

Original Research Article

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## Bacteriological Profile and Clinical Outcome in Cases of Neonatal Sepsis

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### ABSTRACT

Neonatal septicemia is a clinically diagnosed condition having diverse etiology and changing antibiotic sensitivity pattern. The knowledge of common bacterial agents causing septicemia and their antibiogram is useful in deciding empirical antimicrobial therapy. The neonatal blood cultures were studied to know the bacterial species and their antibiotic sensitivity pattern. Aim of the study is to evaluate sensitivity pattern of bacterial isolates causing neonatal septicemia and to know clinical outcome of the cases. Automated blood cultures of 250 neonates, clinically diagnosed to have septicemia were performed using BacT/ALERT 3D (BioMerieux). Bottles flagging positive were processed for identification and antibiotic susceptibility tests by Kirby Bauer disc diffusion method. Of the 250 cases studied, blood culture was positive in 117 (46.8%). Gram positive organisms were predominant (42.2%), followed by Gram negative organisms (29.7%). CoNS, *Klebsiella pneumoniae*, *Acinetobacter* spp., *Escherichia coli* were commonest organisms isolated accounting for 29.7%, 10.2%, 6.2% and 5.4% of cases respectively. All gram positive isolates were susceptible to vancomycin and linezolid. Most of gram negative isolates were susceptible to imipenem. Mortality rate was 10.4% with 65.4% blood culture positivity. Blood culture helps in rapid and early diagnosis of neonatal sepsis with early shift from empirical therapy to definitive treatment.

#### Keywords

Blood culture, Gram negative bacilli, Mortality, Neonatal sepsis, Treatment.

#### Article Info

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### Introduction

Children are always precious. Neonatal septicemia is an important and common cause of morbidity and mortality in neonates. World Health Organisation (WHO) estimates about four million neonatal deaths every year worldwide (WHO, 2005). The condition can be rapidly fatal if not treated promptly, aggressively and specifically. Treatment of sepsis is becoming more and more complicated because of increasing

antimicrobial resistance. Laboratory evaluation is the only way to reach accurate diagnosis as it is difficult to precisely diagnose the etiology of the condition clinically.

The main objectives of this study includes to identify the sensitivity pattern of the bacterial isolates and to correlate clinical outcome of the cases.

## Materials and Methods

This study was conducted at tertiary care hospital from October 2010 to July 2011 after taking clearance from institutional ethical committee. Blood samples were collected from 250 clinically diagnosed cases of neonatal septicemia admitted to Neonatal Intensive Care Unit. Sample was directly inoculated into pediatric automated blood collection bottle “pediatric” (yellow, 20 ml, BacT/Alert PF). The bottle was loaded in the BacT/ALERT 3D Microbial Detection System (bioMerieux, Durham, North Carolina). After bottle flagged positive, identification of bacteria was done by gram stain, culture and biochemical tests. Sensitivity of the bacterial isolates was performed by Kirby-Bauer’s disk diffusion method as per the Clinical Laboratory Standards Institute (CLSI) guidelines (CLSI Guidelines, 2011).ESBL detection was done by disc potentiation test (ceftazidime and ceftazidime - clavulanic acid combination). MRSA were identified by measuring zone around cefoxitin (30 µg) disk.

## Results and Discussion

Of the 250 cases studied, 117 cases yielded a positive blood culture giving a yield of 46.8%. A total number of 128 isolates were recovered from 117 positive blood cultures. Bacterial isolates accounted for 71.9% (92/128) while the yeasts accounted for 28.1% (36/128) isolations.

Among the bacterial isolates, gram positives outnumbered the gram negatives. 42.2% (54) isolates were gram positive while 29.7% (38) isolates were gram negative.

MRCoNS was the most common isolate among gram positive organisms (23.4%), followed by CoNS (6.3%). *Klebsiella pneumoniae* was the commonest gram negative isolate accounting for 10.2%,

followed by *Acinetobacter* spp. (6.2%) and *E. coli* (5.4%) (Table 1).

Table 2 shows the sensitivity pattern of Staphylococcal isolates in the study. All the isolates were sensitive to linezolid and vancomycin. Chloramphenicol and clindamycin were the next two better options. From the 30 MRCoNS, 27 were sensitive to chloramphenicol and 25 to clindamycin. Amongst the CoNS, all the eight isolates were sensitive to clindamycin, while seven were resistant to chloramphenicol. Linezolid, vancomycin, clindamycin and chloramphenicol were found to be the best for MRSA.

Table 3 shows in vitro sensitivity pattern of gram negative bacilli isolated from the samples. Imipenem was found to be the most dependable antibiotic. Chloramphenicol was the next antibiotic showing satisfactory sensitivity profile. For most of the other antibiotics, the sensitivity in the organisms was less than 50%.

All the *Klebsiella pneumoniae* and *Enterobacter cloacae* strains were found to produce ESBL. For all the other bacilli, 50 or more than 50% of the strains were ESBL producers (Table 4).

### Sensitivity pattern of *Streptococcus* spp. and *Enterococcus* spp.

Both the isolates were only five each in number and were all sensitive to linezolid and vancomycin. Chloramphenicol was effective in 80% of the isolates (4/5 isolates each).

The clinical outcome in all the 250 cases shows that 205 babies recovered and discharged however, 26 of the babies succumbed to infection. The fatality among the cases admitted with septicemia was 10.4%. Of the babies who succumbed to the infection, 65.4% had positive blood cultures.

Of the 205 babies recovered, 45.4% were positive for bacterial and fungal isolates (Table 5).

Sepsis, itself was a cause in nine babies (34.6%) with 66.6% blood culture positivity, followed by respiratory distress syndrome (26.9%) showing 42.8% positivity. Four babies were categorised under septic shock

with 100% positivity of blood culture and three babies as birth asphyxia.

Of the 26 babies died, 17 had shown positive blood culture. A total of 19 isolates were recovered from 17 blood culture samples. 78.9% of the isolates were bacterial, of which gram positive bacteria accounted for 36.8%, while gram negative for 42.1%.

**Table.1** Bacterial isolates in culture proven sepsis

Sl. No	Bacterial isolates (n = 128)	Number	%
<b>GRAM POSITIVE COCCI</b>			
1	CoNS	38	29.6
	MRCoNS	(30)	(23.4)
	CoNS	(8)	(6.3)
2	MRSA	6	4.7
3	<i>Streptococcus</i> spp.	5	3.9
4	<i>Enterococcus</i> spp.	5	3.9
	<b>Total</b>	<b>54</b>	<b>42.2</b>
<b>GRAM NEGATIVE BACILLI</b>			
1	<i>Klebsiella pneumoniae</i>	13	10.2
2	<i>Acinetobacter</i> spp.	8	6.2
3	<i>E. coli</i>	7	5.4
4	<i>Pseudomonas aeruginosa</i>	4	3.2
5	<i>Enterobacter cloacae</i>	3	2.4
6	NFGNB	2	1.6
7	<i>Sphingomonas</i> spp.	1	0.8
	<b>Total</b>	<b>38</b>	<b>29.7</b>
	<b>Yeasts</b>	<b>36</b>	<b>28.1</b>

MRCoNS was the most common isolate among gram positive organisms (23.4%), followed by CoNS (6.3%). *Klebsiella pneumoniae* was the commonest gram negative isolate accounting for 10.2%, followed by *Acinetobacter* spp. (6.2%) and *E. coli* (5.4%).

**Table.2** Sensitivity of *Staphylococcal* isolates

Sensitivity of Staphylococcal isolates			
	MRCoNS (%) (n=30)	CoNS (%) (n=8)	MRSA (%) (n=6)
Penicillin	0 (0%)	2 (25%)	0 (0%)
Erythromycin	5 (16.7%)	5 (62.5%)	2 (33.3%)
Clindamycin	25 (83.3%)	8 (100%)	6 (100%)
Gentamicin	5 (16.7%)	4 (50%)	1 (16.7%)
Tetracycline	16 (53.3%)	2 (25%)	5 (83.3%)
Ciprofloxacin	18 (60%)	6 (75%)	2 (33.3%)
Cotrimoxazole	11 (36.7%)	4 (50%)	2 (33.3%)
Chloramphenicol	27 (90%)	7 (87.5%)	6 (100%)
Vancomycin	30 (100%)	8 (100%)	6 (100%)
Linezolid	30 (100%)	8 (100%)	6 (100%)

**Table.3** Sensitivity pattern among gram negative bacilli

	Kp (n=13)	Aci (n=8)	Ec (n=7)	Ps (n=4)	Ecl (n=3)	NFGNB (n=2)	S (n=1)
<b>IPM</b>	11 (84.6%)	5 (62.5%)	7 (100%)	3 (75%)	3 (100%)	1 (50%)	1 (100%)
<b>AT</b>	0 (0%)	-	3 (42.9%)	1 (25%)	0 (0%)	1 (50%)	1 (100%)
<b>CTX</b>	0 (0%)	0 (0%)	2 (28.6%)	-	0 (0%)	1 (50%)	1 (100%)
<b>CX</b>	0 (0%)	-	2 (28.6%)	-	0 (0%)	1 (50%)	1 (100%)
<b>CTR</b>	0 (0%)	0 (0%)	3 (42.9%)	-	0 (0%)	1 (50%)	1 (100%)
<b>CAZ</b>	0 (0%)	0 (0%)	3 (42.9%)	1 (25%)	0 (0%)	1 (50%)	1 (100%)
<b>CAC</b>	2 (15.4%)	1 (12.5%)	4 (57.1%)	-	1 (33.3%)	1 (50%)	1 (100%)
<b>AMP</b>	0 (0%)	-	1 (14.3%)	-	2 (66.7%)	0 (0%)	1 (100%)
<b>CXM</b>	0 (0%)	-	2 (28.6%)	-	0 (0%)	1 (50%)	1 (100%)
<b>CIP</b>	6 (46.2%)	3 (37.5%)	5 (71.4%)	2 (50%)	2 (66.7%)	2 (100%)	1 (100%)
<b>GEN</b>	2 (15.4%)	0 (0%)	2 (28.6%)	1 (25%)	0 (0%)	1 (50%)	0 (0%)
<b>AK</b>	3 (23.1%)	0 (0%)	3 (42.9%)	2 (50%)	0 (0%)	1 (50%)	1 (100%)
<b>TE</b>	1 (7.7%)	2 (25%)	3 (42.9%)	-	0 (0%)	2 (100%)	1 (100%)
<b>COT</b>	5 (38.5%)	2 (25%)	2 (28.6%)	-	3 (100%)	1 (50%)	1 (100%)
<b>PI</b>	0 (0%)	1 (12.5%)	2 (28.6%)	1 (25%)	0 (0%)	1 (50%)	1 (100%)
<b>CPM</b>	0 (0%)	0 (0%)	3 (42.9%)	1 (25%)	0 (0%)	1 (50%)	1 (100%)
<b>C</b>	9 (69.2%)	-	5 (71.4%)	-	2 (66.7%)	1 (50%)	1 (100%)

**Table.4** ESBL production

ESBL production			
Organism	Number	ESBL	%
<i>Klebsiella pneumoniae</i>	13	13	100.0
<i>E. coli</i>	7	4	57.1
<i>Enterobacter cloacae</i>	3	3	100.0
<i>Pseudomonas aeruginosa</i>	4	2	50.0
NFGNB	2	1	50.0
<i>Sphingomonas</i> spp.	1	0	0.0

*Acinetobacter* spp. was not tested for ESBL production

**Table.5** Clinical outcome of the cases

Clinical outcome	Number	%	Culture Positive	%	Culture negative	%
Death	26	10.4	17	65.4	9	34.6
Recovered	205	82.0	93	45.4	112	54.6
DAMA	19	7.6	7	36.8	12	63.2
<b>Total</b>	<b>250</b>	<b>100</b>	<b>117</b>	<b>-</b>	<b>133</b>	<b>-</b>

**Table.6** Cause of death

Cause of death	Number	%	Blood culture positive (%)
Sepsis	9	34.6	66.6%
Respiratory distress syndrome	7	26.9	42.8%
Septic Shock	4	15.4	100%
Birth asphyxia	3	11.5	33.3%
Others (occipital encephalitis, congenital heart disease and congenital anomaly)	3	11.5	33.3%
<b>Total</b>	<b>26</b>	<b>100</b>	

**Table.7** Organisms responsible for death

Organism	Death	%
<b>Bacteria</b>	<b>15</b>	<b>78.9</b>
<input type="checkbox"/> <b>Gram positive</b>	<b>7</b>	<b>36.8</b>
MRSA	2	10.5
MRCoNS	2	10.5
<i>Streptococcus</i> spp.	2	10.5
<i>Enterococcus</i> spp.	1	5.3
<input type="checkbox"/> <b>Gram-negative</b>	<b>8</b>	<b>42.1</b>
<i>E. coli</i>	3	15.8
<i>Acinetobacter</i> spp.	2	10.5
<i>Klebsiella pneumoniae</i>	1	5.3
<i>E. cloacae</i>	1	5.3
<i>Pseudomonas aeruginosa</i>	1	5.3

Neonatal sepsis is a life threatening emergency and any delay in treatment may lead to fatality. The organisms causing neonatal sepsis vary from hospital to hospital and from one community to other. These differences could be due to the characteristics of the population, their religious faiths, customs and habits, pattern of antimicrobial agents used and local and nursery practices. Sepsis contributes to 30-50% of neonatal deaths in developing countries. Ballot *et al.*, (2012) showed CoNS to be the predominant organisms accounting for 19.1% followed by *Klebsiella pneumoniae* 12.1% and *Acinetobacter* spp. 10.9%. Ghotaslou *et al.*, (2007), showed CoNS as the most frequent

isolate followed by *Klebsiella pneumoniae* (16.6%).

The study done by Macharashvili *et al.*, (2009), showed 100% sensitivity to vancomycin for *S. aureus* and *S. epidermidis* isolates. Ballot *et al.*, (2012), reported a similar picture with 100% vancomycin sensitivity. The emergence of MRSA poses a serious threat to use of routinely administered antimicrobials which necessitates the use of vancomycin as the useful drug for management of these cases. Vancomycin is an exclusive drug in the treatment of gram positive infections especially against MRSA and *Enterococci*. In the present study,

linezolid has shown excellent efficacy that is complete absence of resistance in staphylococci including MRSA.

Vancomycin needs to be protected. It should be used exceptionally. Linezolid which is well tolerated in neonates is a better alternative. Therefore, for multi drug resistant staphylococcal infections linezolid may be considered instead of vancomycin. Coagulase negative Staphylococci are emerging as causative organisms of neonatal septicemia and recovery of these pathogens should no longer be considered as a contaminant especially in preterm and low birth weight babies with longer hospital stay and exposure to diagnostic and supportive procedures (Baptiste *et al.*, (2011).

The present study showed the emerging carbapenem resistance in the gram negative bacilli. The study by Viswanathan *et al.*, (2008) also showed 6.6% of emerging carbapenem resistance among gram negative isolates. In that study, Gram-negative organisms showed 100% resistance to ampicillin, cefotaxime, and gentamicin. According to Vinodkumar *et al.*, (2008), majority of gram negative organisms showed resistance to one or more antibiotics. According to the study done by Prabhu *et al.*, (2003), gram negative bacilli showed high resistance to ampicillin (64.28%). Cefotaxime or ceftazidime with amikacin was the most appropriate drug for multi resistant strains of gram negative bacilli.

Resistant bacteria are emerging worldwide as a real threat to the favourable outcome of common infections in community and hospital settings. High morbidity, prolonged hospital stay as well as high mortality are observed in resistant gram negative infections. There is a tendency among clinicians to directly begin the therapy with carbapenems like imipenem. A variety of

mechanisms like ESBL production, Amp - C, metallo  $\beta$ -lactamase etc, have led us to a frightening situation. Organisms harbouring multiple mechanisms simultaneously are not uncommon. Acquisition of carbapenem resistance by recalcitrant organisms has created pan-resistant strains. Pan resistance is not a mere fiction but is a reality being experienced by many tertiary care centres. In the present study, we encountered two *Klebsiella pneumoniae*, three *Acinetobacter* spp. and one *Pseudomonas aeruginosa* and one NFGNB that were resistant to imipenem. This is a clear indication of futility of using carbapenems for empirical therapy for septicemia.

According to Shakil *et al.*, (2010), 36.5% of *Klebsiella pneumoniae* and 28.6% of *E. coli* isolates were ESBL producers.<sup>10</sup> Sehgal *et al.*, (2007) showed that 61.3% of gram negative bacteria were showing ESBL production. The problem of antibiotic resistance is worsening worldwide. The widespread and injudicious use of broad-spectrum beta-lactam antibiotics has led to a marked increase in the incidence of ESBL-producing gram-negative organisms. This has necessitated the use of expensive drugs such as carbapenems. Thus, management of such cases is a major challenge to the clinicians.

According to Viswanathan *et al.*, (2008), out of 216 suspected cases, 29 babies died with mortality rate of 13.4%.<sup>7</sup> According to Chacko *et al.*, (2005), case fatality rate was 19.4% with 13.3% culture positivity rate. Early positive blood culture helps us to identify high risk babies.

According to Khatun *et al.*, (2012), birth asphyxia was the main cause contributing to 42% followed by sepsis and pneumonia (27%) and birth trauma (7%). The study done by Lahariya *et al.*, (2010), showed 24.9% of deaths due to sepsis and pneumonia followed



by asphyxia in 18.5% and prematurity/LBW in 16.8% of cases. Neonatal sepsis is the single most important cause of death in the community. Sepsis related neonatal deaths are preventable if diagnosed and treated early.

According to the study done by Sriram *et al.*, (2011), 57.1% of deaths were caused by gram negative organisms in late onset sepsis cases and 44.4% of the deaths were caused by gram positive organisms in early onset sepsis. According to the study done by Ballot *et al.*, (2012), mortality was significantly associated with gram negative organisms. Study by Jun Ho Wu *et al.*, (2009), showed that high mortality rate was contributed by *Candida* spp. (15%) followed by gram positive and gram negative organisms (8% and 7%) respectively. The majority of the death was contributed by gram negative bacilli, which were multi resistant to commonly used drugs, indicating their high degree of virulence (Tables 6 and 7).

Conclusions of the study are as follows

Antimicrobial agents are the most important tool for managing infectious diseases. The wide availability of antibiotics and inappropriate use of broad spectrum antibiotics in the community has led to the development of multidrug resistant bacteria. The antibiotic resistance is a global problem. The emerging multidrug resistant organisms like *Acinetobacter* spp., ESBL, AmpC, metallo  $\beta$ -lactamase producing gram negative bacteria pose a threat in treatment of infections due to these isolates.

The number of *Acinetobacter* spp. recorded in the present study were eight, accounting for 6.2% of the isolates. Two babies succumbed to the infection. Though the number is small, 25% of the *Acinetobacter* spp. infected babies could not be saved. High vigilance to rapidly

identify these infections is critical in the management of neonatal sepsis.

There is requirement of simple and sustainable intervention to reduce the burden of multidrug resistant pathogens causing sepsis. The implementation of hand washing, barrier nursing and antibiotic stewardship policies is need of time.

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