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Original Article

Bacteriological Profile and Antibiotic Susceptibility Pattern of the Isolates among the Neonatal Septicemia in Northeast India

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Abstract

Background: Bacterial resistance to antibiotics was a global problem. Multidrug-resistant bacteria causing neonatal septicemias were increasing in the world. It was difficult to compare the bacterial profile and antibiotic susceptibility pattern of the isolates among the neonatal septicemia between countries because the epidemiology of neonatal septicemia was extremely variable.

Objective: Timely identification of bacterial profile and antibiotic susceptibility pattern of the isolates among the neonatal septicemias are essential to guide the clinicians regarding both the empirical and definitive treatments of neonatal septicemia.

Materials and Methods: Based on the AIIMS protocol 2014 of neonatal sepsis-World Health Organization newborn CC, an operational definition of clinically diagnosed neonatal septicemia was established for the selection of participants in the study for blood culture and sensitivity test (CST). Hence, in this study, blood CST was done only among the selected patients for clinically diagnosed neonatal septicemia as recommended in the National Committee for Clinical Laboratory Standards.

Results: This study observed that there was a shift from the predominance of Gram-negative organisms to Gram-positive organisms, especially *Staphylococcus aureus*. Acinetobacter and Citrobacter were emerging organisms.In this study, aminoglycosides and fluoroquinolones were sensitive to organisms, especially in Gram-negative organisms. Imipenem and meropenem were also sensitive in both Gram-positive and Gram-negative organisms. Imipenem was more sensitive to organisms than meropenem. Tobramycin, doxycycline, gatifloxacin, and chloramphenicol were more sensitive to organisms than erythromycin, azithromycin, and clindamycin.

Conclusion: Early clinical diagnosis and prompt initiation of empirical antimicrobials therapy to patients of pending culture sensitivity reports for definitive therapy may be life-saving. Hence, periodic surveillance for bacteriological profile and antibiotic susceptibility pattern of the isolates among the neonatal septicemia for appropriate choice of antimicrobials for empirical therapy can be outlined and reevaluated in a timely manner to save the life of 5 million neonatal deaths a year, with 98% occurring in developing countries and limited resource rural areas. This study concluded that empiric therapy for clinically diagnosed neonatal septicemia should cover both Gram-negative and Gram-positive organisms. Hence, the combination of one antibiotic from each of the following two groups, (1) Imipenam/piperacillin/cefotaxime and (2) amikacin/gentamicin/netilmicin, can be included as an initial therapy for neonatal septicemia.

Key words: Antibiotic resistance, Antimicrobial susceptibility pattern, Bacterial isolates, Blood stream infection, Neonatal sepsis

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INTRODUCTION

Septicemia in neonates refers to generalized bloodstream bacterial infection documented by positive blood culture in the first 4 weeks of life and is one of the four leading causes of neonatal mortality and morbidity in India.[1]

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The incidence of neonatal septicemia varies from country to country, but it is much higher in developing countries.[2]

According to the World Health Organization estimates, there are about 5 million neonatal deaths a year, with 98% occurring in developing countries.[3]

The spectrum of organisms that cause neonatal sepsis changes over times and varies from region to region. This is due to the changing pattern of antibiotic use and changes in lifestyle.[4]

According to the AIIMS Protocol 2014, neonates with sepsis may present one or more of the following symptoms and signs (1) hypothermia or fever; (2) lethargy, poor cry, and refusal to suck; (3) poor perfusion and prolonged capillary refill time; (4) hypotonia and absent neonatal reflexes; (5) bradycardia or tachycardia; (6) respiratory distress, apnea, and gasping respiration; (7) hypoglycemia and hyperglycemia; and (8) metabolic acidosis.

Antibiotic resistance is a global problem. Reports of multiresistant bacteria causing neonatal sepsis in developing countries are increasing. It is difficult to compare antibiotic resistance between countries because the epidemiology of neonatal sepsis is extremely variable.

Hence, periodic surveillance for bacteriological profile and antibiotic susceptibility pattern for appropriate choice of antimicrobials for empirical therapy can be outlined and reevaluated in timely manner to guide the clinicians.

The evaluation of organisms responsible for neonatal septicemia will be essential for the appropriate management of neonatal septicemias in the society and to save 5 million neonatal deaths a year, with 98% occurring in developing countries, especially in the limited resource rural areas.

Hence, it is rational to take up this study to determine the changing pattern of bacterial profile and antibiotic susceptibility of isolates among the neonatal septicemia in the Northeast India.

MATERIALS AND METHODS

This study was conducted in the neonatal intensive care unit (NICU), Department of Pediatrics, JNIMS, over 2 years from October 2016 to September 2018. The study was approved by the Institutional Ethical Committee of JNIMS. Based on the AIIMS protocol 2014 of neonatal sepsis-WHO newborn CC, an operational definition (OD) of clinically diagnosed neonatal septicemia was established for the selection of participants. The neonate presented with one or more of the following symptoms and signs (1) hypothermia or fever (former is more common in preterm), (2) lethargy, poor cry, and refusal to suck, (3) poor perfusion and prolonged capillary refill time, (4) hypotonia and absent neonatal reflexes, (5) bradycardia or tachycardia, (6) respiratory distress, apnea, and gasping respiration, (7) hypoglycemia or hyperglycemia, and (8) metabolic acidosis, but cannot be diagnosed clinically by any other possible causes than neonatal sepsis which was selected for clinically diagnosed neonatal septicemia of this study.

The following inclusion and exclusion criteria were used.

Inclusion Criteria

Those neonates presented with one or more of the above symptoms and signs of OD were selected for blood culture and sensitivity test (CST) of the study. Premature and matured babies of both the sexes in the age group of 1–28 days were included in the study.

Exclusion Criteria

Those neonates presented with one or more of the above symptoms and signs but can be diagnosed clinically by any other possible causes than neonatal septicemias were excluded from the study. Babies above 28 days of age were excluded from the study. The gender, caste, ethnicity, and race were not used as inclusion or exclusion criteria.

Those neonates fulfilled the above OD of clinically diagnosed neonatal septicemia were selected for blood CST. The trained doctors in the NICU draw blood from the selected neonates for culture blood CST under strict aseptic and antiseptic precaution before starting the antibiotics. The local site to draw the blood was cleaned with povidone iodine (1%) and washed by 70% alcohol. 3 ml of blood sample was only collected from a peripheral vein under aseptic and antiseptic precautions and inoculated into 20 ml of Brain Heart Infusion broth (HiMedia, India). The blood in the culture media was immediately sent to the Microbiology Department, JNIMS, for CST. Then, the selected neonate was treated with systemic antibiotics to save the life. Antimicrobial susceptibility testing was performed for all blood culture isolates by Kirby–Bauer disc diffusion method as recommended in the National Committee for Clinical Laboratory Standards.[5,6]

The investigator obtains the informed consent from the parents/guardian of the selected neonate. Then, only the selected neonates were enrolled in the study. The appropriate antibiotic of the neonatal septicemia was changed according to the blood CST report.

Data collection was done by the investigators. Bacteriological profile and antibiotic susceptibility pattern of the isolates

in the blood CST reports of the selected neonates were collected in a pre-designed pro forma, for observation and analysis of the study. Evaluations were done at the end of every 6 months and at the end of the study.

Data analysis was done by the statistician.

RESULTS

A total of 360 clinically diagnosed neonatal septicemia patients of both the sexes in the age group of 1–28 days were investigated for blood CST.

Table 1 shows that 33.33% (120 of 360) of blood CST were found to be blood culture positive neonatal septicemias. Of these, 61.66% (74 of 120) had early onset sepsis (EOS) and 38.33% (46 of 120) had late onset sepsis (LOS).

Table 2 shows that, of the 120 positive blood CST reports, *Staphylococcus aureus* 55% (66 of 120) was positive and was the most common isolated organism, followed by *Pseudomonas aeruginosa* 15% (18 of 120), *Acinetobacter* 15% (18 of 120), *Citrobacter* 6.66% (8 of 120), *Escherichia coli* 5% (6 of 120), and *Klebsiella pneumoniae* 3.33% (4 of 120).

Table 3 shows that *S. aureus* was the most common Gram-positive isolate. Gram-positive *S. aureus* isolates were sensitive to erythromycin (81.81%), tobramycin (78.78%), imipenem (78.78%), linezolid (72.72%), levofloxacin (75.75%), ceftriaxone (54.54%), vancomycin (48.48%), and cefotaxime (33.33%).

P. aeruginosa 15% (18 of 120), *Acinetobacter* 15% (18 of 120), *Citrobacter* 6.66% (8 of 120), *E. coli* 5% (6 of 120), and *K. pneumoniae* 3.33 % (4 of 120) were the most common isolated Gram-negative organisms in this study.

Gram-negative isolates were sensitive to aminoglycosides (gentamicin, amikacin, netilmicin, and tobramycin) and fluoroquinolone (gatifloxacin, ciprofloxacin, and levofloxacin).

P. aeruginosa 15% (18 of 120) is one of the most common Gram-negative organisms and is sensitive to imipenem (88.88%), meropenem (77.77%), amikacin (77.77%), and gentamicin (77.77%).

E. coli 5% (6 of 120) is sensitive to amikacin (100%), levofloxacin (100%), tobramycin (100%), gatifloxacin (100%), imipenem (100%), and doxycycline (100%).

K. pneumoniae 3.33% (4 of 120) is sensitive to amikacin (100%), gentamicin (100%), netilmicin (100%), piperacillin (100%), tobramycin (100%), gatifloxacin (100%), imipenem (100%) meropenem (100%), and chloramphenicol (100%).

Acinetobacter 15% (18 of 120) and Citrobacter 6.66% (8 of 120) were emerging organisms.

Acinetobacter 15% (18 of 120) is sensitive to amikacin (44.44%), gentamicin (100%), netilmicin (88.88%), piperacillin (44.44%), tobramycin (100%), gatifloxacin (88.88%), imipenem (100%), meropenem (55.55%), and chloramphenicol (77.77%).

Citrobacter 6.66% (8 of 120) is sensitive to amikacin (100%), gentamicin (100%), netilmicin (50%), piperacillin (75%), ceftriaxone (75%), gatifloxacin (75%), levofloxacin (75%), imipenem (75%), doxycycline (100%), and chloramphenicol (100%).

Aminoglycosides and fluoroquinolones were sensitive to organisms, especially in Gram-negative organisms.

Table 1: Distribution of blood CST results into two age groups of (1) 1–7 days of age, as EOS and (2) 8–28 days of age, as LOS

Sl no.	Total number of blood CST	Total number of positive blood CST (%) *n*=120	Positive blood CST in EOS (%) *n*=74	Positive blood CST in LOS (%) *n*=46
1	360	33.33 (120 of 360)	61.66 (74 of 120)	38.33 (46 of 120)

CST: Culture and sensitivity test, EOS: Early onset sepsis, LOS: Late onset sepsis

Table 2: Distribution of the isolated organisms among the 120 positive blood CST in neonatal septicemia

Isolated organisms in blood CST	Total number of the specific organism out of 120 positive blood CST	Frequency of isolates (%)
Staphylococcus aureus	66	55 (66 of 120)
Pseudomonas aeruginosa	18	15 (18 of 120)
Acinetobacter	18	15 (18 of 120)
Citrobacter	8	6.66 (8 of 120)
Escherichia coli	6	5 (6 of 120)
Klebsiella pneumoniae	4	3.33 (4 of 120)

CST: Culture and sensitivity test

Table 3: Distribution of the antibiotic sensitivity pattern of the isolates among the 120 positive blood CST in neonatal septicemias

Tested antibiotics in blood CST	1	2	3	4	5	6
	***Staphylococcus aureus* 55 % (66 of 120) %**	***Acinetobacter* 15% (18 of 120) %**	***Pseudomonas aeruginosa* 15% (18 of 120) %**	***Citrobacter* 6.66% (8 of 120) %**	***Klebsiella pneumoniae* 3.33 % (4 of 120) %**	***Escherichia coli* 5% (6 of 120) %**
Penicillin						
Ampicillin			33.33 (6 of 18)	75 (6 of 8)	50 (2 of 4)	33.33 (2 of 6)
Amoxicillin/ clavulanic	54.54 (36 of 66)					
Cefaclor	48.48 (32 of 66)					
Cefazolin						
Cefuroxime	60.60 (40 of 66)	44.44 (8 of 18)		75 (6 of 8)		33.33 (2 of 6)
Erythromycin	81.81 (54 of 66)					
Gentamicin	69.69 (46 of 66)	100 (18 of 18)	77.77 (14 of 18)	100 (8 of 8)	100 (4 of 4)	66.66 (4 of 6)
Cefixime	27.27 (18 of 66)	22.22 (4 of 18)	55.55 (10 of 18)	50 (4 of 8)		
Ceftriaxone	54.54 (36 of 66)	44.44 (8 of 18)	44.44 (8 of 18)	75 (6 of 8)	100 (4 of 4)	33.33 (2 of 6)
Ceftazidime		11.11 (2 of 18)	11.11 (2 of 18)			33.33 (2 of 6)
Cefotaxime	33.33 (22 of 66)					33.33 (2 of 6)
Levofloxacin	75.75 (50 of 66)	77.77 (14 of 18)	66.66 (12 of 18)	75 (6 of 8)	50 (2 of 4)	100 (6 of 6)
Clindamycin	57.57 (38 of 66)	11.11 (2 of 18)				33.33 (2 of 6)
Amikacin	69.69 (46 of 66)	44.44 (8 of 18)	77.77 (14 of 18)	100 (8 of 18)	100 (4 of 4)	100 (6 of 6)
Azithromycin	51.51 (34 of 66)					33.33 (2 of 6)
Vancomycin	48.48 (32 of 66)					33.33 (2 of 6)
Cefepime		33.33 (6 of 18)				33.33 (2 of 6)
Cefpirome		33.33 (6 of 18)	33.33 (6 of 18)	25 (2 of 8)		33.33 (2 of 6)
Imipenem	78.78 (52 of 66)	100 (18 of 18)	88.88 (16 of 18)	75 (6 of 8)	100 (4 of 4)	100 (6 of 6)
Meropenem	33.33 (22 of 66)	55.55 (10 of 18)	77.77 (14 of 18)	50 (4 of 8)	100 (4 of 4)	33.33 (2 of 6)
Teicoplanin	57.57 (38 of 66)					
Linezolid	72.72 (48 of 66)					
Tobramycin	78.78 (52 of 66)	100 (18 of 18)	33.33 (6 of 18)	50 (4 of 8)	100 (4 of 4)	100 (6 of 6)
Gatifloxacin	57.57 (38 of 66)	88.8 (16 of 18)	66.66 (12 of 18)	75 (6 of 8)	100 (4 of 4)	100 (6 of 6)
Piperacillin		44.44 (8 of 18)	44.44 (8 of 18)	75 (6 of 8)	100 (4 of 4)	66.66 (4 of 6)
Chloramphenicol		77.77 (14 of 18)	77.77 (14 of 18)	100 (8 of 8)	100 (4 of 4)	66.66 (4 of 6)
Doxycycline		66.66 (12 of 18)	55.55 (10 of 18)	100 (8 of 8)	50 (2 of 4)	100 (6 of 6)
Cefoperazone/ sulbactam	12.12 (8 of 66)	55.55 (10 of 18)	33.33 (6 of 18)	25 (2of 8)	50 (2 of 4)	
Netilmicin	12.12 (8 of 66)	88.88 (16 of 18)	66.66 (12 of 18)	50 (4 of 8)	100 (4 of 4)	66.66 (2 of 6)
Ciprofloxacin	27.27 (18 of 66)	66.66 (12 of 18)	66.66 (12 of 18)	50 (4 of 8)		33.33 (2 of 6)
Ticarcillin/ clavulanate			44.44 (8 of 18)	75 (6 of 8)		33.33 (2 of 6)
Ofloxacin		55.55 (10 of 18)	33.33 (6 of 18)	50 (4 of 8)	50 (2 of 4)	66.66 (4 of 6)
Nalidixic acid		33.33 (6 of 18)				
Aztreonam			22.22 (4 of 18)	25 (2 of 8)	50 (2 of 4)	
Tetracycline		22.22 (4 of 18)				
Cotrimoxazole						

CST: Culture and sensitivity test

Imipenem and meropenem were also sensitive to both Gram-positive and Gram-negative organisms. Imipenem was more sensitive to organisms than meropenem.

Piperacillin, ceftriaxone, cefoperazone/sulbactam, and cefixime were more sensitive to organisms than cefotaxime, ceftazidime, cefepime, cefpirome, cefuroxime, cefaclor, cefazolin, amoxicillin/clavulanic, and ampicillin.

Tobramycin, doxycycline, gatifloxacin, and chloramphenicol were more sensitive to organisms than erythromycin, azithromycin, and clindamycin.

DISCUSSION

Resistance of bacteria to antibiotics was a global problem. Multidrug-resistant bacteria causing neonatal septicemia were increasing in the world. It was difficult to compare the bacterial profile and antibiotic susceptibility pattern of the isolates among the neonatal septicemias between countries because the epidemiology of neonatal septicemia was extremely variable.

Shipra *et al.* reported that, in culture-proven septicemia, 55% of neonates presented with EOS and 45% presented with LOS. Gram-positive isolates were more as compared with Gram-negative isolates. The most common isolates were *S. aureus*, *S. epidermidis,* and *E. coli*. All Gram-positive isolates were sensitive to vancomycin and linezolid, while carbapenems and polymyxin B were the most effective drugs in the Gram-negative isolates. Mortality was higher in LOS as compared with EOS cases. Moreover, the difference was statistically significant.[7]

The present study observed that proven sepsis was 33.33% (120 of 360). Of these, 61.66% (74 of 120) had EOS and 38.33% (46 of 120) had LOS. This study showed that, of the 120 positive blood CST reports, *S. aureus* 55% (66 of 120) was positive and was the most common organism followed by *P. aeruginosa* 15% (18 of 120), *Acinetobacter* 15% (18 of 120), *Citrobacter* 6.66% (8 of 120), *E. coli* 5% (6 of 120), and *K. pneumoniae* 3.33% (4 of 120). In this study, aminoglycosides and fluroquinolones were sensitive, especially, in Gram-negative organisms.

West and Peterside reported that the antibiotic sensitivity testing showed that Gram-negative isolates were sensitive to meropenem and Gram-positive isolates to linezolid, netilmicin, and chloramphenicol. The most common bacteria causing neonatal sepsis was found to be Klebsiella. The Gram negative organisms showed the highest sensitivity to meropenem. Cefazolin was found to be most resistant antibiotic to Gram negative organisms. The Gram-positive samples showed highest sensitivity for linezolid, and penicillin and ampicillin were found to be most resistant. Best overall sensitivity among Gram-negative isolates was to imipenem (93%), followed by amikacin (52%) and netilmicin (41%). Gram-positive isolates had a sensitivity of 91% to linezolid, 68% to tetracycline, 64% to piperacillin/tazobactam, and 52% to ciprofloxacin.[8]

In this study, *S. aureus* was the most common Gram-positive isolate. Gram-positive *S. aureus* isolates were sensitive to erythromycin (81.81%), tobramycin (78.78%), imipenem (78.78%), linezolid (72.72%), levofloxacin (75.75%), ceftriaxone (54.54%), vancomycin (48.48%), and cefotaxime (33.33%). Aminoglycosides and fluoroquinolones were still sensitive to organisms, especially in Gram-negative organisms. Imipenem and meropenem were also sensitive in both Gram-positive and Gram-negative organisms. Imipenem was more sensitive than meropenem. Piperacillin, ceftriaxone, cefoperazone/sulbactam, and cefixime were more sensitive to organisms than cefotaxime, ceftazidime, cefepime, cefpirome, cefuroxime, cefaclor, cefazolin, amoxicillin/clavulanic,, and ampicillin. Tobramycin, doxycycline, gatifloxacin, and chloramphenicol were more sensitive than erythromycin, azithromycin, and clindamycin.

Antibiotic resistance is a global problem. Reports of multiresistant bacteria causing neonatal sepsis in developing countries are increasing. The wide availability of over-the-counter antibiotics and the inappropriate use of broad-spectrum antibiotics in the community may explain this situation.[3] This study observed that it was difficult to compare the bacterial profile and antibiotic susceptibility pattern of the isolates among the neonatal septicemias between countries because the epidemiology of neonatal septicemia was extremely variable.

There has been a shift from the predominance of Gram-negative organisms to Gram-positive organisms *S. aureus* in the past decade throughout the world, and the reason for which is not clear.[9-11] This present study observed a shift from the predominance of Gram-negative organisms to Gram-positive organisms (*S. aureus*).

Bhat *et al.* shown that although the Gram-negative organisms were most common in both EOS and LOS, but the incidence of Gram-positive sepsis was higher in LOS (21.89%) when compared to EOS (15.7%). *S. aureus* was the most common Gram-positive microbe in both EOS (7.3%) and LOS (17.41%). A low rate (2.24%) of enterococci infection was positive.[11] In the present study also, *S. aureus* was the most common microbe. A low rate of enterococci infection was also observed.

Ballot *et al.*, Kaufman and Fairchild, and Hoogen *et al.* reported the isolation of general purpose buffer in 54.9%,

68.2%, and 75%, respectively.[12-14] This study showed a preponderance of Gram-positive *S. aureus* 55% (66 of 120), which was in concordance with the previous studies.

The present study showed that *S. aureus* 55% (66 of 120) was the most common Gram-positive organisms which was high as compared to studies conducted by Agnihotri *et al.* and Sundaram *et al*.[15,16]

Shrestha *et al*., Jyothi *et al*., and Nepal *et al.* reported that Klebsiella and Acinetobacter were the most common organisms attributing to LOS.[17-19] This study showed that *S. aureus* 55% (66 of 120) was positive and was the most common isolated organism followed by *P. aeruginosa* 15% (18 of 120), *Acinetobacter* 15% (18 of 120), *Citrobacter* 6.66% (8 of 120), *E. coli* 5% (6 of 120), and *K. pneumoniae* 3.33% (4 of 120).

Tallur *et al.* observed that, among the maternal risk factors, the difficult delivery (32%) in the form of cesarean, forceps, or vacuum was the risk factors.[20] In our study, history of fetal distress, premature rupture of membrane, prolong labor, home delivery, and instrumental deliveries were the main risk factors for neonatal septicemia.

Draz *et al.* and Tsering *et al.* reported that the greater prevalence of resistance to commonly used antibiotics has also been observed.[1,21] Similar observations were seen in this study.

CONCLUSION

The resistance of bacteria to antibiotics was a global problem. Multidrug-resistant bacteria causing neonatal septicemias were increasing in the world. Early clinical diagnosis and prompt initiation of empirical antimicrobials therapy to patients of pending culture sensitivity reports for definitive therapy may be life-saving. Hence, a periodic surveillance for bacteriological profile and antibiotic susceptibility pattern of the isolates among the neonatal septicemias in different areas for appropriate choice of antimicrobials for empirical therapy can be outlined and reevaluated in timely manner to save the life of 5 million neonatal deaths a year, with 98% occurring in developing countries and limited resource rural areas.

This study observed that there was a shift from the predominance of Gram-negative organisms to Gram-positive organisms, especially *S. aureus*. *Acinetobacter,* and *Citrobacter* were emerging organisms. In this study, aminoglycosides and fluoroquinolones were sensitive to organisms, especially in Gram-negative organisms. Imipenem and meropenem were also sensitive to organisms in both Gram-positive and Gram-negative organisms. Imipenem was more sensitive to organisms than meropenem. Tobramycin, doxycycline, gatifloxacin, and chloramphenicol were more sensitive to organisms than erythromycin, azithromycin, and clindamycin. This study concludes that empiric therapy for suspected neonatal septicemia should cover both Gram-negative and Gram-positive organisms. Hence, the combination of one antibiotic from each of the following two groups such as (1) imipenem/piperacillin/cefotaxime and (2) amikacin/gentamicin/netilmicin can be a choice as an initial therapy for neonatal septicemia.

This study concluded that empiric therapy for clinically diagnosed neonatal septicemia should cover both Gram-negative and Gram-positive organisms. Hence, the combination of one antibiotic from each of the following two groups, (1) imipenem/piperacillin/cefotaxime and (2) amikacin/gentamicin/netilmicin, can be included as an initial therapy for neonatal septicemia.

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