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Original Article

Paediatrics Section

# Bacteriological Profile of Sepsis among Low Birth Weight Neonates: A Hospital-based Cross-sectional Study from Northern India

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## ABSTRACT

**Introduction:** Septicaemia in neonates is characterised by the generalised bacteriological infection which is characterised with a blood culture positivity after 28 days of birth along with clinical presentation of systemic infection. It is one of the common causes of death among Low Birth Weight (LBW) neonates. Incidence of sepsis varies based on different variables, such as hospitals, obstetric and nursing procedures, gender, period of gestation, weight of the baby at birth, place of delivery status, mother's health and nutrition and perinatal care.

**Aim:** To know the bacteriological profile and antibiotic susceptibility patterns of organisms causing sepsis in LBW neonates.

**Materials and Methods:** This cross-sectional hospital-based observational study was conducted in tertiary care hospital, Moradabad, Western Uttar Pradesh, India. LBW newborns (Birth weight <2.5 kg) hospitalised in the Neonatal Intensive Care Unit (NICU) over a period of one year from June 2019 to May 2020 with a diagnosis of clinical sepsis were enrolled. Relevant investigations and treatment were started as per our NICU protocol. Data was entered sequentially in Microsoft Excel spreadsheet and analysed in Statistical Package for the Social Sciences (SPSS) (version 20.0) using Chi-square test.

**Results:** During the study period, total 145 LBW neonates were admitted in NICU. Out of whom, 83 neonates were admitted with clinical sepsis. A 87.9% of LBW neonates were preterm. Blood culture reports were positive in 30 (36.2%) LBW neonates. In culture proven sepsis, 20 (67%) neonates had early onset neonatal sepsis and 10 (33%) had late onset neonatal sepsis. In culture proven early onset neonatal sepsis, 13 (65%) LBW neonates had gram negative sepsis and 7 (35%) had gram positive sepsis. The most frequently isolated organisms were *Acinetobacter baumannii* (23.3%) and *Klebsiella pneumoniae*-(16.7%). These organism were resistance to cefotaxime, ampicillin, amikacin and ciprofloxacin.

**Conclusion:** *Acinetobacter* sepsis in LBW neonates is surging rapidly and is associated with high degree of Antimicrobial Resistance (AMR). Therefore, knowledge and awareness of multidrug resistant organism causing sepsis in LBW neonates and their latest antimicrobial sensitivity pattern is essential to choose most appropriate antibiotics.


**Keywords:** Antibiotic sensitivity, Blood culture, Microorganism, Neonatal sepsis

## INTRODUCTION

Sepsis remains as one of the common causes of mortality in LBW newborns [1]. The birth weight of the neonates is one of the determining factors for development of sepsis. LBW neonates are two times more likely to develop sepsis than Normal Birth Weight (NBW) newborns. This results from either prematurity, prolonged hospital stay and increased invasive procedures such as endotracheal incubation, indwelling catheters and multiple pricks [2]. Although advances in medical field have improved the survival of LBW neonates but they remain at a high risk for sepsis [3]. LBW is defined as a birth weight of less than 2500 g (upto and including 2499 g) irrespective of gestational age as per World Health Organisation (WHO) [4]. Depending on the onset of symptoms, neonatal sepsis is of two types; onset before 72 hours of life (Early Onset Neonatal Sepsis (EONS)) or after 72 hours (Late Onset Neonatal Sepsis (LONS)) [5]. The established risk factors associated with neonatal sepsis are LBW, preterm birth/prematurity, Prolonged Rupture Of Membranes (PROM), foul smelling amniotic fluid, multiple per vaginal examination, maternal septicaemia and bottle feeding [6]. Microorganisms causing sepsis and their antibiotic susceptibility patterns may change over time and differ among countries [7]. As per National Neonatal Perinatal Database (NNPD), *Klebsiella pneumoniae* was reported as the most common pathogen followed by *Staphylococcus aureus* and *Pseudomonas* species in India, whereas group B streptococci is the commonest cause of neonatal sepsis in developed countries [8,9].

In India, most of studies had discussed the culture positivity rate, microbiological profile and antibiotics susceptibility patterns of microorganism causing sepsis in all suspected newborns [10-21]. But, only one study have discussed all the parameters (culture positivity rate, microbiological profile and antibiotics susceptibility patterns) in high risk group such as LBW and preterm neonate [22]. This study was done with an aim to know the clinical presentation, culture positivity, pathogens causing EONS and LONS and their antibiotic susceptibility patterns in LBW neonates.

## MATERIALS AND METHODS

The present study was a cross-sectional observational study which was carried out in LBW neonates admitted to NICU. The study was conducted over a period of one year (June 2019 to May 2020) after obtaining approval from Institutional Ethical Research Committee (TMMC and RC/IEC/18-19/013).

**Inclusion criteria:** All the eligible LBW (birth weight <2.5 kg) neonates suspected of having clinical sepsis and admitted in NICU were included. Diagnosis of clinical sepsis was based on presence of atleast one of the following clinical features of sepsis; Refusal to feed, grunting, chest retraction, lethargy, respiratory rate >60/min, apnea, temperature instability such as hyperthermia/fever or hypothermia, vomiting, abdominal distension, abnormal gastric residual, convulsion, hypotonia, irritability, pus draining umbilicus, multiple (>10) pustules and bleeding diathesis [6].

**Exclusion criteria:** Asymptomatic LBW neonates, neonates born with gross congenital anomalies, at risk neonates and babies of all non consenting parents were excluded from the study.

**Sample size:** Minimum sample size was calculated using the formula $n=Z^2\alpha/2\ P\ (100-P)/E^2P$ (Prevalence rate)=42% [13]. As

per sample size calculation, a figure of 83 was arrived at to be the minimum sample size. Enrollment of neonates was done after obtaining written informed consent from parents.

### Study Procedure

A detailed history including gestational age, birth weight, age on admission, mode of delivery, place of delivery, sex and day of onset of symptoms were noted in predesigned proforma. After admission in NICU, relevant investigations including blood sugar, sepsis screen (Total Leucocyte Count (TLC), Absolute Neutrophil Count (ANC), C-Reactive Protein (CRP) I/T Ratio, Micro Erythrocyte Sedimentation Rate (ESR)), X-ray chest and blood/Cerebrospinal Fluid (CSF) culture were send. Antibiotics therapy and supportive treatment were started as per our NICU protocol. With aseptic precautions about 2 mL of venous blood was drawn by a venous puncture and aseptically inoculated into blood culture bottles. BACTEC system was used for blood culture and bacterial growth. The isolated organisms were identified and tested for antimicrobial susceptibility patterns using Kirby-Bauer disc diffusion susceptibility method and Clinical and Laboratory Standards Institute (CLSI) guidelines [23]. Antibiotic sensitivity patterns were interpreted and reported by the microbiologist.

## STATISTICAL ANALYSIS

Research data were entered sequentially in Microsoft Excel spreadsheet and analysed in SPSS software version 20. Group comparisons were done by applying Chi-square test. Frequency and percentage were calculated. The p-value <0.05 was taken as significant.

## RESULTS

During the study period, total 145 LBW neonates were admitted in the NICU. Out of whom, 83 LBW neonates were admitted with clinical sepsis. Of these 83 neonates, 64 were inborn and 19 were outborn and 57 (68.6%) newborn were male and 26 (31.4%) newborn were female. A 60 (72.3%) newborns were presented in early neonatal period (<7 days) and 23 (27.7%) newborns presented in late neonatal period (8-28 days). Caesarean delivery was done in 25 (30.2%) neonates and normal delivery in 58 (69.8%) LBW newborns. There were 2 (2.4%) neonates who had birth weight less than 1000 gm Extremely Low Birth Weight (ELBW), 7 (8.4%) had birth weight between 1000-1499 gm Very Low Birth Weight (VLBW) and rest 74 (89.2%) had birth weight between 1500-2499 gm. Home delivery was reported among 9% and hospital delivery among 91% subjects. Preterm delivery was found among 73 (87%) and term delivery among 10 (13%) subjects [Table/Fig-1].

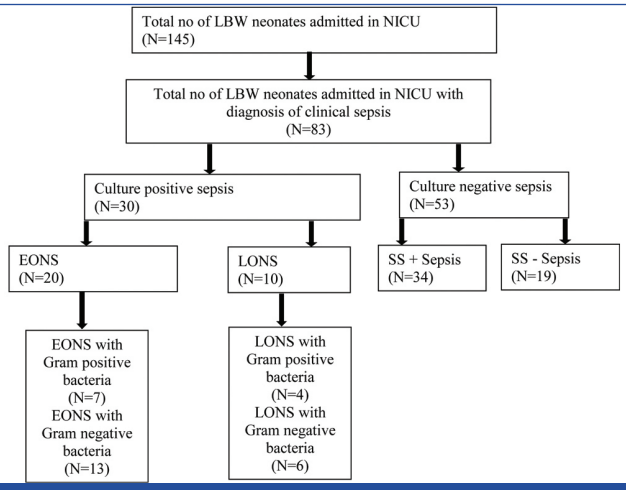
The mean age and mean weight of neonates were 3.63±5.65 days and 1975±410 gm, respectively. Blood culture positivity was seen in 30 (36.2%) neonates only. In culture proven sepsis, 20 (67%) neonates had EONS and 10 (33%) had LONS. In culture proven EONS, 13 (65%) LBW neonates had gram negative sepsis and 7 (35%) had gram positive sepsis. Similarly in LONS, 6 (60%) LBW neonates had gram negative sepsis and 4 (40%) had gram positive sepsis. In culture negative clinical sepsis, 34 (64.1%) neonates had sepsis screen positive (≥2 sepsis screen parameter positive) sepsis while 19 (35.9%) had sepsis screen negative sepsis [Table/Fig-2].

Out of 83 newborns, breathing difficulty (67.4%) and hypothermia (57.8%) were the most common clinical presentation of early onset sepsis while refusal to feed (62.6%) and lethargy (57.8%) were the most common clinical presentation of late onset sepsis. Detailed clinical presentation of EONS and LONS is shown in [Table/Fig-3].

Among culture positive cases, the most frequently isolated organisms in LBW neonates were *Acinetobacter baumannii* 7 (23.3%), *Klebsiella pneumoniae* 5 (16.7%), *Staphylococcus aureus* 4 (13.3%), *E. Coli* 3 (10%), *Pseudomonas* 3 (10%), *MRSA* 2 (10%) *CONS* 2 (6.6%) as shown in [Table/Fig-4]. It was seen that *Acinetobacter baumannii* was sensitive to colistin (100%), meropenem (85%) and piperacillin-tazobactam (57%) while *Klebsiella pneumoniae* was sensitive to

| Characteristics of LBW neonates | | | Numbers | (%) |
|---|---|---|---|---|
| Age of newborns (days) | Early neonatal period | <7 | 60 | 72.3 |
| | Late neonatal period | 7-14 | 10 | 12.1 |
| | | 15-21 | 9 | 10.8 |
| | | 22-28 | 4 | 4.8 |
| Gender | Male | | 57 | 68.6 |
| | Female | | 26 | 31.4 |
| Type of cases | Inborn | | 64 | 77.1 |
| | Out born | | 19 | 22.9 |
| Mode of delivery | NVD | | 58 | 69.8 |
| | LSCS | | 25 | 30.2 |
| Place of delivery | Home delivery | | 07 | 9 |
| | Institutional delivery | | 76 | 91 |
| Gestational age (weeks) | <37 wks (PT) | | 73 | 87.9 |
| | >37 wks (FT) | | 10 | 12.1 |
| Birth weight (gm) | 1500-2499 | | 74 | 89.2 |
| | 1499-1000 | | 7 | 8.4 |
| | <1000 | | 2 | 2.4 |

**[Table/Fig-1]:** Demographic characteristics of Low Birth Weight (LBW) neonates (n=83).
FT: Full term; PT: Preterm; NVD: Normal vaginal delivery; LSCS: Lower segment caesarean




**[Table/Fig-2]:** Flow diagram of distribution of sepsis in LBW Newborns.
LBW: Low birth weight; EONS: Early onset neonatal sepsis; LONS: Late onset neonatal sepsis; SS: Sepsis screen

| Presenting complaints | | Early onset neonatal sepsis | Late onset neonatal sepsis |
|---|---|---|---|
| Difficulty in breathing | n | 56 | 5 |
| | % | 67.4 | 6 |
| Hypothermia | n | 48 | 16 |
| | % | 57.8 | 19.2 |
| Refusal to feed | n | 18 | 52 |
| | % | 21.6 | 62.6 |
| Lethargy | n | 25 | 48 |
| | % | 30.1 | 57.8 |
| Jaundice | n | 0 | 18 |
| | % | 0 | 21.6 |
| Fever | n | 0 | 16 |
| | % | 0 | 19.2 |
| Seizures | n | 2 | 14 |
| | % | 2.4 | 16.8 |
| Gastrointestinal bleeding | n | 5 | 10 |
| | % | 6.02 | 12.1 |

**[Table/Fig-3]:** Distribution of clinical presentation among the LBW neonates with clinical sepsis (n=83).
$\chi^2$ value=8.098, p-value=0.008* A p-value <0.05 is considered to be statistically significant

| Bacterial isolates | Onset of sepsis | | Frequency | Percentage |
|---|---|---|---|---|
| | EONS | LONS | | |
| *Acinetobacter baumannii* | 5 | 2 | 7 | 23.3 |
| *Klebsiella pneumoniae* | 3 | 2 | 5 | 16.7 |
| *Staphylococcus aureus* (MSSA) | 3 | 1 | 4 | 13.3 |
| *Pseudomonas* species | 2 | 1 | 3 | 10 |
| *E. Coli* | 2 | 1 | 3 | 10 |
| MRSA | 1 | 1 | 2 | 6.7 |
| *Staphylococcus* epidermidis | 1 | 1 | 2 | 6.7 |
| Coagulase negative Staphylococci (CONS) | 1 | 1 | 2 | 6.7 |
| *Enterococcus* | 1 | 0 | 1 | 3.3 |
| *Citrobacter* | 1 | 0 | 1 | 3.3 |
| Total | 20 | 10 | 30 | 100 |

**[Table/Fig-4]:** Frequency and distribution of bacterial isolates causing early onset and late onset sepsis in LBW neonates (n=30).
$\chi^2$ value=1.139, p-value=0.951

| Antibiotic | *Acinetobacter* (n=7) | | *Klebsiella* (n=5) | | *Staphylococcus aureus*# (n=4) | | *Pseudomonas* (n=3) | | *E. coli* (n=3) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | S/S+R | S% | S/S+R | S% | S/S+R | S% | S/S+R | S% | S/S+R | S% |
| Cefotaxime | 3/7 | 49 | 3/5 | 60 | 2/6 | 33 | 1/3 | 33 | 1/3 | 33 |
| Cefoxitin | NT | | NT | | 4/6 | 67 | NT | | NT | |
| Ampicillin | 0/7 | 0 | 0/5 | 0 | 1/6 | 17 | 1/3 | 33 | 1/3 | 33 |
| Amikacin | 1/7 | 15 | 1/5 | 20 | 0/6 | 0 | 1/3 | 33 | 0/3 | 0 |
| Gentamycin | 2/7 | 29 | 1/5 | 20 | 1/6 | 17 | 1/3 | 33 | 1/3 | 33 |
| Piperacillin-tazobactam | 4/7 | 57 | 4/5 | 80 | NT | - | 2/3 | 66 | 2/3 | 67 |
| Oxacillin | NT | - | NT | - | 4/6 | 67 | NT | - | NT | - |
| Ciprofloxacin | 3/7 | 43 | 3/5 | 60 | 1/6 | 17 | 2/3 | 66 | 1/3 | 33 |
| Meropenem | 6/7 | 85 | 5/5 | 100 | NT | - | 3/3 | 100 | 3/3 | 100 |
| Vancomycin | NT | - | NT | - | 6/6 | 100 | NT | - | NT | - |
| Linezolid | NT | - | NT | - | 6/6 | 100 | NT | - | NT | - |
| Colistin | 7/7 | 100 | 5/5 | 100 | NT | - | 3/3 | 100 | 3/3 | 100 |

**[Table/Fig-5]:** Antibiotics sensitivity patterns among the major bacterial isolates causing sepsis in LBW neonates.
S: Number of sensitive isolates; S: % Percentage of sensitive isolates; R: Number of resistant isolates; NT: Not tested
#*Staphylococcus aureus* include Both MSSA and MRSA

meropenem (100%) and piperacillin-tazobactam (80%). Details of the bacterial isolates and their antibiotics sensitivity patterns is shown in [Table/Fig-5].

## DISCUSSION

The LBW neonates are risk of several morbidities, of which sepsis is the most common and devastating morbidity leading to high mortality. LBW babies are at the highest risk of infection because of prolonged hospitalisation, impaired immunity, central venous line catherisation and invasive ventilation. This study provides the most updated data about this high risk group. According to previous studies done in India, the blood culture positivity rate in NICU vary from centre to centre and time to time. It has ranged from 7.8% [24] to 64.87% [25] in all newborns admitted with suspected sepsis. In this study, blood culture positivity rate among the LBW neonates with clinical sepsis was 36.2%. Almost similar culture positivity rate in LBW neonates were reported by Rawat A and Shukla OS (40%) and Hoque M et al., (29.8%) [22,26]. However, positivity rate in this study was high as compared to the results reported by Simiyu DE (13.9%) and Lee SM et al., (21.1%) [27,28].

In this study it was found that EONS (67%) was more common than LONS (33%) in LBW neonates, which was similar to a study done in Kenya where the frequency of EONS and LONS was 56.7% and 25.4%, respectively [27]. On contrary, LONS was more common in studies done by Lim WH et al., [29]. In this study, gram negative bacilli were predominant organism as compared with gram positive cocci. *Acinetobacter baummanii* (23.3%) and *Klebsiella pneumoniae* (16.7%) was the most common isolates identify in both EONS and LONS in LBW neonates. Similar microbiological profile of sepsis in preterm LBW neonates was reported by Hoque M et al., where *Acinetobacter* (41.2%) and *Klebsiella pneumoniae* (23.5%) were the most common organisms in both types of sepsis [26]. Another study done in Kenya reported by Simiyu DE found *Klebsiella and Citrobacter* as the most common organism causing sepsis in LBW neonates [27]. In a study done in Taiwan by Lim WH et al., in VLBW neonates where *E. coli* (40%) was the most common organisms in EONS and coagulase negative staphylococci (54.7%) was the most common bacteria in LONS [29]. Another study done in Gujarat, India reported by Rawat A and Shukla OS found that common bacteria for EONS were *Klebsiella, Pseudomonas* and Methicillin Resistant *Staphylococcus aureus* (MRSA) [22]. In this study, the frequency of *Acinetobacter* was 23.3% which was higher to the studies which were conducted by Nazir A (13.7%), Arora U and Jaitwani J (12.3%), and Mondal GP et al., (15.2%) [30-32].

Antibiotic susceptibility pattern was studied for all isolates causing sepsis in LBW neonates. The analysis of drug resistance pattern showed that, *Acinetobacter baumannii* resistant to ampicillin (100%) and lowest to colistin (0%), meropenem (15%) and piperacillin-tazobactam (43%) *Staphylococcus aureus* was sensitive to cefoxitin (67%) and cloxacillin (67%). Most of organisms were resistance to commonly used antibiotics such as ampicillin, amoxiclav, amikacin, and cefotaxime. In this study, colistin (100%) and meropenem (100%) were found to be most sensitive antibiotics for gram negative sepsis while for gram positive sepsis, linezolid (100%) and vancomycin (100%) were found to be most sensitive antibiotics. Almost similar antibiotics resistance patterns have been reported

| Sl. No. | Author and publication year | Place | Culture positivity | Microbiological profile | Antibiotic sensitivity patterns |
|---|---|---|---|---|---|
| 1 | Hoque M et al., [26] (2010) | Bangladesh | 29.8% | *Acinetobacter* (41.2%)<br>*Klebsiella* (23.5%)<br>*E. coli* (23.5%) | Not discussed |
| 2 | Rawat A and Shukla OS [22] (2019) | Gujarat, India | 40% | *Klebsiella* (17.5%)<br>MRSA (17.5%)<br>*Pseudomonas* (17.5%) | Gentamycin (100%)<br>Colistin (80%) |
| 3. | Simiyu DE [27] (2005) | Kenya | 13.9% | *Klebsiella* (23%)<br>*Citrobacter* (19.2%)<br>*Enterobacter* (11%) | Gentamycin (86%)<br>Cefotaxime (82%)<br>Amoxicillin (76%) |
| 4. | Lee SM et al., [28] (2015) | Korea | 21.1% | CoNS (26%)<br>*E. coli* (10%) | Not discussed |
| 5 | Lim WH et al., [29] (2012) | China | 15.1% | CoNS (50.5%)<br>*E. coli* (40%) | Not discussed |
| 6. | Nazir A [30] (2019) | North India | 13.7% | *Acinetobacter* | Colistin (100%)<br>Tigecycline (87%)<br>Meropenem (4.08%) |
| 7. | DeNIS [33] (2016) | New Delhi | 14.3% | *Acinetobacter* (22%)<br>*Klebsiella* (17%)<br>*E. coli* (14%) | Carbepenems (22%)<br>Cephalosporins (62%)<br>Colistin (93%) |
| 8. | Jatsho J et al., [34] (2020) | Bhutan | 14% | CoNS (31%)<br>*K. pneumoniae* (27%)<br>*Acinetobacter* (18.8%) | Imipanem (100%)<br>Ampicillin (0%) |
| 9. | Present study (2022) | Uttar Pradesh, India | 36.2% | *Acinetobacter* (23.3%)<br>*Klebsiella* (16.7%) | Meropenem (85%)<br>Colistin (100%) |

**[Table/Fig-6]:** Comparison of bacteriological profile and antibiotic sensitivity patterns of organisms causing sepsis in LBW neonates [22,26-30,33,34].

by Delhi Neonatal Infection Study (DeNIS) where most of isolated pathogens showed a high degree of AMR, not only to commonly used antibiotics but also to so-called reserve antibiotics such as extended-spectrum cephalosporins and carbapenems [33]. Recently, similar antibiotic resistance patterns in LBW neonates were reported by Nazir A where *Acinetobacter baummanii* had resistance to reserve antibiotics such as carbapenems [30]. A comparison of studies on microbiological profile and antibiotic sensitivity patterns of organism causing sepsis in LBW newborns is discussed in [Table/Fig-6] [22,26-30,33,34].

The AMR is today, a global problem and it is surging rapidly in India. There are certain risk factors for emergence of AMR such as irrational use of broad spectrum antibiotics, poor infection control practice, lack of antibiotics stewardship policy, lack of nurse patient ratio and overcrowding [35]. Hence, in any NICU, it is very essential to have annual review to define the current bacteriological profile and their sensitivity pattern of organisms causing sepsis in high risk group such as LBW neonates. This situation is alarming because these are the reserve antibiotics. If we do not follow rational antibiotics policy and continue using these antibiotics as empirical treatment, multidrug resistance organisms will naturally develop. For prevention of neonatal infection, strict infection control practices should be followed and for appropriate effective management, rational antibiotics policy of NICU should be place in all NICUs.

### Limitation(s)

The limitations of this study were small sample size and study has enrolled LBW neonates which were only admitted to our hospital. There is also a need for more epidemiological and clinical studies to track changes in microorganisms that cause sepsis in high risk group.

## CONCLUSION(S)

Gram negative bacilli were the most common organism causing sepsis in LBW neonates. Most of these organisms were resistant to commonly used antibiotic such as cefotaxime, amikacin, ampicillin and ciprofloxacin. In order to minimise the AMR in neonatal sepsis, successful prophylactic steps, timely and accurate diagnosis, and subsequent administration of antibiotics therapy are crucial. The terrifyingly high level of AMR, calls for an urgent review and implementation of antimicrobial guidelines and rules for neonatal sepsis.

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