

# Bacterial Isolates and its Antibiotic Susceptibility Pattern in NICU

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## ABSTRACT

### Background

Neonatal sepsis is one of the major causes of morbidity and mortality among the newborns in the developing world.

### Objectives

To determine the common bacterial isolates causing sepsis in neonatal intensive care unit and its antibiotic susceptibility pattern.

### Methods

A one year descriptive prospective study was conducted in neonatal intensive care unit to analyse the results of blood culture and to look into the sensitivity of the commonly used antibiotics.

### Results

The blood culture yield by conventional method was 44.13% with nosocomial sepsis accounting for 10.79%. 84.08% were culture proven early onset sepsis and 15.95% were late onset sepsis. Klebsiella infection was the commonest organism isolated in early, late and nosocomial sepsis but statistically not significant. Gram positive organisms were 39.36% in which Staphylococcus aureus was the leading microorganism followed by coagulase negative staphylococcus aureus. Gram negative organisms were 60.64% amongst them Klebsiella was the most often encountered followed by Pseudomonas. The most common organism Klebsiella was 87.5% and 78.3% resistance to ampicillin and gentamycin respectively. Among gram negative isolates 87.5% and 77.2% were resistance to ampicillin and gentamycin respectively. Among gram positive isolates 58.5% and 31.5% resistance were noted to ampicillin and gentamycin respectively. Resistance to cefotaxim to gram negative and gram positive isolates were 87.34% and 59.35% respectively.

### Conclusion

Klebsiella is most common organism which is almost resistance to first line antibiotics. Resistance to both gram negative and gram positive isolates among firstline antibiotics and even with cefotaxim is emerging and is a major concern in neonatal intensive care unit.

## KEY WORDS

*Antibiotic susceptibility, bacterial isolates, neonates*

## INTRODUCTION

Neonatal sepsis is one of the major causes of morbidity and mortality among the newborns in the developing world.<sup>1</sup>

Babies are more susceptible than mothers and infections in infants are more difficult to detect.<sup>2</sup> It is estimated that 26% of newborn infants who die do so as a result of infections that occur around birth.<sup>2</sup> After the first week of life, infections are the main cause of neonatal death in many countries. These are mostly acquired either in hospital as a complication of treatment for other perinatal conditions, or at home.<sup>2</sup> Zaidi et al review information about pathogens associated with neonatal sepsis in developing countries.<sup>3</sup> Community-based data are almost completely lacking for the early neonatal period (ie, newborns in the first week of life). Most published data are from hospitals where *Klebsiella* species, *Escherichia coli* (*E. Coli*), and *Staphylococcus aureus* (*S. aureus*) are the most common causes of infection.

Antimicrobial resistance patterns of neonatal pathogens are reported in another paper by Thaver et al.<sup>4</sup> These authors cite concerns about the emerging resistance of *Klebsiella* and *E. coli* to commonly used antibiotics.<sup>3,4</sup> The surveillance of blood stream pathogens in a hospital is important in monitoring the spectrum of microorganisms that invade the bloodstream and the types of organisms associated with a particular clinical discipline. Such data are often used to alert clinicians to emerging pathogens that may pose a threat to the community.

## METHODS

A descriptive prospective study was conducted in neonatal intensive care unit (NICU) of Dhulikhel Hospital, Kathmandu University Hospital from January 2010 to December 2010. Institutional approval was taken for the study. All the case of positive blood cultures was included in the study and skin commensals and contaminants were excluded. Case of early onset neonatal sepsis was defined as an infant who had clinical signs of sepsis if it presents during the first seven days of life and "Late onset" if it occurs after the first week and nosocomial sepsis (nosocomial infection as infections occurring after three days of life which are not directly acquired from mothers genital tract).<sup>5</sup> If there is deteriorating clinical picture or no response to antibiotic therapy over a reasonable period then repeat cultures was performed. Blood culture sample included a single sample collected from peripheral vein under aseptic conditions. A single specimen of two to three milliliter of blood was collected aseptically for culture from all cases. The specimens were inoculated directly into Brain Heart Infusion broth. The inoculated broth bottles were transported immediately to the department of microbiology and incubated at 37°C. The subcultures were done on blood and MacConkey agar plates on days 1, 2, 3, 5, and 7. The colonies isolated were identified by their colonial

morphology. Gram's stain, conventional biochemical tests. Antibiotic resistance pattern of the isolates was studied by using Modified Kirby Baur disc diffusion technique. Proven sepsis was diagnosed, when in addition to the clinical and laboratory signs of sepsis a true pathogen (except coagulase-negative staphylococci species (CoNS) that had to be isolated from at least two different specimens or with only one positive culture adequate antibiotic treatment had to be given for more than 72 hours) was isolated from a normally sterile body fluid.<sup>6</sup>

After obtaining blood for culture, the neonates were administered intravenous ampicillin and gentamycin as the first line of antibiotics. Antibiotic therapy was continued or changed based on the isolation of organisms in the blood culture and sensitivity pattern. This study was undertaken to determine the profile and antibiotic sensitivity pattern of aerobic isolates from blood culture of neonates in NICU. Data were analysed using SPSS 17.

## RESULTS

During the one year study period 213 neonates (male:female=1.5:1) were screened for sepsis.

Among our neonate, low birth weight (LBW) was 115 (54%) and birth weight >2500 gram were 98(46%). There were 71(33.3%) preterm and 142(66.7%) term. Inborn were 166(77.93%) and out born 47(22.06)%. Out of 213 neonates, 107 had positive blood culture but when the contamination / skin commensal were excluded the total number of organism isolated was 94. Among culture positive neonates, 26 were diagnosed as nosocomial culture proven sepsis. The overall incidence of bacteremia was 44.13%. Nosocomial sepsis accounted for 10.79% of the isolates. Among the 94 culture positive patient 84.08% were early onset sepsis and 15.95% were late onset sepsis. Gram negative organisms were 57(60.64%) and gram positive organisms were 37(39.36%).

**Table 1. Pattern of micro-organisms isolated from blood culture.**

Micro-organism	Number (%)	Early onset sepsis (n=79)	Late onset sepsis (n=15)	p value
<i>Klebsiella</i>	27 (28.72)	24	3	0.735
<i>S. aureus</i>	17 (18.1)	16	1	1
<i>Enterobacter</i>	10 (10.6)	8	2	0.257
CoNS	9 (9.57)	7	2	0.219
<i>Pseudomonas</i>	9 (9.57)	8	1	1
<i>E. coli</i>	6 (6.38)	4	2	0.109
<i>Acinetobacter</i>	5 (5.3)	4	1	0.408
MRSA	5 (5.3)	5	0	1
<i>Enterococcus</i>	3 (3.2)	3	0	1
<i>Streptococcus</i> sp.	3 (3.2)	0	3	1
Total	94 (100)	79	15	

Table 1 shows distribution of organisms in early and late onset sepsis.

In the early onset sepsis, Klebsiella infection is the most common (p=0.735) followed by S. Aureus (p=1), where as in late onset sepsis, Klebsiella and Streptococcus species were the commonest followed by Enterobacter, Coagulase negative staphylococcus species (CoNS) and E coli.

In the gram positive cocci, S. aureus was the leading microorganism followed by CoNS. In the gram negative bacilli Klebsiella was the most often encountered followed by Pseudomonas and Enterobacter.

Among 94 patients, 89 had one episode and five had two episodes of positive blood culture.

Antimicrobial susceptibility of gram positive and negative isolates are shown in table 2 and 3.

**Table 2. Antimicrobial sensitivity pattern of gram negative isolates.**

Antibiotics	E.coli %	Klebsiella %	Pseudomonas %	Enterobacter %	Acinetobacter %
Ampicillin	NT	12.5	NT	25	0
Gentamycin	50	21.7	0	22.2	20
Amikacin	80	88.9	11.1	100	20
Cloxacillin	NT	NT	NT	NT	NT
Ciprofloxacin	50	69.9	10	62.5	25
Imepenem	100	100	88.9	100	100
Cefotaxime	33.3	5	0	25	0
Vancomycin	100	NT	NT	NT	NT
Ceftazidime	NT	0	40	0	NT
Piperacillin	NT	0	37.5	NT	0
Penicillin	NT	NT	NT	NT	NT
Aztreonam	0	0	0	0	0

NT-Not tested

Klebsiella had 87.5% and 78.3% resistance to first line antibiotic ampicillin and gentamycin respectively, however it was 100% sensitive to imepenem and 88.9% sensitive to amikacin.

Similarly Enterobacter, Enterococcus, Acinetobacter were resistant to ampicillin and gentamycin. Gram negative isolates were mostly sensitive to amikacin except Pseudomonas and Acinetobacter, both mostly sensitive to imepenem. All gram negative isolates were resistant to Aztreonam.

Antibiotic susceptibility of overall gram positive and negative organisms are shown in table 4.

Amikacin has relative better sensitivity to CoNS, S. aureus, methicillin resistant staphylococcus aureus (MRSA) and enterococcus except streptococcus.

All gram positive isolates had 100% sensitivity to vancomycin except CoNS where 75% sensitivity was seen. Ciprofloxacin was other alternative for gram positive isolates except for

**Table 3. Antimicrobial sensitivity pattern of gram positive isolates.**

Antibiotics	CoNS %	S. aureus %	Enterococcus %	MRSA %	Streptococcus sp %
Ampicillin	60	23.1	0	0	0
Gentamycin	90	85.7	50	66.7	50
Amikacin	88.9	92.3	100	83.3	0
Cloxacillin	57.10	91.7	NT	0	NT
Ciprofloxacin	100	80	100	0	100
Imepenem	0	100	100	NT	100
Cefotaxime	75	67.6	0	20	-
Vancomycin	75	100	100	100	100
Piperacillin	0	100	NT	NT	100
Penicillin	0	0	NT	NT	0
Aztreonam	0	NT	0	NT	NT

NT-Not tested

**Table 4. Overall Resistance of Gram negative and Gram positive organisms.**

Antibiotics	Gram (-)ve %	Gram (+)ve %
Ampicillin	87.5	58.5
Gentamycin	77.22	31.5
Amikacin	40	27.1
Cloxacillin	NT	50.4
Ciprofloxacin	56.58	24
Imipenem	2.22	25
Cefotaxime	87.34	59.35
Vancomycin	0	5
Ceftazidime	86.7	NT
Piperacillin	87.5	33.4
Penicillin	NT	100
Aztreonam	100	100

NT-Not tested

MRSA.

## DISCUSSION

The blood culture yield was (99/213) 46.47%. The culture yield is higher than study done in India (18%) but comparable to the similar study conducted in Western Nepal (44.9%) but lower than in study in Kathikeyan ( 51%) and Tallur et al (64%).<sup>7-10</sup>

Gram negative bacteria were the most common organisms isolated in present study accounting 60.64% and amongst them Klebsiella was the most often encountered followed by Pseudomonas. There are earlier study in agreement to present findings, which reported Klebsiella pneumoniae as the most common cause of bacteremia.<sup>11,12</sup> Mehta et al have reported the incidence of 80.96% for gram negatives.<sup>13</sup> Hence, in most of the studies, gram negative bacilli have taken over the gram positive organisms especially in

hospital settings.

In the early onset neonatal sepsis *Klebsiella* was commonest followed by *S. aureus* but statistically not significant. Similar results were shown in Karachi where *Klebsiella* (39.41%) and *Staphylococcus* (24.11%) were isolated.<sup>14</sup> Study done by Bhat Y R showed most common organism in early onset sepsis to be CoNS.<sup>7</sup> Study by Lee NC showed most common organism in early onset sepsis to be Group B *Streptococcus* (GBS) however culture was done by Bact/ALERT culture media.<sup>15</sup> *Streptococcus agalactiae* (Group B streptococcus, GBS) is the most common cause of neonatal sepsis in many countries, though low rates are reported from many low-income countries, especially those in south Asia. In gram-negative bacilli (*Escherichia coli*, *Klebsiella* spp., *Pseudomonas* spp., *Acinetobacter* spp.) and gram-positive cocci (such as *Staphylococcus aureus* and *Staphylococcus epidermidis*) are other important causes.<sup>3,16</sup> However, there are many difficulties in interpreting aetiological neonatal sepsis data, because many studies report selected populations of high-risk infants. Specimens from infants in the first 24 hours of life are also seriously under-represented, especially those from low birth-weight babies and babies born outside health facilities.<sup>3,17,18</sup> Intrapartum antibiotic prophylaxis against *S. agalactiae* has also led to a substantial change in the bacteria responsible for early onset neonatal sepsis; gram-negative bacilli and *Staphylococcus* spp. predominate in countries implementing these programs.<sup>19</sup> However, in our study no cases of GBS has been isolated during the study period.

In late onset neonatal sepsis, in our study *Klebsiella* and *Streptococcus* species were common followed by *Enterobacter*, CoNS and *E. coli*. Study in Karachi showed *Klebsiella* species (57.14%) followed by *Klebsiella pneumoniae* (14.28%) as commonest organism in late onset sepsis.<sup>14</sup>

Study in Kanti Children Hospital showed most common organism as *E. coli* followed by *S. aureus*.<sup>20</sup> However, there was statistically significant predominance of *S. aureus* in late onset sepsis.<sup>20</sup> Study in Patan hospital showed most common organism as CoNS (31%) followed by *Acinetobacter* (26.7%).<sup>21</sup> Study in Western Nepal showed most common organism in nosocomial as *Klebsiella* 46% followed by *Enterobacter* (15.5%).<sup>8</sup> However in our study most common organism causing nosocomial as *Klebsiella* 36.36%, *S. aureus* 31.5%. But many authors have reported CoNS to be the most common bacteria in nosocomial infection.<sup>7,22,23</sup>

Study in Patan Hospital showed among gram positive isolates, a significant proportion of both CoNS and nonhemolytic streptococcus were resistant to penicillin, amoxicillin, cloxacillin and cefotaxim. Among gram negative isolates in blood culture significant proportion of the isolates were resistant to commonly used antibiotics cefotaxim. More than 30% are resistant to cefotaxim and

more 50% are resistant to gentamycin.<sup>21</sup> In our study among gram negative isolates 87.5% and 77.2% were resistance to ampicillin and gentamycin respectively. 58.5% and 31.5% of gram positive isolates were resistance ampicillin and gentamycin respectively.

Resistance to cefotaxim to gram negative and gram positive isolates were 87.34% and 59.35% respectively hence resistance to third generation cephalosporin is also emerging.

Imipenem had less resistance pattern of only 2.2% and 25% in gram positive and negative isolates respectively. All of the gram positive isolates were sensitive to vancomycin and most gram negative organisms were susceptible to amikacin and imipenem similar to the study done by Lee et al.<sup>15</sup>

Polymicrobial sepsis was seen in 5.3% in our study which is slightly lower than 6.8% in study by Kumhar GD et al.<sup>24</sup> A combination of ciprofloxacin and amikacin appears to best choice for infection due to *Klebsiella* as by study done by Kumhar GD et al.<sup>24</sup>

The sensitivity pattern of *S. aureus* was similar to study done in Western Nepal where 100% sensitivity to imipenem and vancomycin. Contrast to study in Western Nepal resistant to ciprofloxacin was noted, but only 20% result was seen in our study and there was negligible resistance to aztreonam but in our study almost all isolates were 100% resistant.<sup>8</sup>

Treatment by ciprofloxacin is also indicated in multidrug resistant *S. aureus* in pediatric age group, but its use in neonates is still experimental due to lack of safety and data.<sup>9,25</sup> Aggarwal P et al concluded that the evaluated dose resulted in sufficient serum concentrations to eliminate common gram-negative pathogens but would have been insufficient for the treatment of *S. aureus* and *Pseudomonas aeruginosa* infections.<sup>26</sup> Multiple regression models adjusting for common neonatal characteristics like weight and gestational age at birth, were used in two studies and found no significant relation between the use of ciprofloxacin and the cartilage size of the right knee measured in ultrasound examination or the height at 12 months corrected age.<sup>25,27,28</sup>

In neonatology, the use of ciprofloxacin in life-threatening infections, although rare, is justified by the fact that clinical benefits largely outweigh the potential risks.<sup>29</sup>

## CONCLUSION

Most common organism in early, late and nosocomial sepsis in our set up was *Klebsiella* infection. A low susceptibility to commonly used antibiotics and gentamycin is a cause for concern. The antibiotic susceptibility profile suggested that for a given cohort the initial empirical choice of cloxacillin and in combination with amikacin was more rational. Vancomycin is still the drug of choice for gram positive organisms. Imipenem has good coverage for all gram

negative and most of the gram positive organisms.

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