

## Original Article

### Bacteriological Profile of Neonates Admitted with Suspected Sepsis in NICU of Tertiary Care Hospital of Western Nepal

Badri Kumar Gupta<sup>1\*</sup>, Amit Kumar Shrivastava<sup>2,3</sup>, Laxmi Shrestha<sup>2</sup>, Pradeep Chetri<sup>4</sup> and Rita Khanal<sup>5</sup>

<sup>1</sup>Department of Pediatric, Universal College of Medical Sciences, Bhairahawa, Nepal

<sup>2</sup>Department of Pharmacology, Universal College of Medical Sciences, Bhairahawa, Nepal

<sup>3</sup>Department of Pharmacy, Universal College of Medical Sciences, Bhairahawa, Nepal

<sup>4</sup>Department of Community Medicine, Universal College of Medical Sciences, Bhairahawa, Nepal

<sup>5</sup>Department of Microbiology, Universal College of Medical Sciences, Bhairahawa, Nepal

#### Abstract

Septicemia in neonates refers to generalized bacterial infection documented by positive blood culture in the first four weeks of life and is one of the four leading causes of neonate's mortality. To study and identify the bacterial etiologic agents responsible for neonatal sepsis and to determine the susceptibility pattern of isolates in a tertiary care hospital in Universal College of Medical Sciences and Teaching Hospital (UCMS-TH). The pathogens most often implicated in neonatal sepsis in developing countries differ from those seen in developed countries.

#### Materials and methods

All clinically suspected cases of neonatal sepsis admitted to Neonatal Intensive Care Unit (NICU) from Jan 2019 - April 2019 were included in the study. The data were analyzed statistically. Two hundred ninety six blood samples were collected and processed from patients in accordance with international protocol. Antibiotic susceptibility of isolates was done by disc diffusion method according to National Committee for Clinical Laboratory Standards (NCCLS) guideline.

\*Corresponding author: Badri Kumar Gupta, Department of Pediatric, Universal College of Medical Sciences Bhairahawa, Nepal, Tel: +977 9847042676; E-mail: drbadrikrgupta@gmail.com

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#### Results

A total number of 296 blood cultures were taken from clinically suspected cases of neonatal sepsis. Among these patients, caesarean section was carried out in 60.8 % and 39.2 % were delivered by normal vaginal delivery. Out of total patients, only 17.96 % were culture positive. The culture showed the presence of gram negative bacteria in 22 (42.3 %) and gram positive bacteria in 30 (57.69 %) cases. 40 (13.5 %) neonates were born preterm, 244 (82.4 %) full term and 12 (4.1 %) were post term. The most common organisms were Coagulase negative 31.8 % followed by Klebsiella. The isolated organisms were sensitive to meropenam, vancomycin and Amikacin.

#### Conclusion

Gram positive organism (Coagulase negative) and Gram negative (Klebsiella) are the leading cause of neonatal sepsis in this study and sensitive to meropenam, vancomycin and amikacin antibiotic. Careful selections of antimicrobials helps in early recovery reduced stay in neonatal intensive care unit and reduced risk for emergence multidrug resistant organism in NICU. The causative diverse microbial flora and their changing antibiotic susceptibility pattern warrant for continuous monitoring

#### Introduction

Septicemia is the significant cause of morbidity and mortality in the neonates and is responsible for 30 - 50 % of total neonatal deaths each year in developing countries. It is estimated that up to 20 % of neonates develop sepsis and approximately 1 % die of sepsis related cause [1]. Early diagnosis and appropriate therapy of septicemia is of utmost importance to prevent morbidity and mortality [2]. The present study was undertaken to determine the bacteriological profile and their antimicrobial susceptibility pattern of prevalent pathogens isolated from the blood of septicemic neonates from Neonatal Intensive Care Unit (NICU).

Neonatal sepsis defined as a clinical syndrome of bacteremia with systemic signs and symptoms of infection in the first 28 days of life. When pathogenic bacteria gain access into the bloodstream, they may cause overwhelming infection without much localization (septicemia) or may be predominantly localized to the lung (pneumonia) or the meninges (meningitis).

Neonatal sepsis (sepsis neonatorum or neonatal septicemia): Are terms that are used to describe the systemic response to infection in the newborn infant. The criteria of neonatal sepsis should include the documentation of infection in a newborn infant with a serious systemic illness in which non-specific explanations for the abnormal pathophysiologic state are excluded or unlikely. Neonatal sepsis can be classified into two subtypes depending upon whether the onset of symptoms is before 72 hours of life (early-onset neonatal sepsis - EONS) or later (late-onset neonatal sepsis - LONS). These definitions have contributed greatly to diagnosis and treatment by identifying which microorganisms are likely to be responsible for sepsis during

these periods and the expected outcomes of infection. Common risk factors associated with the increased severity of the two syndromes are the birth weight and gestational age.

Many focal infections as meningitis, pneumonia and urinary tract infection that can occur in other age groups may occur in neonates as well, but infections in neonates have unique elements that differ from those in older age groups. In neonates focal symptoms and signs due to localized infections may be clinically imperceptible and difficult to differentiate on initial presentation from generalized blood stream infections [3]. Neonatal sepsis is one of the major causes of morbidity and mortality in the newborn. Surviving infants can have significant neurological sequelae as a result of Central Nervous System (CNS) involvement. Septic shock or hypoxemia can occur secondary to severe parenchymal lung disease [4]. An early, sensitive and specific laboratory test would be helpful to avoid the unnecessary treatment of uninfected patients that may result in over treatment or contribute to antibiotic resistance. Several leukocyte indices and acute-phase protein levels have been evaluated for the diagnosis of sepsis, and measurement of multiple plasma cytokines and leukocyte activation markers have showed promising results [5]. However, to date, no single laboratory test has provided rapid and reliable identification of early infected neonates. This inability has led to a search for new diagnostic markers [6]. Because of this, we planned to describe the main clinical and bacteriological profile of neonatal sepsis from Universal College of Medical Sciences and Teaching Hospital (UCMS-TH) and the associated pathogens.

Diagnosis of septic screening test: Total leucocytic count more than 20000/cumm, or less than 5000/cumm, platelets count less than 150000/cumm, band neutrophil ratio more than 0.2, absolute neutrophil count, C-reactive protein positive (more than 1 mg/dl), micro ESR > 15 mm/hr, blood cultures and sensitivity by standard methods.

## Risk Factor

S.N.	Risk Factor
1	Low birth weight (< 2500 g) or Prematurity
2	Febrile illness in the mother with the evidence of bacterial infection within 2 weeks prior to delivery
3	Foul smelling liquor
4	Rupture of the membrane > 24 hrs.
5	Single unclean or > 3 sterile vaginal examination during labour
6	Prolong labour (sum 1st and 2nd stage of labour > 2 hrs.)
7	Perinatal asphyxia (APGAR score < 4 at 1 minute)

## Methods and Methodology

This study is a cross sectional prospective study was conducted between January 2019 to April 2019 neonates admitted in Neonatal Intensive Care Unit in UCMS-TH. Inclusion criteria were all newborn admitted to UCMS-TH NICU with screening positive sepsis or clinically suspected sepsis and Neonates presence of 2 or more risk factor positive were consider as suspected sepsis were included. Neonates were excluded from the study any new born with sepsis who have received prior antibiotics and any newborn with congenital anomalies. Blood culture was done in all neonates suspected to have neonatal septicemia.

Blood culture sample included a single sample collected from a peripheral vein or artery under aseptic conditions. The local site was cleansed with 70 % alcohol and povidone iodine (1 %), followed by 70 % alcohol again. Blood cultures were done in a brain heart infusion biphasic medium. Approximately, 3 ml of blood was inoculated into the brain heart infusion broth and incubated at 37°C. Subcultures were done on sheep blood agar and MacConkey agar at the earliest visual detection of turbidity or blindly on days 1, 4, and 7 if the bottles did not show turbidity. Isolate was identified by their characteristic appearance on their respective media, Gram staining and confirmed by the pattern of biochemical reactions using the standard method [7]. Members of the family Enterobacteriaceae were identified by indole production, H<sub>2</sub>S production, citrate utilization, motility test, urease test, oxidase, carbohydrate utilization tests, and other tests. For gram-positive bacteria, coagulase, catalase, bacitracin and optochin susceptibility tests and other tests were used. Blood culture broth that showed no microbial growth within seven days was reported as culture negative, only after result of routine subculture on blood, MacConkey, and chocolate agar [7].

Antimicrobial susceptibility testing was performed for all blood culture isolates by Kirby - Bauer disc diffusion method as recommended in the National Committee for Clinical Laboratory Standards (NCCLS) guidelines [8]. The drugs for disc diffusion testing were in the following concentrations: Ampicillin (10 µg), cloxacillin (1 µg), lomefloxacin (10 µg), amoxiclav (20/10 µg), cephalexin (30 µg), cefuroxime (30 µg), ciprofloxacin (5 µg), erythromycin (15 µg), gentamicin (10 µg), (30 µg), penicillin (10 units), tetracycline (30 µg), co-trimoxazole (1.25 µg trimethoprim/23.75 µg sulfamethoxazole), amikacin (30 µg), ofloxacin (5 µg), sparfloxacin (5 µg), pefloxacin (5 µg), cefoperazone (75 µg), netilmicin (30 µg), imipenem (10 µg), piperacillin/tazobactam (100/10 µg), azithromycin (15 µg), and linezolid (30 µg). The discs were obtained from Himedia (India) Laboratories.

## Ethical Clearance

The approval of Institutional Review Committee of Universal College of Medical Sciences, Bhairahawa, Nepal was taken before the initiation of experiment. Registration No. UCMS/IRC/073/19. All the protocols and experiments were conducted in compliance with the ethical principles and guidelines.

## Statistical Analysis

Data analysis was done using Statistical Package for Social Sciences (SPSS) software version 14.0. The level of significance for tests was set at  $P < 0.05$

## Results

During the study period, a total n = 296 newborn with clinical sepsis were admitted. Blood culture reports were positive in 52 (17.6 %). Among the culture positive cases. There were 30 (15.2 %) male and 22 (22.4 %) female neonate with male to female ratio of 1.3:1. However 32 (27.6 %) were delivered by normal vaginal delivery and 20 (11.1 %) were delivered by caesarian section. 44 (61.1 %) neonate were born low birth weight and 8 (3.5 %) were normal weight (> 2.5 kg). 12 (30 %) were preterm 40 (16.4 %) were term baby. This p-value suggested that except gender others, mode of deliver, weight and maturity of baby, sepsis was affected.

S. no	Neonatal risk factors	Culture positive (%)	Culture negative (%)	p value
1	Gender			
	Male	30 (15.2 %)	168 (84.8 %)	> 0.05
	Female	22 (22.4 %)	76 (77.6 %)	
2	Mode of delivery			
	NVD	32 (27.6 %)	84 (72.4 %)	< 0.05
	LSCS	20 (11.1 %)	160 (88.9 %)	
3	Low birth weight			
	Yes	44 (61.1 %)	28 (38.9 %)	< 0.05
	No	8 (3.5 %)	216 (96.5 %)	
4	Maturity of baby			
	Preterm	12 (30)	28 (70)	
	Term	40 (16.4)	204 (83.6)	< 0.05
	Postterm	0 (0)	12 (100)	

**Table 1:** Demographic data of neonatal sepsis.

Organisms	Number	Percentage
Coagulase negative	16	30.8
<i>Klebsiella</i>	14	26.9
<i>Staphylococcus aureus</i>	2	3.8
<i>Actinobacter</i> species	8	15.4
<i>Staphylococcus epidermidis</i>	2	3.8
Methicillin resistant CONS	4	7.7
<i>Enterococcus</i> species	2	3.8
MR <i>Staphylococci</i> species	3	5.8
Grampositive coccus CONS	1	1.9
Total	52	100

**Table 2:** Types of the Micro-organism present in proven neonatal sepsis.

The data showed that the Coagulase Negative Staphylococci (CONS) 16 (30.8 %) and *Klebsiella* species 14 (26.9 %) were the most common Gram positive and Gram negative organisms in neonatal sepsis.

Organisms: CONS		
Medicine	Frequency	Percentage
Gentamicin	7	33.33
Amikacin	8	38.10
Ceftriaxone	3	14.29
Cefoperazone	2	9.52
Tobramycin	1	4.76
Total	21	100.00

**Table 3:** Sensitivity pattern of CONS Micro-organism.

Table 3 shows the Antibiotics susceptibility pattern in CONS. Best overall sensitivity among Gram positive isolates was to Amikacin (38.10 %) followed by Gentamicin (33.33 %) and for ceftriaxone (14.29 %).

Table 4 showed *Klebsiella* had sensitivity of Meropenam (34.48 %), Cefixime (17.24 %) and Gentamicin (13.79 %).

Organisms: <i>Klebsiella</i>		
Medicine	Frequency	Percentage
Cefotaxime	3	10.34
Gentamicin	4	13.79
Tobramycin	1	3.45
Cotrimazole	1	3.45
Imipenam	1	3.45
Meropenam	10	34.48
Amikacin	2	6.90
Cefixime	5	17.24
Doxycycline	1	3.45
Levofloxacin	1	3.45
	29	100.00

**Table 4:** Antibiotic sensitivity pattern on *Klebsiella*.

Table 6 showed that maternal risk factor of neonatal sepsis were PROM 20 (6.8 %), Chorioamnionitis 4 (1.4 %) foul smelling liquor 4 (1.4 %), Prolonged labour 6 (2 %) were found in culture positive cases and Perinatal asphyxia were 20 (6.8 %).

## Discussion

The uncertainty surrounding the clinical approach to treatment of neonatal septicemia can be minimized by periodic epidemiological surveys of etiological agents and their antibiotic sensitivity patterns leading to recognition of their most frequently encountered pathogens in a particular geographical area. For effectual management of septicemia cases, study of bacteriological profile along with the antimicrobial sensitivity pattern plays a noteworthy role. Out of the 296 clinically suspected cases of sepsis in our study, 52 were culture positive with a blood culture positivity rate of 17.56 %. The incidence of Gram-negative and Gram-positive organisms was 42.3 % and 57.69 %, respectively [9-11].

In this study, a male predominance with male-to-female ratio of 1.3:1 was found in our study, which agrees with previous reports. This might be because of the importance given to the male infants and also because of more number of male infants born compared to female infants born. Culture-positivity for aerobic organisms in neonates varies from 25 % to 60 % [12-14]. In this study, blood culture-positivity rate is 17.56 %. This finding is comparable with other reports [15]. However, a high blood culture-positivity rate in septicemic children (56 %) had been reported by Sharma et al., [16] and Jain et al., [17]. A low blood culture isolation rate could be due to administration of antibiotic before blood collection from the primary centers or the possibility of infection with anaerobes. A negative blood culture does not exclude sepsis and about 26 % of all neonatal sepsis could be due to anaerobes [15].

The pathogens most often implicated in neonatal sepsis in developing countries differ from those seen in developed countries. Overall, Gram-negative organisms are more common and are mainly represented by *Klebsiella*, *Escherichia coli*, *Acenobacter*, and Methicillin resistance CONS. Of the Gram-positive organisms, *Staphylococcus aureus*, CONS, *Streptococcus pneumoniae*, and *S. pyogenes* are most commonly isolated [18]. Gram-negative and gram - positive septicemia was encountered in 42.30 % and 57.69 % of the culture-positive cases in this study, which is comparable to a study conducted by Ag

nihotri et al., [19]. Which reported that Gram-negative and Gram-positive organisms were responsible for 59 % and 41 % of the septicemia cases, respectively. Similar observations were made by other workers [20,21].

S.N	Organisms	Drugs sensitivity	Cases	percentage
1	<i>Staphylococcus aureus</i> (N-2)	Vancomycin	2	100
2	<i>Actinobacter</i> Species (N-30)	Meropenam	5	16.67
		Amikacin	4	13.33
		Levofloxacin	2	6.67
		Azithromycin	3	10.00
		Ciprofloxacin	2	6.67
		Lomefloxacin	2	6.67
		Tetracycline	2	6.67
		Cefoperazone	2	6.67
		Doxycycline	2	6.67
		Meropenam	1	3.33
		Piperacillin	1	3.33
3	<i>Staphylococcus epidermidis</i> (N-4)	Vancomycin	2	50
		Co-trimoxazole	2	50
4	<i>Enterococcus</i> Species (N-4)	Vancomycin	2	50
		Amikacin	2	50
5	Methicillin Resistance <i>Staphylococcal</i> species (N-9)	Vancomycin	1	11.11
		Gentamicin	3	33.33
		Ciprofloxacin	1	11.11
		Levofloxacin	2	22.22
		Amikacin	2	22.22
6	Gram Positive Coccus CONS (N-3)	Vancomycin	1	33.33
		Gentamicin	1	33.33
		Ciprofloxacin	1	33.33
7	MRCONS (N-8)	Vancomycin	4	50
		Erythromycin	2	25
		Cotrimoxazole	2	25

S.N 1 showed the antibiotic susceptibility pattern of *Staphylococcus aureus* sensitive to Vancomycin 2 (100 %)

S.N 2 showed the antibiotic susceptibility pattern of *Actinobacter* sensitive to Meropenam (16.67 %), Amikacin (13.33 %) and Azithromycin and Cefixime (10 %)

S.N 3 showed the isolated four cases of *Staphylococcus epidermidis* sensitivity to Vancomycin 2 (50%) and Cotrimoxazole 2 (50 %)

S.N 4 showed the isolated four cases of *Enterococcus* species sensitive to Vancomycin 2 (50 %) and Amikacin 2 (50 %)

S.N 5 showed the isolated 9 cases of staphylococcal species sensitive to Vancomycin 1 (11.1 %), Gentamicin 3 (33.33 %), Ciprofloxacin 1 (11.11 %), Levofloxacin 2 (22.22 %) and Amikacin 2 (22.22 %)

S.N 6 showed isolated 3 cases of Gram positive coccus (CONS) sensitive to Vancomycin 1 (33.33 %), Gentamycin 1 (33.33 %) and Ciprofloxacin 1 (33.33 %)

S.N 7 showed isolated 8 cases of MRCONS sensitive to Vancomycin 4 (50 %), Erythromycin 2 (25 %) and Cotrimoxazole 2 (50 %)

**Table 5:** Sensitivity pattern of anti-microbialagents in different types of micro-organism.

The report of the National Neonatal-Perinatal database showed *Klebsiella* as the predominant (29 %) pathogen [22]. *Klebsiella* spp. (26.9 %) was the predominant Gram - negative species isolated in this study, which agrees with previous reports [23,24]. Antibiotic resistance is today a global problem. Reports of multi-resistant bacteria causing neonatal sepsis in developing countries are increasing. The wide availability of over-the-counter antibiotics and the in appropriate

use of broad-spectrum antibiotics in the community may explain this situation. It is difficult to compare antibiotic resistance between countries because the epidemiology of neonatal sepsis is extremely variable.

Characteristics	Culture positive (%)	Culture negative (%)
PROM (more than 18 hours)	20 (6.8 %)	276 (93.2 %)
Chorioamnionitis	4 (1.4 %)	292 (98.6 %)
Foul smelling liquor	4 (1.4 %)	292 (98.6 %)
Prolonged labour	6 (2 %)	290 (98 %)
Perinatal asphyxia	20 (6.8 %)	276 (93.2 %)

**Table 6:** Univariate analysis of factors associated with Culture positive - sepsis (N - 296).

## Conclusion

Careful selections of antimicrobials helps in early recovery, reduced stay in neonatal intensive care unit and reduced risk for emergence multidrug resistant organism in NICU .The causative diverse microbial flora and their changing antibiotic susceptibility pattern warrant for continuous monitoring. Also, an antibiotic policy should be formulated in the hospital. Depending on the antibiotic sensitivity pattern of the isolates, antibiotic should be used. Furthermore, we advise the health education be provided to the public on the danger of indiscriminate use of antibiotics. Which is currently considered to be menace in our society and which has been responsible for ineffectiveness. It is evident from this study that Gram-negative organisms (*Klebsiella*, *Acinetobacter*), CONS, and Methicillin resistance CONS are the leading cause of neonatal sepsis in this study. Furthermore, we advise that health education be provided to the public on the dangers of indiscriminate use of antibiotics, which is currently considered to be a menace in our society and which has been responsible for the ineffectiveness of most commonly used antibiotics.

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