

# A Prospective Study on Neonatal Sepsis in a Tertiary Hospital, Nepal

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## Abstract

About 20% of neonates develop sepsis and among them approximately 1% die due to sepsis-related causes. Bacterial pathogens are the commonest cause of neonatal sepsis which is either early onset ( $\leq 72$  hours of age) or late onset ( $> 72$  hours). Little is known about the epidemiology and antimicrobial susceptibility pattern of sepsis causing bacterial pathogens in Nepal. A prospective study was carried out among neonates suspected to have sepsis and admitted to Tribhuvan University Teaching Hospital from January to December 2016. Clinical suspicion of sepsis was made based on clinical findings and laboratory parameters, later confirmed by isolation of organisms in blood culture. Drug resistance pattern of Gram-positive and Gram-negative bacteria were studied by standard methods. Meropenem resistant Gram-negative bacteria were processed for the detection of  $\beta$ -lactamases and resistant genes were detected by X-pert Carba-R (Cepheid) Assays. Of 372 neonates with clinically suspected sepsis, 132 (35.4%) had blood culture positivity, with 47% early onset and 53% late onset sepsis. Coagulase negative *Staphylococcus aureus* (CONS) was the most common (37.9%) etiological agent followed by *Klebsiella pneumoniae* (12.9%). Of all 132 isolates, 81 (61.3%) were Gram-positive of which 22 (27.2%) were multi-drug resistant (MDR), three (3.7%) were methicillin-resistant *S. aureus* (MRSA), and 14 (17.2%) were methicillin-resistant CoNS; and 50 (37.8%) were Gram-negative of which 26 (52%) were MDR and 29 (58%) were resistant to  $\beta$ -lactamases. The  $bla_{KPC}$  gene was detected in four isolates of *K. pneumoniae*, two of *E. coli*, one ABC (*Acinetobacter baumannii* complex), and one *Enterobacter aerogenes* whereas  $bla_{NDM}$  gene was detected in one isolate of *K. pneumoniae*, two of *E. coli*, two *Pseudomonas aeruginosa*, one *Acinetobacter baumannii* complex, and one *Enterobacter aerogenes*. Overall mortality due to sepsis-related causes was 7.6% (10 of 132). One-third of clinically suspected neonatal sepsis cases were culture positive. Late onset sepsis was more common than early onset. CoNS was the predominant bacterial isolate followed by *Klebsiella pneumoniae*, with high rates of multi-drug resistance.

**Keywords:** Neonatal Sepsis, MDR, Nepal

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## INTRODUCTION

Neonatal sepsis (NS) is a clinical syndrome presented with clinical features of infection with or without underlying bacteremia in the first month of life.<sup>5</sup> It is a leading cause of neonatal morbidity and mortality worldwide.<sup>1</sup> About 30-50% of total neonatal deaths are due to NS in developing countries.<sup>2,3</sup> According to the World Health Organization (WHO) reports, about 20% of all neonates develop sepsis and approximately 1% die due to sepsis-related causes.<sup>3</sup> Those who survive are prone to short and long-term morbidity (neurodevelopmental).<sup>4</sup> Sepsis-related morbidity and mortality is largely preventable with the prevention of sepsis itself, with timely recognition of the disease and by appropriate and aggressive antimicrobial therapy and supportive care.

Neonatal sepsis (NS) is classified as early onset and late onset sepsis.<sup>5</sup>

Early onset sepsis (EOS): Presentation within the first 72 hours of life. Infants with EOS mostly present with pneumonia and respiratory distress. Maternal genital tract is the primary source of infection.<sup>5</sup> An increased risk of EOS is seen with some maternal-perinatal conditions.<sup>5</sup> Early diagnosis of sepsis can be done with the knowledge about these potential risk factors.<sup>5</sup>

Based on the studies in India, an increased risk of early onset sepsis has been seen with the following risk factors: (a) Febrile illness in the mother within two weeks prior to delivery with evidence of bacterial infection; (b) Prematurity or Low birth weight (<2500 grams); (c) Rupture of membranes >24 hours; (d) Single unclean or >3 sterile vaginal examinations during labor; (e) Prolonged labor (sum of first and second stage of labor >24 hours); (f) Foul smelling and/or meconium stained liquor; (g) Perinatal asphyxia (Apgar score <4 at one minute).<sup>5</sup>

Antibiotic treatment should be initiated for the newborn if there is foul-smelling liquor or three of the above-mentioned risk factors. If only two risk factors are present then infants should be investigated for possible infection and then treated accordingly.<sup>5</sup>

### Late onset sepsis (LOS)

Presentation after 72 hours of life. Nosocomial (hospital-acquired) or community-acquired can be source of infection in LOS.<sup>5</sup> Neonates' usual presentation are with pneumonia,

septicemia or meningitis. Predisposing factors that increased risk of nosocomial sepsis include prematurity, low birth weight, invasive procedures, mechanical ventilation, admission in intensive care unit, administration of parenteral fluids, and use of stock solutions.<sup>5</sup>

Neonatal sepsis can be classified into three major groups according to the criteria for the diagnosis of neonatal sepsis.<sup>6</sup>

- Proven sepsis: Infants with clinical signs and symptoms of infection plus a positive blood culture or PCR. For coagulase-negative *Staphylococcus aureus* (CoNS) either two positive blood cultures are required or one positive blood culture plus an increase in inflammatory markers like positive (i.e. >6 mg/dL) C-reactive protein (CRP).<sup>6</sup>
- Probable Sepsis: When blood culture is negative, presence of clinical signs and symptoms of infection with at least two abnormal laboratory results are required.<sup>6</sup>
- Possible Sepsis: When blood culture is negative, presence of clinical signs and symptoms of infection plus raised in inflammatory markers like CRP or IL-6/IL-8 level.<sup>6</sup>

The organisms that cause EOS of term and preterm infants together are Group B Streptococcus (GBS) and *E. coli*, which is responsible for approximately 70% of infections combined.<sup>5,6</sup> Intrapartum antibiotic prophylaxis which has decreased dramatically the incidence of early-onset GBS infection but it still remains a major cause of neonatal sepsis. Others organism that cause EOS are *Streptococcus viridans*, *Streptococcus pneumoniae*, *Enterococcus* spp., *Staphylococcus aureus* and Gram-negative enteric bacteria such as *Enterobacter* spp., *Haemophilus influenza* (virtually all non-typeable *Haemophilus* spp.), and *Listeria monocytogenes*.<sup>6</sup> The organisms associated with LOS are CoNS, *S. aureus*, *E. coli*, *K. pneumoniae*, *Enterobacter* spp., *Pseudomonas aeruginosa*, and *Acinetobacter* spp.<sup>7</sup>

Neonatal sepsis continues to remain a common and significant health care burden in low-resource settings, especially in vulnerable groups such as very-low-birth-weight infants (VLBW:<1500 grams). Despite ongoing efforts for early diagnosis, treatment, and prevention, it remains a challenge for clinicians due to frequent changes in epidemiology, non-specific symptoms,

lack of ideal diagnostic markers, and emergence of multi-drug resistant (MDR) pathogens.<sup>8,9</sup>

## METHODS

This study was conducted among neonates, both in-born and brought-in cases, admitted to the Neonatal Intensive Care Unit (NICU) and non-NICU units (neonatal unit and neonatal ward) at the Department of Pediatrics in Tribhuvan University Teaching Hospital (TUTH) from January to December 2016. All newborns with clinical suspicion of sepsis were enrolled into the study and their body samples (blood, urine, cerebrospinal fluid, pus, sputum) were processed for sepsis work-up. In this study, blood cultures were only analyzed because blood culture positivity is a prerequisite for labelling any case a 'proven sepsis'.<sup>6</sup> Clinical suspicion of sepsis was made on the basis of following variables.<sup>6,10</sup>

Clinical variables – a) Temperature instability; b) tachypnea plus grunting or desaturations; c) tachycardia or bradycardia; d) plasma glucose >10 mmol/L; e) feeding intolerance; f) lethargy/altered mental status.

Hemodynamic variables – a) Blood pressure two Standard Deviation below normal for age (Systolic pressure <50 mmHg for newborn on Day-1, <65 mmHg for infants ≤1 month).

Tissue perfusion variables – a) Capillary refill time >3 seconds; b) plasma lactate >3 mmol/L.

In clinically suspected cases, additionally, the following inflammatory variables were investigated. Positivity for any two of them would make the case 'probable sepsis' in absence of blood culture positivity.

- I. Semi quantitative CRP test performed by Latex agglutination method. CRP level >6 mg/dL was considered positive.
- II. Total leukocyte count <5,000 or >30,000 cells per mm.<sup>3</sup>
- III. Micro-ESR >15 mm in first hour
- IV. Platelet count <100,000 cells/μL

Micro-ESR was performed in the bed side; total leucocyte count (TLC), differential count (DC), and platelet count were tested in the hematology laboratory; and blood culture and C-reactive protein (CRP) tests in microbiology laboratory by following the standard methods.<sup>11</sup>

One ml of blood was mixed with nine ml of Brain Heart Infusion (BHI) broth under aseptic condition then transported to the microbiology laboratory. BHI broth with blood samples were incubated at 37°C aerobically and observed for turbidity and other signs of bacterial growth. Sub-culture was done in Chocolate, Blood and MacConkey agar plates from turbid BHI broths and incubated at 37°C for 16-18 hours. The organisms were isolated and identified as Gram positive or Gram negative according to the American Society for Microbiology (ASM) guidelines.<sup>11</sup>

Results of Gram staining test were informed to the clinicians. Antibiotic susceptibility testing (AST) was done simultaneously with isolate identification and reports were dispatched accordingly. Patterns of MDR (i.e. organism is resistant to at least three groups of antimicrobial agents) were also noted.

In Gram positive organisms, methicillin resistance was identified according to Clinical and Laboratory Standard Institute (CLSI) guidelines. In Gram negative cases, cefotaxime resistant



**Fig. 1.** 9ml of BHI broth from left to right showing only BHI broth, BHI broth with 1ml of blood but without turbidity and BHI broth with 1ml of neonatal blood with turbidity.



**Fig. 2.** Tube coagulase test showing Negative and Positive result.

organisms were tested for Extended Spectrum  $\beta$ -lactamase (ESBL) and AmpC type  $\beta$ -lactamase. Modified Hodge test was performed in meropenem resistant organisms following the CLSI guidelines. Organisms that tested positive for Modified Hodge test were processed for the detection of Metallo- $\beta$ -lactamase (MBL), *Klebsiella pneumoniae* carbapenemase type  $\beta$ -lactamases (KPCs), and Oxacillinase type  $\beta$ -lactamases (OXA) drug resistance patterns according to the standard

guidelines.<sup>7,9</sup> For genetic confirmation of drug resistance mechanism, all of the isolates were processed for X-pert Carba-R (Cepheid) Assay. (Figures 1-10)

The patients included into the study were prospectively followed up until discharge or death. All reports were recorded in the case report forms (Performa).

IRB of Institute of Medicine, TUTH has approved this study. Informed written consent was taken from each patient's parent or guardian before enrollment into the study. Descriptive analysis of data was done by SPSS Inc. version



**Fig. 3.** Biochemical reaction of *Burkholderia cepacia* complex (BCC).



**Fig. 4.** Methicillin resistant *Staphylococcus aureus* (MRSA) showing resistant to ceftazidime 30 microgram.



**Fig. 5.** Organism producing ESBL detected by combined disk method (zone size increased by >5mm in combination disk of ceftazidime plus clavulanic acid in comparison to ceftazidime alone).

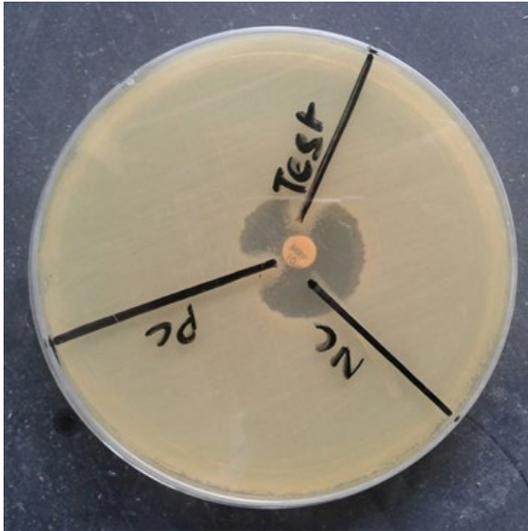


**Fig. 6.** Organism producing AmpC detected by ceftazidime (CX 30) and ceftazidime (CX 30) plus phenyl boronic acid (zone size increased by >5mm in CX 30 plus phenyl boronic acid in comparison to CX 30 alone).

21.0.<sup>12</sup> Results were interpreted as frequency distribution and percentages.

## RESULTS

During one-year study period, 372 neonates were admitted to the neonatal units with the diagnosis of suspected sepsis, 59.1% were male and 40.9% were female; (52.4%) were  $\leq 72$  hours age and (47.6%) were  $>72$  hours age.



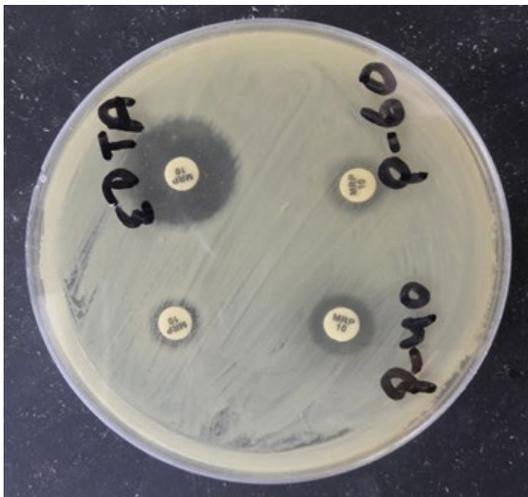
**Fig. 7.** Modified Hodge test showing positive result. (Test and positive control show indentation).

Of 372 suspected sepsis cases, 132 (35.5%) had a positive and 240 (64.5%) had a negative blood culture. Nearly 47% of culture positive cases fell into early-onset category and 53% (70 of 132) into late-onset sepsis category.

Overall, the most common isolates were coagulase negative *Staphylococcus* (CoNS) (37.9%, 50 of 132) followed by *Klebsiella pneumoniae* (12.9%, 17 of 132). Fig. 1. In EOS, CoNS and *Klebsiella pneumoniae* were most common isolates, whereas *Staphylococcus aureus* and *Enterococcus* species were most common in LOS. Table 1.

Following treatment, 92.4% (122) of culture positive neonates got improved and discharged from the hospital, whereas 7.6% (10) got expired. Of 122 improved cases, 56 (45.9%) were EOS and 66 (54.1%) were LOS; of 10 expired cases, 6 (60%) were EOS and four (40%) were LOS. Of all culture-positive sepsis cases, 33 (25%) were preterm, five (3.8%) were low birth weight, six (4.5%) had perinatal asphyxia, and five (3.8%) were preterm with premature rupture of membranes (PROM). Nearly half (48.5%) of the neonatal sepsis cases had no risk factors of neonatal sepsis. Table 2.

As high as 36.6% of bacterial organisms causing neonatal sepsis were found to be multi-drug resistant (MDR): 16.8% (22/131) of Gram



**Fig. 8.** Detection of MBL by using Meropenem and EDTA (Meropenem plus EDTA disk showing increase in zone diameter by  $>5$ mm in comparison to Meropenem disk only).



**Fig. 9.** Detection of KPCs by using Meropenem and phenyl boronic acid (Meropenem plus phenyl boronic acid disk showing increase in zone diameter by  $>5$ mm in comparison to Meropenem disk only).

positive and 19.84% (26/131) of Gram negative bacteria isolated. Table 3.

Among 16 *Staphylococcus aureus* isolates, three (18.7%) were methicillin resistant (MRSA) and among 50 CoNS isolates, 14 (18%) were methicillin resistant. Out of 50 Gram negative isolates of blood culture, eight (16%) were KPCs; ESBL, AmpC, and MBL were seven (14%) each. Xpert Carba-R Assays confirmed KPCs and New Delhi Metallo- $\beta$ -lactamase (NDM) by detecting bla<sub>KPC</sub> and bla<sub>NDM</sub> drug resistance genes respectively. The bla<sub>KPC</sub> gene was detected in four isolates of *K. pneumoniae*, two of *E. coli*, one ABC, and one *Enterobacter aerogenes*. bla<sub>NDM</sub> gene was detected in one isolate of *K. pneumoniae*, two isolates of *E. coli*, two isolates of *Pseudomonas aeruginosa*, one isolates of *Acinetobacter baumannii* complex (ABC), and one isolates of *Enterobacter aerogenes*.

## DISCUSSION

Neonatal sepsis continues to remain a major clinical problem with high rates of morbidity and mortality, especially in resource-poor countries like Nepal. In our study, more than one third (35.4%) of neonates had microbiologically confirmed sepsis. This finding is in agreement with other studies done in Nepal in which rates of culture-proven sepsis were 30.8% and 28.3%.<sup>7,13</sup>

The clinical signs and symptoms in neonates are rather subtle and nonspecific which makes it difficult for an early diagnosis of neonatal sepsis. Sepsis diagnosis may also be interfered by other life-threatening diseases in newborns such as perinatal asphyxia and necrotizing enterocolitis.<sup>14,15</sup> Blood culture is still the gold standard test for the definitive diagnosis of neonatal sepsis despite its drawbacks such as low sensitivity, time-consuming, and possible contamination especially with commensal CoNS.<sup>4</sup>

The rate of culture-proven neonatal sepsis (35.5%) is comparable to those reported in other developing countries of Asia and Africa: Nigeria (45.9%), Ethiopia (44.7%), Bangladesh (34.9%) and Uganda (37%)<sup>16-19</sup> However, negative blood culture does not rule out sepsis because about 26% of all neonatal sepsis could be due to anaerobic organism.<sup>20</sup> Other pathogens potentially causing sepsis such as viruses (for e.g. rubella, cytomegalovirus), protozoa (for e.g. *Toxoplasma gondii*), and treponema (for e.g. *Treponema pallidum*).<sup>4</sup>

The incidence of septicemia has been reported to be higher in males than in females.<sup>21</sup> In our study too, septicemia was found to be higher in males (59.1%) compared to females (40.9%), with male-to-female ratio of 1.4:1. In another study

Assay	Assay Version	Assay Type
Xpert Carba-R	2	In Vitro Diagnostic

Test Result:	IMP1 NOT DETECTED;
	VIM NOT DETECTED;
	NDM DETECTED;
	KPC NOT DETECTED;
	OXA48 NOT DETECTED

Analyte Name	Ct	EndPt	Analyte Result	Probe Check Result
SPC	35.4	59	NA	PASS
IMP1	0.0	2	NEG	PASS
VIM	0.0	2	NEG	PASS
NDM	35.9	117	POS	PASS
KPC	0.0	-7	NEG	PASS
OXA48	0.0	8	NEG	PASS

**Fig. 10.** Detection of NDM by using Xpert Carba-R Assay (Genetic confirmation by detecting bla<sub>NDM</sub> drug resistance gene).

from Nepal, male-to-female ratio was 1.2:1 in 2015 and 2:1 in 2003.<sup>7,13</sup>. Prevalence of gender bias in the society (especially while seeking health care), place of study, and sample-related factors could be the potential reasons for male preponderance.<sup>7</sup>

The proportion of late onset sepsis was higher than early onset sepsis in our setting (incidence of LOS vs. EOS: 53% and 47%, respectively). This is in agreement with previous studies.<sup>22,23</sup> Nagaveni et al., were reported that

LOS and EOS were 53.3% and 46.7%, respectively<sup>2</sup>, which is consistent with our results. In contrast, another study from India by Varsha et al. reported higher rate of EOS than LOS, which could be justified by a larger cohort of neonates in  $\leq 72$  hours age group (74.6%) compared to  $>72$  hours age group (25.3%).<sup>23</sup>

The most common organism isolated from infected neonates was CONS (37.9%) followed by *Klebsiella pneumoniae* (12.9%). This

**Table 1.** Age-wise distribution of blood culture isolates in 132 culture-positive cases

Culture isolates	>72 hours (LOS)	$\leq 72$ hours (EOS)	Total	
	No. (%)	No. (%)	No.	%
CoNS	22 (31.4%)	28 (45.2%)	50	37.9%
<i>Staphylococcus aureus</i>	12 (17.1%)	4 (6.5%)	16	12.1%
<i>Enterococcus faecalis</i>	6 (8.6%)	3 (4.8%)	9	6.8%
<i>Enterococcus faecium</i>	5 (7.1%)	1 (1.6%)	6	4.5%
<i>Escherichia coli</i>	4 (5.7%)	2 (3.2%)	6	4.5%
<i>Klebsiella pneumoniae</i>	8 (11.4%)	9 (14.5%)	17	12.9%
<i>Klebsiella oxytoca</i>	0	1 (1.6%)	1	0.8%
BCC	3 (4.3%)	2 (3.2%)	5	3.8%
ACBC	5 (7.1%)	4 (6.5%)	9	6.8%
<i>Citrobacter freundii</i>	2 (2.9%)	2 (3.2%)	4	3%
<i>Enterobacter aerogenes</i>	1 (1.4%)	2 (3.2%)	3	2.3%
<i>Pseudomonas aeruginosa</i>	2 (2.9%)	3 (4.8%)	5	3.8%
<i>Candida albicans</i>	0	1 (1.6%)	1	0.8%

CoNS: Coagulase-negative *Staphylococcus aureus*; BCC: *Burkholderia cepacia complex*; ACBC: *Acinetobacter calcoaceticus baumannii complex*.

**Table 2.** Risk factors of neonatal sepsis in 132 culture-positive cases

Risk factors	Frequency (%)
No risk factor	64 (48.5%)
Pre-term	33 (25.0%)
Perinatal asphyxia	6 (4.5%)
Pre-term with premature rupture of membranes (PROM)	5 (3.8%)
Low birth weight (LBW)	5 (3.8%)
Congenital anomaly with surgical intervention	4 (3.0%)
Congenital anomaly	4 (3.0%)
Meconium stained liquor	4 (3.0%)
LBW with mechanical ventilation	2 (1.5%)
Other interventions	2 (1.5%)
PROM with perinatal asphyxia	1 (0.8%)
Mechanical ventilation	1 (0.8%)
PROM	1 (0.8%)

finding is in agreement with studies done in India and Egypt which showed CONS as the predominant isolate in both EOS and LOS.<sup>21,4</sup> Contrary to our findings of CoNS and *Klebsiella pneumoniae* as the most common isolates in EOS, a six-year analysis of neonatal sepsis cases in Taiwan have reported Group B Streptococci (GBS) (36%) and

**Table 3.** Proportion of multi-drug resistant organisms among 131 blood culture isolates

Bacterial isolates	Total	MDR isolates (%)
Gram positive organisms	81	22 (16.8%)
Gram negative organisms	50	26 (19.84%)
Total	131	48 (36.6%)

Note: One of the isolates of blood culture was *Candida albicans*.

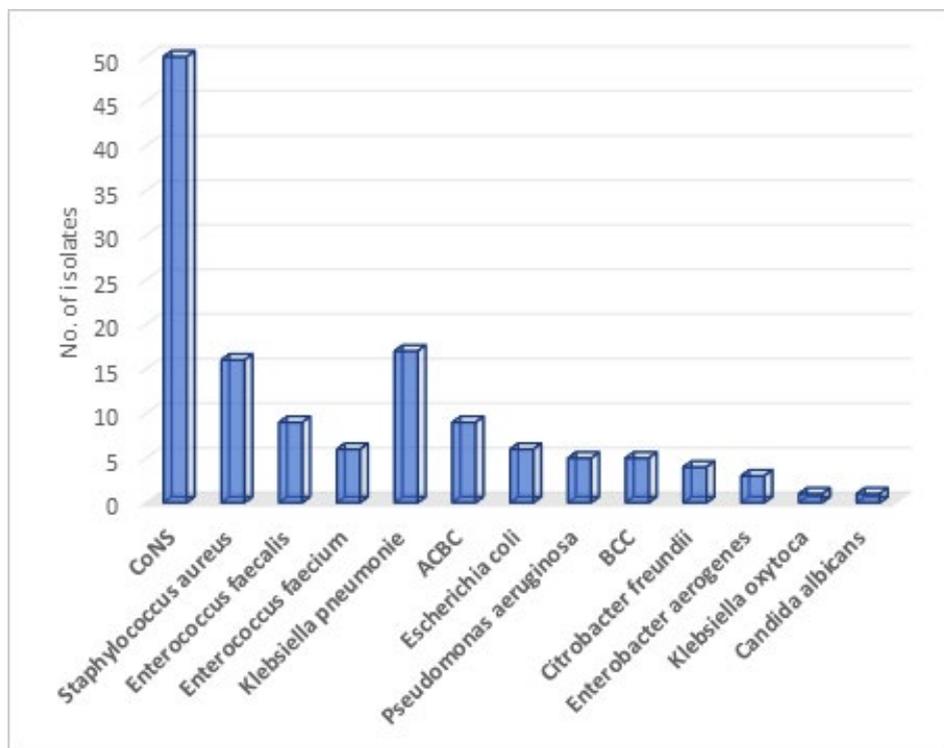
*Escherichia coli* (26%). Similarly, the most common organisms in LOS were *Staphylococcus aureus* and *Enterococcus* spp. whereas CoNS (40%) and *Candida* (15%) were most common in Taiwan.<sup>24</sup>

Prematurity, low birth weight, and perinatal asphyxia were reported in neonates with culture-positive sepsis. High mortality rates have been reported in neonates with these factors, especially in resource-poor settings.<sup>25</sup>

Sepsis is a leading cause of neonatal morbidity and mortality. In developing countries it accounts for 30-50% of total neonatal deaths.<sup>2,3</sup> It is estimated that about 1% of all neonates die due to sepsis-related causes.<sup>3</sup> In our study, neonatal mortality rate in probable sepsis cases was 7.6 % (10/132), of which 60% were EOS and 40% were LOS. The mortality rate in proven sepsis cases was 8.3% (10/120). These findings are in agreement with a study done in India by Nagaveni et al. which reported neonatal mortality rate of 14.6%, of which 56.3% had EOS and 43.7% had LOS.<sup>21</sup>

It was found that 90.9% (120 of 132) of culture-positive cases in this study were also positive for at least two of five inflammatory laboratory parameters—i.e. proven sepsis. Remaining 12 cases did not fulfil the criteria of sepsis although they had bacteremia. The lab parameters tested were: Total leucocyte count (TLC), platelet count, C-reactive protein (CRP), and micro-ESR. In a study done by Shah B. et al., sensitivity and specificity for TLC, platelet count, and CRP was found to be 44% and 92%, 22% and 99%, and 70-93% and 78-94% respectively. Shah B. et al. used CRP, procalcitonin, Interleukin-6 (IL-6), IL-8, tumor necrosis factor (TNF) alpha, and cell surface markers such as Cd11Beta, CD64 as the biomarkers of sepsis.<sup>8</sup>

Out of 132 blood culture isolates in our study, 81 were Gram positive, 50 were Gram negative, and one was *Candida albicans*. Among Gram positives, CoNS, *Staphylococcus aureus*, *Enterococcus faecalis* and *Enterococcus faecium*



**Fig. 11.** Identification of microorganisms isolated from Blood culture.

CoNS: Coagulase-negative *Staphylococcus aureus*; BCC: *Burkholderia cepacia* complex; ACBC: *Acinetobacter calcoaceticus baumannii* complex

were isolated, of which 22 (27.2%) were MDR. Of 16 *Staphylococcus aureus* isolates, 3 (18.7%) were MRSA and out of 50 CoNS isolates, 14 (18%) were methicillin resistant. In proven sepsis cases with Gram positive isolates, vancomycin and teicoplanin are the most effective drug of choice.

Among 50 Gram negative isolates of blood culture, 26 (52%) were MDR, eight (16%) were KPCs, and the rest were ESBL, AmpC, and MBL at 14% (seven each). The Gram-negative isolates were *Escherichia coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Burkholderia cepacia complex*, *Acinetobacter calcoaceticus baumannii complex*, *Citrobacter freundii*, *Enterobacter aerogenes*, and *Pseudomonas aeruginosa*.

Our study found a high rate of antimicrobial resistant bacterial isolates causing neonatal sepsis. The burden of multidrug-resistant organisms is rapidly emerging and it could be potentially disastrous in many settings like ours. Infection with such organisms could lead to treatment failure, higher morbidity and mortality rates, increased health costs, and prolonged hospitalization of patients.<sup>7,26</sup>

There are few limitations of the study. Blood samples were processed aerobically only, therefore, anaerobic and fastidious slow growing organisms were not grown. Only the phenotypic drug resistance mechanism was studied, so the genetic makeup of the resistant bacteria could not be established. Additionally, for sepsis screening, only four inflammatory markers were included out of eight recommended markers (variables like immature neutrophil, immature: total neutrophil ratio, pro-calcitonin, IL-6 or IL-8, and 16s PCR were not included). The inclusion of all markers could help in detecting sepsis even with blood culture negativity.

## CONCLUSION

In this study, just above one-third of clinically suspected sepsis cases were culture positive and CoNS was the predominant isolate followed by *Klebsiella pneumoniae*. Rate of multi-drug resistance was also high. Because clinical signs and symptoms are subtle in neonates, a clinical diagnosis of sepsis can be the challenge. Therefore, routine blood cultures along with early information about antibiotic susceptibility and

multi-drug resistance patterns could supplement clinical guidance to effective treatment.

## Summary of findings

- Out of 372 suspected sepsis cases, 32.3% (120 of 372) were proven sepsis.
- Blood culture positivity was higher among neonates admitted to NICU (43.8%) in comparison to non-NICU admissions (31.9%)
- The most common organism grown in blood culture was CoNS followed by *Klebsiella pneumoniae* and *Staphylococcus aureus*.
- LOS was more common than EOS (53% vs. 47%).
- *Staphylococcus aureus* was more commonly isolated in LOS than in EOS.
- MDR rate in the bacterial isolates of blood culture was 36.6% (48 of 131).
- Along with MDR pattern, ESBL, AmpC, KPCs, and MBL were also detected in Gram negative bacteria, and MRSA and methicillin resistant CoNS in Gram positive bacteria.
- Pre-term birth (25%), perinatal asphyxia (4.5%), low birth weight (3.8%) and PROM (3.8%) were reported in neonates with culture-positive sepsis.
- Mortality rate among blood culture positive neonatal sepsis cases was 7.6% (10 of 132).

## Research in context

### Evidence before this study

NS is one of the leading causes of neonatal mortality and morbidity worldwide. In developing countries, it is responsible for 30-50% of total neonatal deaths. According to the WHO reports, about 20% of all neonates develop sepsis and among them approximately 1% die due to sepsis-related causes. No uniform data is available in Indian subcontinent including Nepal in relation to etiological agents of NS, mortality trend due to infection, and antimicrobial susceptibility pattern of bacterial isolates. Advanced microbiological tests are rarely available in low-resource countries such as Nepal.

### Added value of this study

This study has assessed the prevalence of culture-proven sepsis among all neonates admitted to the largest tertiary hospital of Nepal (TUTH, Kathmandu) over a one-year study period. Additionally, this study has explored the etiological agents of NS, and tracked the trend

of antimicrobial susceptibility and multi-drug resistance. Drug-resistant genes such as blaKPC and blaNDM have been detected in Gram negative bacterial isolates.

#### Implications of all the available evidence

Because clinical signs and symptoms are subtle in neonates, their clinical diagnosis of sepsis can be the challenge. Our study findings could be a reference for clinicians in resource-limited settings, who would be encouraged to order routine blood cultures in suspected sepsis cases. Additionally, it is important to get information about antibiotic susceptibility and multi-drug resistance patterns of sepsis-causing bacterial agents early on during case management.

#### ACKNOWLEDGEMENTS

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#### CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

#### FUNDING

None.

#### AUTHORS' CONTRIBUTION

RS collected data and processed lab reports. NPS, BMP, BR, and SB supervised the study. RS and SB interpreted the data. SB, SS and SS prepared manuscript drafts and reviewed comments. All authors have read and approved the final manuscript.

#### DATA AVAILABILITY

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

#### ETHICS STATEMENT

Ethical approval was taken from the Institutional Review Committee (IRC), Institute of

Medicine, Tribhuvan University Teaching Hospital, Kathmandu, Nepal.

#### INFORMED CONSENT

Informed consent was taken from the legal guardian of the neonates.

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