

Bacteriological profile in neonatal sepsis in a tertiary care NICU: A one-year prospective observational study

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Abstract

Background: In developing countries, mortality among neonates is very high, among which neonatal sepsis accounts for variable figures ranging from 26 to 50%. Neonatal sepsis contributes to 20% of neonatal mortality in India. The incidence of sepsis in hospital-based studies is 30 per 1000 live births and in community based studies, the incidence is 2.7-17% of all live births. Early diagnosis of neonatal sepsis is very difficult because the signs and symptoms are subtle and nonspecific leading to high rate of empirical antibiotic utilization. After detecting the causative agent of neonatal sepsis and their antibiotic sensitivity patterns, it is easy to choose appropriate therapy. **Objectives:** To determine the bacteriological profile of neonates with sepsis. To establish the culture and sensitivity pattern of the organisms **Materials and Methods:** A one year hospital based observational study was conducted at Tertiary care hospital. All neonates with suspected sepsis were subjected to blood culture and sensitivity. **Results:** A total of 50 sepsis screen positive cases were included of which 18 (36%) were culture positive. In those 18 positive cases 8 babies were found to have Gram Negative sepsis and the 10 babies had Gram Positive sepsis. Among culture positive sepsis, 7 (38.9%) cases were early onset sepsis and 11 (61.1%) cases were late onset sepsis. Among 10 gram positive organisms, majority cases were CONS and enterococci, among 8 Gram Negative organisms majority cases were klebsiella. **Conclusion:** Staphylococcus and Klebsiella were the predominant organisms which were resistant to first line empirical antibiotic therapy. Hence the choice of antibiotics for empirical therapy should be guided by routine antimicrobial surveillance for treatment of Neonatal sepsis.

Key Word: neonatal sepsis.

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INTRODUCTION

In developing countries, mortality among neonates is very high, among which neonatal sepsis accounts for variable figures ranging from 26 to 50%. Neonatal sepsis contributes to 20% of neonatal mortality in India¹. The

incidence of sepsis in hospital-based studies is 30 per 1000 live births and in community-based studies, the incidence is 2.7-17% of all live births^{2,3}. Neonatal sepsis is defined as any sepsis diagnosed during first 28 days of life. It is further classified as early onset sepsis if, signs and symptoms of sepsis appeared within 72 hours and classified as late onset sepsis if clinical features of sepsis are presented after 72 hours². Causes of neonatal sepsis include gram positive and gram negative bacteria and few fungi like Candida species. The spectrum of bacteria causing neonatal sepsis is significantly changing over time even in the same place. In developing countries multidrug resistant bacteria causing neonatal sepsis are increasing particularly in ICU's. Early diagnosis of neonatal sepsis is very difficult because the signs and symptoms are subtle and nonspecific leading to high rate of empirical antibiotic utilization. After detecting the causative agent of neonatal

sepsis and their antibiotic sensitivity patterns, it is easy to choose appropriate therapy.

MATERIALS AND METHODS

A one year hospital based observational study was conducted at NICU in a Tertiary care hospital.

By reviewing literature, a standardized questionnaire was prepared to collect demographic data, risk factors and clinical features of sepsis.

The indications for drawing samples for blood culture were:

1. Clinical features in the neonate (1 or more features): lethargy, apnea, tachypnea, tachycardia, hypotension, instability of temperature, poor feeding, poor perfusion, and abdominal distension².
2. Perinatal risk factors (1 or more features): maternal fever, prolonged rupture of the membranes for more than 24 hours, foul-smelling or meconium-stained liquor, or frequent (>3) unclean vaginal examinations, and/or having severe prematurity, or birth asphyxia necessitating active resuscitation².

RESULTS

During our observational study 50 sepsis screen positive were included in which 36% (18) positive results of neonatal sepsis were isolated. In those 18 positive cases 8 babies were found to have Gram Negative sepsis and the 10 babies had Gram Positive sepsis

Table 1: showing gestation age of the neonates whom are included in study

Sex	Term (%)	Pre term (%)	Total (%)
Male	20 (40)	8 (16)	28 (56)
Female	16 (32)	6 (12)	22 (44)
Total	36 (72)	14 (28)	50 (100)

Table 2: showing gender of the neonates with culture positive sepsis

Sex	EOS (%)	LOS (%)	Total (%)
Male	5 (27.8)	7 (38.9)	12 (66.7)
Female	2 (11.1)	4 (22.2)	6 (33.3)
Total	7 (38.9)	11 (61.1)	18 (100)

From the above table, among culture positive sepsis, 7 (27.7%) cases were early onset sepsis and 11 (72.3%) cases were late onset sepsis.

Table 3: Organisms isolated from blood culture

	Isolated Bacteria	EOS	LOS
1	Staph aureus	0	2
2	CONS	0	3
3	Enterococcus	2	1
4	Klebsiella	4	1
5	Staph hemolyticus	0	1
6	Staph hominis	0	1
7	Pseudomonas	1	1
8	Acinatobacter	0	1

From the above table majority of EOS was caused by gram negative organisms and LOS was caused by gram positive organisms

SAMPLE COLLECTION

Procedure: The sample was collected wearing sterile gloves prior to the procedure. A patch of skin (approximately 5cm in diameter), over the proposed vein puncture site with 70% isopropyl alcohol followed by povidone Iodine and again by alcohol povidine Iodine to be applied in concentric circles moving outward from the centre. The skin was allowed to dry at least 1 minute before the sample is collected. 1 ml blood was collected for blood culture bottle containing 5 to 10 ml of culture broth. Since samples collected from indwelling lines and catheters are likely to be contaminated, cultures were collected only from a fresh vein puncture site. All blood cultures were incubated for at least 7 days before they are reported as sterile. Blood cultures were incubated aerobically at 37°C and sub-cultured every alternate day on to blood agar and MacConkey agar up to 5 days. If there was any growth in the blood agar and MacConkey’s agar colonies were subjected to gram stain. Depending on gram stain it was further sub cultured. Using different techniques like Gram stain reaction and biochemical reaction properties organisms were identified. Antimicrobial sensitivity testing was performed by Kirby Bauer diffusion method using Muller Hinton Agar with incubation of 24 hours at 37°C according to clinical laboratory standard Institute standards.

Table 4: Showing Gram positive and Gram negative organisms

Gram positive	Total (%)	Gram negative	Total (%)
CONS	3 (30)	Klebsiella	5 (62.5)
Enterococcus	3 (30)	Pseudomonas	2 (25)
Staph aureus	2 (20)	Acinetobacter	1 (12.5)
Staph hemolyticus	1 (10)		
Staph hominis	1 (10)		
Total	10 (100)	Total	8 (100)

Among 10 gram positive organisms, majority cases were CONS (30%) and enterococci (30%). Among 8 Gram negative organisms majority cases were klebsiella (62.5%).

Table 5: Anti-microbial sensitivity pattern of isolated gram positive bacteria in NICU

Sl no	Antibiotic	CONS (n=3)	S.aureus (n=2)	Entero-coccus (n=1)	Staph Hemolyticus (n=1)	Staph Hominis (n=1)
1	Ampicillin	33%	0%	0%	0%	0%
2	Gentamicin	66%	50%	33%	0%	100%
3	Ceftriaxone	33%	50%	0%	0%	0%
4	Cotrimoxazole	66%	50%	0%	100%	100%
5	Vancomycin	66%	100%	100%	0%	100%
6	Chloramphenicol	33%	50%	0%	0%	0%
7	Clindamycin	33%	100%	0%	100%	0%
8	Erythromycin	33%	50%	0%	0%	0%
9	Ciprofloxacin	0%	50%	0%	0%	0%
10	Cloxacillin	0%	0%	0%	0%	0%

From the table, majority of Gram positive organisms were sensitive to cephalosporins, vancomycin, clindamycin and cotrimoxazole. 50% of organisms were sensitive to gentamicin.

Table 6: Antimicrobial Sensitivity pattern of isolated gram negative bacteria in NICU

Sl no	Antibiotic	Klebsiella (n=5)	Pseudomonas (n=2)	Acinetobacter (n=1)
1	Ampicillin	0%	0%	0%
2	Gentamicin	60%	100%	0%
3	Cefotaxime	0%	0%	0%
4	Cotrimoxazole	80%	0%	0%
5	Vancomycin	0%	0%	100%
6	Chloramphenicol	0%	0%	0%
7	Clindamycin	0%	0%	0%
8	Erythromycin	0%	0%	0%
9	Ciprofloxacin	40%	100%	0%
10	Colistin	100%	100%	100%
11	Meropenem	80%	100%	100%
12	Piperacillin- Tazobactam	80%	100%	100%

Majority of Gram negative organisms were sensitive to Piperacillin-Tazobactam and Meropenem. 37.5% of organisms were sensitive to both gentamicin and ciprofloxacin. All the organisms were found to be sensitive to colistin

DISCUSSION

For the diagnosis of neonatal sepsis blood culture remains the gold standard investigation to know the bacteriological profile and sensitivity pattern. In this prospective observational study of neonates with clinical sepsis 18 (36%) were culture positive, among them 10 (55.5%) were gram positive and 8 (44.5%) were gram negative. In a study conducted by Rajalakshmi *et al.* conducted in a SNCU at Kolkata with similar demographic profile had

comparable number of positive blood cultures (46.3%)⁴. In another study conducted in Northwest Ethiopia, the culture positive rates were 46.6%⁵ and the results reported from Addis Ababa were 44.7% and Egypt were 40.7%^{6,7} which was comparable to our study but it is lower than study done in Sudan (61.3%) and Yemen (57%)^{8,9}. On the other hand, culture positive rates in the present study were higher than the study done in Nepal (20.3%), Tanzania (19.2%) and Gondar (32.1%)^{10, 11, 12}. In our study

Klebsiella was the predominant gram-negative organism whereas Staphylococcal group was commonly isolated in the Gram positive group. These observations were similar to the study conducted at Northwest Ethiopia and Tanzania^{5, 13}. In NNPD Network, *Klebsiella spp.* (32%) was the leading pathogen, with the proportion of *Acinetobacter spp.* being a low 3%². In another cohort study conducted in multiple tertiary care centers, the commonly isolated organisms were *Acinetobacter* (22%), *Klebsiella* (17%) and *CONS* (15%)¹⁴. In a study conducted in South India tertiary care hospital by Bambala *et al.*, found that *Klebsiella pneumoniae* was isolated from 66% of culture positive cases followed by Coagulase-negative staphylococci in 12% of cases¹⁵. Another study conducted in a tertiary care centre at Topiwala national medical college also found that *Klebsiella pneumoniae* (35.4%) was leading cause of neonatal sepsis among the gram-negative pathogens and *Staphylococcus aureus* (22.9%) was the predominant among gram-positive pathogen¹⁶. Bacteriological profile of our study was similar to the above studies where *Klebsiella* and *Staphylococcus* were the predominant organisms isolated. In our study 94% of the organisms were resistant to Ampicillin, all organisms were resistant to Cloxacillin and 88.8% of the organisms were resistant to Cephalosporin while 50% of the organisms were sensitive to Gentamicin. Among Gram positive organisms 90% were sensitive to Vancomycin. The gram-negative organisms in this study were multi drug resistant (MDR) [Gram-negative isolates resistant to any three of five antibiotic classes (extended-spectrum cephalosporins, carbapenems, aminoglycosides, fluoroquinolones, and piperacillin-tazobactam) is defined as multidrug resistant]. 87.5% of Gram negative organisms were sensitive to Carbapenems and Piperacillin-Tazobactam and organisms resistant to carbapenem were sensitive to colistin (100%), ciprofloxacin (50%) and gentamicin (50%). In an Indian study by Paulin Packiaseli the resistance of gram positive organisms to amoxicillin and cephalixin was significantly increased¹⁷. In another study done by Rajlakshmi *et al.* 100% resistance (60/60) was noted for ampicillin, cefotaxime, and gentamicin and emerging resistance in Carbenepms which is similar to the observations made in our study. Similarly in a South Indian study majority of the *Klebsiella pneumoniae* isolates were resistant to all the antibiotics except Amikacin and Meropenem¹⁸. In a cohort study in delhi the prevalence of carbapenem resistance among *Klebsiella*, *Acinetobacter*, were 38% and 78% respectively. Eighty-one percent *Klebsiella*, 60% *Acinetobacter* and all isolated *Burkholderia cepacia* were multidrug resistant. High rates of multidrug resistance were also observed in 82% *Acinetobacter*, 54% *Klebsiella* and 38% *Escherichia coli*¹⁴. High proportion of resistance to common first and second

line antibiotics like ampicillin (98.5%), gentamicin (84.4%), amikacin (65.6%) and cefotaxime (81.3%) were also noticed in study by Viswanathan *et al.*¹⁹.

The present study also showed similar antibiotic sensitivity and resistance pattern as the above studies.

CONCLUSION

Staphylococcus and *Klebsiella* were the predominant organisms which were resistant to first line empirical antibiotic therapy. Due to advancements in bacterial culture techniques the rate of isolation of organisms has increased which has led to culture driven antimicrobial therapy and thereby preventing occurrence of resistance. Hence the choice of antibiotics for empirical therapy should be guided by routine antimicrobial surveillance for treatment of Neonatal sepsis.

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