

## THE STUDY OF BACTERIOLOGICAL PROFILE AND ANTIBIOGRAM OF NEONATAL SEPTICEMIA

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**ABSTRACT:** Septicemia accounts for a significant proportion of morbidity and mortality in the newborn and is therefore a major problem in pediatric practice worldwide. The objective of this study was to know the bacteriological profile and antibiogram of neonatal septicemia of NICU (Neonatal Intensive Care Unit) of CIMS. Under aseptic precautions, blood was drawn from 500 neonates with suspected septicemia and inoculated in biphasic media. Isolates obtained were identified as per standard protocol and antibiotic susceptibility was done by Kirby Bauer disc diffusion method as per CLSI (Clinical and laboratory standards institute) guidelines. A total number of 78(15.6%) patients had positive blood cultures. The most common pathogens isolated were *Staphylococcus aureus* (n=22, 28.20%) followed by *Escherichia coli* (n=19, 24.3%), *Enterobacter spp* (n=10, 12.82%), *Coagulase negative Staphylococcus* (n=9, 11.53%), *Proteus spp.* (n=8, 10.25%), *Pseudomonas spp.* (n=5, 6.41%), *Acinetobacter spp* (n=4, 5.12%), *Klebsiella spp* (n=1, 1.28%). The Gram negative organisms were more resistance to commonly used antibiotics like Cefotaxime, Ampicillin but highly sensitive to Imipenam. The Gram positive bacteria showed high resistance to Chloramphenicol, Penicillin but they were highly susceptible to Vancomycin and Gentamycin. As the Gram negative organisms were the most common isolates in neonatal septicemia, As there is increasing incidence of resistant strain in common isolates of neonatal septicemia.

**KEYWORDS:** Neonatal sepsis, Blood culture, Antibiogram, Bacteriological profile.

**INTRODUCTION:** Neonatal septicaemia is one of the commonest causes of neonatal mortality and morbidity throughout the world. The world health organization (WHO) estimates that 85% of newborn deaths are due to infections including sepsis, pneumonia and tetanus. It is also estimated that 20% of all neonates develop sepsis and is responsible for 30-50% of total neonatal death in developing countries.<sup>1-3</sup>

Incidence of neonatal septicaemia varies from 2.2/1000 live births in developed countries to 10- 50/1000 live births in developing countries, though underreporting is common on both.<sup>4</sup>

Gram negative neonatal septicemia are more common and are mainly caused by *Klebsiella*, *Escherichia coli*, *Enterobacter*, *Proteus spp.*, *Pseudomonas*. The gram positive organisms, *Staphylococcus aureus*, *Coagulase negative staphylococci (CONS)*, *Streptococcus pneumonia* and *Streptococcus pyogenes* are most commonly isolated.<sup>2,5</sup>

Neonates are considered immunocompromised in view of their relatively immature immune defense mechanisms. Specifically they have quantitative as well as qualitative deficiency in their humoral immunity. The preterm neonate is at further risk, as trans placental transfer of antibodies starts after 32 weeks of gestation and endogenous synthesis does not begin until about 24 weeks after birth.<sup>4</sup>

A number of studies of the microbial flora and sensitivity patterns in neonates from other parts of the world but we aimed to determine the Gram-positive and Gram-negative bacteriological

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profile of bacteremia and antibiotic susceptibilities in Neonatal Intensive Care Unit (NICU) of Chhattisgarh Institute of Medical Sciences Hospital CIMS (Bilaspur)

**MATERIALS AND METHODS:** The study was carried out between March 2013 to July 2015 in the Department of Microbiology, Chhattisgarh Institute of Medical Sciences (CIMS) Hospital Bilaspur. Blood for culture was collected aseptically from 500 clinically suspected septicemia. The predominant clinical presentations reported disclosed following symptoms like respiratory distress, refusal to feed, lethargy, restlessness and irritability, hypothermia or fever and seizures.<sup>6,7</sup> Cases admitted in NICU. One ml neonates blood was collected and inoculated into biphasic media (Pediatric use) Himedia, Mumbai.

The bottle was shaken gently and incubated at 37°C aerobically for a maximum period of 7 days. The bottles were observed daily and as soon as signs of growth like turbidity, air bubbles or colonies over the solid slant portion of the biphasic medium were detected in either medium, subculture was done on blood agar and MacConkey agar on 3<sup>rd</sup> and 5<sup>th</sup> day.

The identification tests for gram positive bacteria were gram stain, coagulase test, catalase test and for gram negative bacteria were gram stain, IMViC (Indole, methyl red, voges-proskauer, citrate), motility, urease, TSI, oxidase were done from an isolated colony. I used amino acid test for *Pseudomonas* and *Acinetobacter* spp.

Antibiotic sensitivity tests of the isolates were performed by the Kirby bauer disc diffusion method on mueller hinton agar for antibiotics according to CLSI (Clinical and laboratory standards institute) guideline.<sup>8</sup>

**RESULT:** Out of the 500 blood culture from neonates, 78(15.6%) showed bacterial growth and 422 samples were negative. Out of 78 bacterial isolate, 31(39.74%) were gram positive cocci and 47(60.25%) were gram negative bacilli.

The most common gram-positive bacteria causing septicemia infection was *Staphylococcus aureus* 22(28.20%) followed by coagulase-negative *Staphylococcus* (CONS) 9(11.53%). In gram-negative bacteria *Escherichia coli* 19(24.35%) was the most common pathogen followed by *Enterobacter* 10(12.82%), *Proteus* spp. 8(10.25%), *Pseudomonas* 5(6.41%), *Klebsiella* 1(1.28%) and *Acinetobacter* 4(5.12%) (Table 1).

Microorganisms	Number of Organisms	Percentage of Total Organisms
Gram-positive organisms	31	39.74%
<i>Staphylococcus aureus</i>	22	28.20%
Coagulase-negative <i>Staphylococcus</i> (CONS)	9	11.53%
Gram-negative organisms	47	60.25%
<i>Escherichia coli</i>	19	24.35%
<i>Enterobacter</i>	10	12.82%
<i>Proteus</i> spp.	8	10.25%
<i>Pseudomonas</i> spp.	5	6.41%
<i>Klebsiella</i> spp.	1	1.28%
<i>Acinetobacter</i> spp.	4	5.12%
Total	78	100%

**Table 1: Number and percentage of organisms isolated from blood culture**

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The tables I have provided represents the antibiotic sensitivity. In gram positive bacteria the antibiotic Sensitivity of Staphylococcus aureus and CONS (coagulase negative staphylococci) were Vancomycin (100%), Gentamycin (90.32%) (Table 2).

Sensitivity in gram negative bacteria were Imipenam (85.10%), Piperacillin/Tazobactum (65.95%). Klebsiella spp were 100% resistant to Ampicillin, Cefotaxime, Gentamycin (Table 3).

MICROORGANISMS			
Antibiotics	Staphylococcus AureusN=22	Coagulase negative StaphylococcusN=9	Total N=31
Vancomycin	22(100%)	9(100%)	31(100%)
Gentamycin	21(95.45%)	7(77.77%)	28(90.32%)
Erythromycin	13(59.09%)	6(66.66%)	19(61.29%)
Cefoxitin	12(54.54%)	2(22.22%)	14(45.16%)
Clindamycin	11(50%)	5(55.55%)	16(51.61%)
Penicillin	10(45.45%)	3(33.33%)	13(41.93%)
Cefazolin	9(40.90%)	5(55.55%)	14(45.16%)
Ciprofloxacin	9(40.90%)	6(66.66%)	15(48.38%)
Chloramphenicol	8(36.36%)	4(44.44%)	12(38.70%)

Table 2: Antibiotic susceptibilities of gram positive organisms

MICROORGANISMS							
Anti- biotics	Esche- richia Coli N=19	Enter obacter Spp. N=10	Proteus Spp. N=8	Pseu- domonas N=5	Kle- bsiella N=1	Acineto- bacter N=4	Total N=47
Amoxicillin/ Sulbactam	-	-	-	-	-	1(25)	1(2.12)
Ciprofloxacin	2(10.52)	3(30)	6(75)	2(40)	1(100)	2(50)	16(34.04)
Piperacillin/ .tazobactum	13(68.42)	4(40)	7(87.5)	3(60)	1(100)	3(75)	31(65.95)
Amikacin	12(63.15)	2(20)	8(100)	3(60)	1(100)	4(100)	30(63.82)
Gentamycin	9(47.36)	2(20)	8(100)	2(40)	0	1(25)	22(46.80)
Imipenam	18(94.73)	7(70)	6(75)	4(80)	1(100)	4(100)	40(85.10)
Ceftriaxone	-	-	-	2(40)	-	4(100)	6(12.76)
Ampicillin	2(10.52)	1(10)	2(25)	-	0	-	5(10.63)
Piperacillin	9(47.36)	5(50)	3(37.5)	2(40)	1(100)	2(50)	22(46.80)
Ticarcillin	-	-	-	4(80)	-	-	4(8.51)

Table 3: Antibiotic susceptibilities of gram negative organisms

**DISCUSSION:** Severe sepsis remains one of the leading causes of death in Neonates. Physical signs and symptoms, though useful in identifying possible cases, have limited specificity. Definitive diagnosis is by bacteriologic culture of blood samples to identify organisms and establish antibiotic susceptibility.

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We processed 500 blood samples from clinically diagnosed septicemia cases. The rate of bacterial isolation in blood culture in this study was 15.6% (78/500), This was in concordance with the other studies by Roy I et al.,<sup>9</sup> and Kayange N et al.<sup>10</sup> The incidence of culture-proven neonatal septicemia was 14.4 per 1000 live births. This was comparable to the study done by Karthi-keyan et al.<sup>11</sup> The weaker immune system in neonates and children explains this higher rate of isolation.<sup>12</sup>

In a report from Karachi, the incidence of gram positive and gram negative were almost equal.<sup>13</sup> In our study Gram negative bacterial isolates (60.25%) were more than Gram positive isolates (39.74%). This is in contrast to developed countries, where Gram positive bacteria were more commonly reported. This was in concordance with National Neonatal Perinatal Database (NNPD) (2003),<sup>14</sup> Aletayeb SMH et al.,<sup>15</sup> and Sundaram V et al.<sup>16</sup> Kamath et al,<sup>17</sup> reported that 71.8% of BSIs in India were caused by Gram negative bacteria, with Klebsiella species accounting for 16.4%, Pseudomonas spp. 13.6%, Escherichia coli 11.8%, Enterobacter spp. 11.4% and Acinetobacter spp. In our study we have found that the presence of Klebsiella spp was 1.28%, Pseudomonas spp 6.41%, Escherichia coli 24.35%, Enterobacter spp 12.82%, Acinetobacter spp 5.12%. Couto,<sup>18</sup> et al reported that 51.6% of blood stream infections in Brazil were caused by Gram-negative bacteria, with Klebsiella spp accounting for 26.6%, Escherichia coli 9.7% and Pseudomonas spp. 6.4%. Bizzarro et al,<sup>19</sup> reported that 32.8% of BSIs in the USA were caused by Gram-negative bacteria, with Escherichia coli accounting for 37%, K.pneumonia 17% and Pseudomonas aeruginosa 12%. Among the Gram negative organism Escherichia coli was the most common organism followed by Enterobacter and Proteus spp.<sup>20, 21, 22, 23</sup> In our country, the most frequent Gram-negative Microorganisms grown in blood cultures are Klebsiella spp. and Escherichia coli.<sup>24</sup> In our study we have found that the predominant isolates was Escherichia coli followed by Enterobacter spp, Proteus spp.

In this study, the predominant isolates was Staphylococcus aureus which is in agreement with other reports.<sup>11, 25</sup> Mustafa M,<sup>26</sup> reported that Gram positive bacteria were having better susceptibility to Amikacin (68%), Cephalosporins and Ciprofloxacin (63%); but were more resistant to Ampicillin (13.6%) and Gentamicin (45%) in their present study (2014). Sudarshan raj<sup>27</sup> reported that Gram negative bacteria were resistant to most commonly used antibiotics like Ampicillin (70.04%), Ofloxacin (56.09%) and Co-trimoxazole (80.48%). Resistance was least with Imipenem (0%), Netilmicin (29.26%) and Ceftazidime (25.60%). In our study, We have found that the gram negative bacteria were resistant to most commonly used antibiotics like Ampicillin (10.63%), Ciprofloxacin (34.04%), Cefotaxime (36.17%). Kamble R,<sup>28</sup> reported that Among the Klebsiella pneumonia (56.25%) were sensitive to Ciprofloxacin (64.28%) and Amikacin (75%), and 62.5% of the Klebsiella isolates was complete resistance to Ampicillin, Amoxyclav, Cefazolin, Cephalothin, Cefuroxime and Cefoperazone. 80% of Acinetobacter and Pseudomonas spp were sensitive to Piperacillin+Tazobactam (95.23%) while 90% of Acinetobacter and 86.67% of Pseudomonas spp. Were sensitive to Imipenem (95.65%). In our study all Gram negative bacteria were having considerable sensitivity to Imipenem (85.10%), Amikacin (83.82%) and Piperacillin/Tazobactam (65.95%). All Gram positive isolates were highly sensitive to Vancomycin (100%), Gentamycin (90.32%), Erythromycin (61.29%).

**CONCLUSION:** In the present study, Gram negative organisms were the predominant cause of neonatal sepsis. Escherichia coli was the most common species, followed by Enterobacter and Proteus species. The organisms were resistant to most commonly used antibiotics. Organisms responsible for neonatal sepsis are different from developed and developing countries. Periodic

antibiotic susceptibility studies will help pediatricians to choose an appropriate antimicrobial for empirical treatment of neonatal septicemia.

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