

# Bacteriological Profile of Neonatal Sepsis in a Tertiary Level Hospital of Nepal

Khanal R<sup>1</sup>, Manandhar S<sup>2</sup>, Acharya GP<sup>3</sup>

## Abstract

**Introduction:** Neonatal sepsis is one of the most common reasons for admission to neonatal units in developing countries. It is also a major cause of mortality in both developed and developing countries. This study was done to determine the bacterial profile causing neonatal sepsis and to assess their susceptibility pattern to various antimicrobial agents.

**Materials and Methods:** A cross-sectional prospective study was conducted in Paropakar Maternity and Women's Hospital, Kathmandu, Nepal among 340 neonates suspected of neonatal sepsis. Blood culture was performed and organisms were identified with Gram staining and conventional biochemical methods. Antimicrobial susceptibility testing was performed by Kirby-Bauer disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI). **Results:** The prevalence rate of neonatal sepsis was 20.3%. Among 340 neonates, 52.17% were males and 47.82% were females. Gram positive cocci were the most predominant isolates (88.40%). Among Gram positive cocci *Staphylococcus epidermidis* was the most common isolates (72.46%) followed by *Staphylococcus aureus* (7.24%), *Staphylococcus saprophyticus* (4.34%) and *Enterococcus fecalis* (4.34%). Gram negative bacilli were found in 11.60% of the growth positive samples of which *E.coli* and *Klebsiella spp* were found in 10.14% and 1.44% respectively. Sensitivity to Amikacin was highest among all types of organisms isolated. Vancomycin and Gentamycin sensitivity was highest for Gram positive and Gram negative organisms respectively. Ampicillin resistance was highest among isolates. Among the 69 isolates 48 were Multiple drug resistant. **Conclusion:** The predominance of Gram positive cocci particularly Coagulase negative Staphylococci is shown. Empirical antibiotic therapy should be reviewed for Multiple drug resistant strains.

**Key words:** Neonatal sepsis, Early onset sepsis, Late onset sepsis, Multiple drug resistant.

## Introduction

Neonatal Sepsis (sepsis neonatorum) is a clinical syndrome resulting from the pathophysiologic effects of local or systemic infection in the first month of life. Neonatal sepsis remains as an important cause of morbidity and mortality among infants in

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developing countries accounting for 30-50% of total deaths each year<sup>1</sup>. The incidence of sepsis in neonatal period is 1-10 per 1000 live births with a mortality rate as high as 15-20%<sup>2</sup>. It is roughly three times more in developing countries like Nepal. This high incidence is mainly due to poor antenatal care and lack of trained staff to conduct deliveries<sup>3</sup>. Classical neonatal infection has been divided into Early or Late onset

infection, depending on the time of onset of infection: early, before 48 hours and late, after 48 hours<sup>4</sup>.

The common organisms responsible are Group B *Streptococcus*, Gram negative enteric organisms particularly *E.coli*, other organisms such as *Staphylococcus*, *Streptococcus*, *Listeria monocytogens*, *Haemophilus influenzae*, Gram negative rods such as *Klebsiella*, *Pseudomonas*, *Serratia* and *Proteus*<sup>5</sup>. Since the spectrum of organisms that cause neonatal sepsis changes overtime and varies from region to region and hospital to hospital even in the same city/country, it is necessary to conduct periodic surveillance to access the changing pattern of organisms causing neonatal sepsis. For effective management of neonatal septicemia with appropriate antibiotics, study of bacteriological profile and their antibiotic sensitivity pattern plays a significant role. In our country pre-existing data on both early and late onset sepsis has been shown great diversity in the changing patterns of the organisms and their sensitivity patterns.

Based on this consideration, a study was carried out to investigate the causative bacterial organisms of Neonatal sepsis and to assess their antibiotic susceptibility pattern in the Neonatal unit of Paropakar Maternity and Women's Hospital, Thapathali, Kathmandu, Nepal.

## Materials and Methods

A cross-sectional prospective study was conducted at the Microbiological Laboratory of Paropakar Maternity and Women's Hospital from the period of December 2010 to April 2011. A total of 340 neonates (0 to 28 days of age) admitted with suspected cases of early onset sepsis (0-2 days of age, n=229) and late onset sepsis (>3-28 days of age, n=71) were investigated. One ml venous blood was drawn from the antecubital or femoral vein taking aseptic precautions by applying Povidone iodine and 70% alcohol at the site of vein puncture, and was inoculated into Brain Heart Infusion Broth (HiMedia, India) for culture. The specimens were transported within one hour to the Microbiology Laboratory. All positive blood cultures were identified by their characteristic appearance on their respective media, Gram staining reaction and biochemical reactions. Antimicrobial susceptibility testing was performed by disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI)<sup>6,7</sup>.

## Results

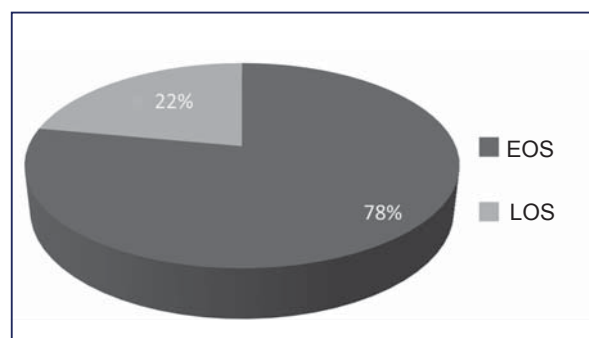
Out of 340 samples 69 were blood culture positive. The prevalence rate of Neonatal septicemia was found to be 20.3%. Among the total neonates 172 were males and 168 were female. Out of which growth positive cultures of male neonates were 36(52.17%) and female were 33(47.82%). There was no significant difference between the sex of the baby and growth of the organism ( $p>0.05$ ). Among the positive blood culture reports 50 of them were of EOS and 19 were LOS type (Figure 1).

Of 69 growth positive blood cultures, Gram positive cocci were the most predominant isolates (88.40%). Among gram positive cocci *Staphylococcus epidermidis* was the most common isolates (72.46%) followed by *Staphylococcus aureus* (7.24%), *Staphylococcus saprophyticus* (4.34%) and *Enterococcus faecalis* (4.34%). Gram negative bacilli were found in 11.60% of the growth positive samples of which *E.coli* and *Klebsiella spp* were found in 10.14% and 1.44% respectively (Table 1).

The predominant organism in both Early and Late onset sepsis was *S.epidermidis* (Table 2). There was no significant difference between the organisms isolated and the onset of Neonatal sepsis ( $p>0.05$ ).

The antibiotic sensitivity testing was performed for 69 isolates. The organisms isolated showed different sensitivity to the drugs used. Amikacin showed the highest sensitivity to all types of organisms isolated from the positive blood culture. Vancomycin sensitivity was highest for Gram positive organism and Gentamicin was most effective for Gram negative organisms isolated. Ampicillin was the resistant drug (Table 3).

Among the 69 isolates 48 (69%) were MDR (Table 4). Of the total MDR, the number of Gram negative MDR was 8 (100%) and Gram positive were 40 (65.57%).



**Fig 1:** Pie chart showing the distribution of neonatal septicemia based on the onset of disease

**Table 1:** Bacteriological profile of the positive blood culture

Organisms	Number	Percentage (%)
<b>Gram negative</b>		
<i>E.coli</i>	7	10.14
<i>Klebsiellaspp</i>	1	1.44
<b>Total</b>	8	11.59
<b>Gram positive</b>		
<i>S. aureus</i>	5	7.24
<i>S. epidermidis</i>	50	72.46
<i>S. saprophyticus</i>	3	4.34
<i>Enterococcus fecalis</i>	3	4.34
<b>Total</b>	61	88.40

**Table 2:** Bacteriological Profile of the EOS and LOS.

Organisms	EOS	LOS	p- value
<i>E.coli</i>	6 (12.00%)	1 (5.26%)	$p>0.05$
<i>Klebsiella spp</i>	1 (2.00%)	0 (0.00%)	
<i>S.epidermidis</i>	35 (70.00%)	15 (78.94%)	
<i>S.aureus</i>	3 (6.00%)	2 (10.52%)	
<i>S.saprophyticus</i>	3 (6.00%)	0 (0.00%)	
<i>Enterococcus fecalis</i>	2 (4.00%)	1 (5.26%)	
<b>Total</b>	50	19	

**Table 3:** Antibiotic Sensitivity Pattern of the Isolated Organisms

Antibiotics		Organisms					
		<i>E.coli</i> (n=7)	<i>Klebsiellaspp</i> (n=1)	<i>S.aureus</i> (n=5)	<i>S.epidermidis</i> (n=50)	<i>S.saprophyticus</i> (n=3)	<i>Str.fecalis</i> (n=3)
<b>Gentamicin</b>	R	4 (57.14%)	0 (0.00%)	5 (100%)	16 (32%)	1 (33.33%)	2 (66.67%)
	S	3 (42.85%)	1 (100%)	0 (0.00%)	34 (68%)	2 (66.67%)	1 (33.33%)
<b>Tobramycin</b>	R	6 (85.71%)	1 (100%)	2 (40%)	26 (52%)	2 (66.67%)	3 (100%)
	S	1 (14.28%)	0 (0.00%)	3 (60%)	24 (48%)	1 (33.33%)	0 (0.00%)
<b>Amikacin</b>	R	4 (57.14%)	0 (0.00%)	1 (20%)	15 (30%)	0 (0.00%)	3 (100%)
	S	3 (42.85%)	1 (100%)	4 (80%)	35 (70%)	3 (100%)	0 (0.00%)
<b>Vancomycin</b>	R	-	-	2 (40%)	17 (34%)	0 (0.00%)	0 (0.00%)
	S	-	-	3 (60%)	33 (66%)	3 (100%)	3 (100%)
<b>Ampicillin</b>	R	4 (57.14%)	1 (100%)	3 (60%)	21 (42%)	2 (66.67%)	2 (66.67%)
	S	3 (42.85%)	0 (0.00%)	2 (40%)	29 (58%)	1 (33.33%)	1 (33.33%)
<b>Cloxacillin</b>	R	-	-	1 (20%)	26 (52%)	0 (0.00%)	2 (66.67%)
	S	-	-	4 (80%)	24 (48%)	3 (100%)	1 (33.33%)
<b>Ciprofloxacin</b>	R	5 (71.42%)	0 (0.00%)	2 (40%)	17 (34%)	0 (0.00%)	2 (66.67%)
	S	2 (28.57%)	1 (100%)	3 (60%)	33 (66%)	3 (100%)	1 (33.33%)

**Table 4:** Multi Drug Resistance Rate

Organisms	Gram positive isolates	Gram negative isolates
Number	40	8
Percentage	65.57%	100%
<b>Total</b>	48	

## Discussion

The spectrum of organisms that cause neonatal sepsis changes over time and varies from region to region<sup>8</sup>. These organisms have also developed increasing multi-drug resistance over the last two decades<sup>9</sup>. Therefore knowledge of the pattern of bacterial isolates and their antimicrobial susceptibility pattern is useful for prompt treatment of patients. Among 340 samples, 69 (20.3%) showed positive blood culture. The growth positivity rate differs depending on the sample size and the time period of the study. Similar previous studies from home and abroad have reported the growth positivity varying from 5% to 70%<sup>10,11,12</sup>. In our study growth positivity rate was high among male neonates (52.17%) than female neonates (47.82%) ( $p>0.05$ ). Similar findings were reported from various places<sup>12,13,14</sup>. Male preponderance this could be because of the priority given to male babies for medical care in our society.

In the present study, 72.46% and 27.53% neonates presented with EOS and LOS, respectively. Similar results have been reported in Iran (77.5% vs. 22.5%)<sup>7</sup> and Bangladesh (70.7% vs. 29.3%)<sup>15</sup>, but in contrast with reports from Saudi Arabia (39% vs. 61%)<sup>16</sup>, Pakistan (42% vs. 58%)<sup>17</sup> and Nepal where late onset sepsis is more common<sup>13</sup>. This could be due to referral cases.

Gram Positive Cocci (88.40%) were predominant isolate followed by Gram negative bacilli (11.60%). In various other hospitals the organisms isolated differ from time to time. Similar findings were reported in Gaza hospital<sup>18</sup>. Coagulase-negative *Staphylococcus* was predominant in Taipei hospital<sup>19</sup>. In Johannesburg hospital Gram negative bacilli were isolated more in EOS and Coagulase negative *Staphylococcus* in LOS<sup>20</sup>. In New Delhi tertiary Hospital, *Klebsiella* (33.8%), *Enterobacter* (7.5%), *Alcaligenes faecalis* (4.9%), and *Escherichia coli* (4.6%) being the common microbes. *Staphylococcus aureus* (24.4%), followed by Coagulase-negative staphylococci (7.9%), were the major Gram-positive isolates<sup>21</sup>. Comparing the neonatal blood culture results in different studies, the common isolates may vary from place to place and institution to institution.

In early onset sepsis, gram positive and gram negative bacteria accounted for 86% and 14% respectively ( $p>0.05$ ). In late onset sepsis, only one isolate was Gram negative and remaining were gram positive bacteria. Comparable findings have been reported in other studies<sup>22,23,24</sup>.

In the present study the predominant organism from EOS and LOS are Coagulase negative staphylococci (CoNS). Studies from different countries report CoNS as the predominant organisms in LOS and among infants with indwelling central venous catheters from intensive care units<sup>25</sup>. Recovery of CoNS from blood of septicemic neonates needs to be reviewed with caution since most of them are regarded as contaminants. CoNS especially *Staphylococcus epidermidis* are the major normal flora of the skin and they can contaminate blood at the venipuncture site during collection of blood<sup>26, 27</sup>.

Classical initial (empiric) treatment of neonatal sepsis consists of a combination of penicillin (Benzylpenicillin, Ampicillin or Cloxacillin) and Aminoglycoside (most commonly Gentamicin)<sup>28, 29</sup>. In this study, Amikacin was found most efficient for all the isolates. Vancomycin sensitivity was highest for Gram positive organism and Gentamicin was most effective for Gram negative organisms isolated. Gram-negative bacteria showed high-level resistance to ampicillin. This observation is comparable to that of other researchers<sup>7,30,31</sup>.

Antimicrobial sensitivity patterns differ in studies and at different times. This is due to emergence of resistant strains as a result of indiscriminate use of antibiotic<sup>16,31</sup>. The high resistance rates in this study may be associated with frequent use of antibiotics for both prophylaxis and treatments of neonates in hospital. Multiple drug resistance (resistance to two or more drugs) was observed. In gram negative group, MDR was 8 (100%) and Gram positive were 40 (65.57%). Among the Gram positives, MDR was observed among *Staphylococcus aureus*, Coagulase negative staphylococci and while among the Gram negatives, MDR was observed in *Klebsiella spp* and *Escherichia coli*. Emerging multiple drug resistance has also reported in other parts of the world<sup>32, 33</sup>.

## Conclusion

The study showed the predominance of Gram positive cocci particularly coagulase negative Staphylococci as the causative agent of Neonatal sepsis. This may be a probable indication of nosocomial infection. The antimicrobial susceptibility pattern showed high occurrence of MDR strains. This suggests that empirical antibiotic therapy should be reviewed based on continuous antibiotic resistance surveillance.

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