | 95 | 117 | | | 2.17 (1.31-3.59) | 1.17 (0.65-2.13) |
| | 8-11 | 12 | 53 | 65 | 21.417 | <0.05 | 2.57 (1.38-4.71) | 2.50 (1.19-5.24) |
| | 12-15 | 4 | 54 | 58 | | | 2.91 (1.01-8.37) | 2.67 (0.82-8.74) |
| | >16 | 4 | 54 | 58 | | | 4.28 (1.50-12.18) | 13.28 (3.36-52.59) |
| 5 | TWBC | | | | | | | |
| | >12000/mm$^3$ | 30 | 140 | 170 | | | | |
| | 5000-12000/mm$^3$ | 26 | 136 | 162 | 0.886 | 0.89 | 0.74 (0.17-3.15) | 1.1 (0.25-4.68) |
| | <5000/mm$^3$ | 3 | 15 | 18 | | | 0.81 (0.2-3.51) | 1.2 (0.28-5.19) |
| 6 | Duration of stay | | | | | | | |
| | <1 week | 45 | 279 | 324 | | | | |
| | 2 weeks | 5 | 11 | 13 | 70.991 | <0.05 | 0.45 (0.20-1.0) | 0.61 (0.21-1.69) |
| | 3 weeks | 3 | 2 | 5 | | | 0.14 (0.05-0.48) | 0.16 (0.03-1.14) |
| | >3 weeks | 6 | 2 | 8 | | | 0.03 (0.009-0.118) | 0.03 (0.01-0.12) |

## DISCUSSION

In the present study nearly one fourth (16.9%) of neonates had microbiologically confirmed sepsis. The finding was in agreement with other studies conducted in Nepal.[4,5] However other findings conducted in Nepal and another part of the developing world shows a higher incidence of neonatal sepsis.[3,6,7,8] The low yield in the present study could be due to the number of referral cases from other hospitals and health facilities. These babies could have received antibiotics prior to referral.

The study finding shows male cases 31 (52.5%) were greater than female cases 28 (47.5%) with male to female ratio1.4:1 which was incongruent with the study of Khinchi et al and Woldu et al.[9,2] This study didn't show any significant difference between sex and neonatal sepsis. In the crude odds analysis also, females were less likely (COR=0.90; 95%CI (0.595–1.360)) to develop neonatal sepsis than males. The reason for an increased number of male cases in these studies may be due to gender bias in the presentation to hospital for care, place of study and other factors.[3]

The highest preterm cases 22 (31.42%) followed by term cases 37 (13.21%) was found in the study to be statistically associated with neonatal sepsis. The result is in accordance with results obtained by Shrestha et al where preterm neonates have shown highest infection 57.8% than term neonates (25.7%).[3] Along with various literature reviews, our study also shows that the incidence of neonatal sepsis is inversely proportional to gestational age and birth weight.[3] Percentage of neonates who had good birth weight above 2.5 kg was higher in controls 240 (93%) than cases 20 (7%). LBW babies had greater 37 cases compared to VLBW and GBW. Statistically, there was a significant difference between birth weight and growth of organisms ($p$ value=0.01). This result is in concordance with the study done by Khinchi et al.[9] However, there was no discernible pattern in the probability of developing neonatal sepsis based on birth weight.

The study also revealed that late onset of sepsis constituted two and half of early onset of sepsis; 17 (29%) cases of EOS and 42 (71%) cases of LOS. This finding was similar to another study done at Kanti Children's Hospital where early onset and late onset of sepsis were 16% and 84% respectively.[10] The present study is in contrast with studies conducted in Pakistan and Bangladesh.[11,12] This is true for the hospital which don't possess birth center and most of cases are being referred from other facilities a few days after birth and time consumed by out of city patients to arrive at this hospital.[13]

Current study findings show that cesarean cases were 38 (63.3%) compared to normal delivery 21 (7.24%) cases, and cesarian cases appeared to be significantly associated with the risk of developing sepsis. The study is in congruent with study of Utomo.[14] It is noted that newborns delivered through CS are not exposed to vaginal and fecal bacteria, but they often experience prolonged hospital stay and late initiation of breastfeeding.[14]The present study findings disagree with Siakwa, et al and Shrestha et al,[15,3] where they found mode of delivery not to be statistically related with neonatal sepsis.

Furthermore, our study results showed that PROM was significantly associated with the risk of neonatal sepsis ($p$<0.001). Several other study findings are consistent with the current study findings. Maternal parity was also found in this study to be significantly associated with the risk of the index neonate developing sepsis ($p$<0.027). The current study is

consistent with Siakwa et al,[15] where they also found parity to be statistically associated with the risk of developing neonatal sepsis ($p \leq 0.001$). It was further observed that as parity increases their index neonates are less likely to develop sepsis according to the bivariate logistic regression.

## CONCLUSION

It is concluded that both maternal and neonatal factors as possible independent risk factors to have a strong association with the risk of neonatal sepsis. The most common risk factors identified were staying at health facility, neonatal age, parity, CS and PROM.

## LIMITATION OF THE STUDY

Since the study was done on only admitted neonates born in selected tertiary care pediatric hospital, the results might lack generalizability to the total population of sepsis cases.

## RECOMMENDATION

Therefore, it is recommended further to study large number of neonates from different hospitals from different areas.

## CONFLICT OF INTEREST

None

## ACKNOWLEDGMENT

The authors wish to thank all the participants and their families for cooperating in this study.

## REFERENCES

1. Yadav NS, Sharma S, Chaudhary DK. Bacteriological profile of neonatal sepsis and antibiotic susceptibility pattern of isolates admitted at Kanti children's Hospital, Kathmandu, Nepal. BMC Research Notes. 2018 May;11:301.
2. Woldu MA, Guta MB, Lenjisa JL, Tegegne GT, Tesafye G. Assessment of the incidence of neonatal sepsis, its risk factors, antimicrobials use and clinical outcomes in Bishoftu General Hospital, Neonatal Intensive Care Unit, Debrezeit-Ethiopia. Pediat Therapeut. 2014 Sep;4(4):214.
3. 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 Mar;15(1):71-73.
4. Gyawali N, Sanjana RK. Bacteriological profile and antibiogram of neonatal septicemia. Indian J Pediatr. 2013 May;80(5):371-4.
5. Khanal R, Manandhar S, Acharya GP. Bacteriological profile of neonatal sepsis in a tertiary level hospital of Nepal. J Nepal Paediatr Soc. 2014 Apr;34(3):175-180.
6. Shaw CK, Shaw CP, Thapalia A. Neonatal sepsis bacterial isolates and antibiotic susceptibility patterns at a NICU in a tertiary care hospital in Western part of Nepal: a retrospective analysis. KUMJ. 2007 Apr;5(2):153-160.
7. Zakariya BP, Bhat V, Harish BN, Arun Babu T, Joseph NM. Neonatal sepsis in a tertiary care hospital in South India: bacteriological profile and antibiotic sensitivity pattern. Indian J Pediatr. 2011 Apr;78(4):413-7.
8. Shitaye D, Asrat D, Woldeamanuel Y, Worku B. Risk factors and etiology of neonatal sepsis in Tikur Anbessa University Hospital, Ethiopia. Ethiop Med J. 2010 Jan;48(1):11–21.
9. Khinchi YR, Kumar A, Yadhav S. Profile of neonatal sepsis. J Coll Med Science. 2010 Aug;6(2):1-6.
10. Shrestha NJ, Subedi KU, Rai GK. Bacteriological profile of neonatal sepsis: A hospital based study. J Nep Paediatr Soc. 2011 Jan;31(1):1-5.
11. Aftab R, Iqbal I. Bacteriological agents of neonatal sepsis in NICU at Nishtar Hospital Multan. J Coll Physicians Surg Park. 2006 Mar;16(3):216-9.
12. Begum S, Baki MA, Kundu GK, Islam I, Kumar M, Haque A. Bacteriological profile of neonatal sepsis in a tertiary hospital in Bangladesh. J Bangladesh Coll Phys Surg. 2012 Apr;30(2):66-70.
13. Boghossian NS, Page GP, Bell EF. Late-onset sepsis in very low birth weight infants from singleton and multiple-gestation births. J Pediatr. 2013 Jun;162(6):1120–4.
14. Utomo MT. Risk Factors of Neonatal Sepsis: A Preliminary Study in Dr. Soetomo Hospital. Indonesian Journal of Tropical and Infectious Disease. 2010 Apr;1(1):23–26.
15. Siakwa M, Kpikpitse D, Mohamed SS. Neonatal sepsis in rural Ghana: A case control study of risk factors in a birth cohort. International Journal of Research In Medical and Health Science. 2014 Sep;4(5):77–88.