Original Article

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Isolation of Pathogenic Aerobic Organism from the Blood of Septicemic Neonates and the Susceptibility of Isolates to Various Antibiotics Attending in TMMC & RC, Moradabad, India

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Abstract

Introduction: Neonatal septicemia can be described as, systemic bacterial infection in neonates documented by positive blood culture. Neonatal septicemia is a major cause of morbidity and mortality among the neonates. It may be classified on the basis of onset of sepsis, <3 days as early onset sepsis (EOS) and >3-28 days as late onset sepsis (LOS). EOS and LOS are caused by organisms that are prevalent in the maternal genital tract and environmental factors, respectively.

Materials and Methods: Venous blood was collected aseptically and inoculated in the blood culture bottle. Further isolation and identification was done by Standard Microbiological Guidelines. Antibiotic susceptibility was detected according to CLSI guidelines.

Results: In our study, out of 388 clinically suspected septicemic neonatal cases, 140 (36.08%) was blood culture positive. In our study, out of 140 positive blood culture, 92 (65.71%) were males and 48 (34.29%) were female. Among positive blood culture 97 (69.29%) were EOS and 43 (30.71%) were LOS and out of 140 positive blood culture 83 (59.29%) were Gram-positive organism and 57 (40.71%) were Gram-negative organisms. In our study, the most common organism was *Staphylococcus aureus* 60 (42.85%) and *Klebsiella pneumoniae* 23 (16.43%) was a second most common causative organism of neonatal septicemia. Among Gram-positive organism, the most sensitive antibiotic was vancomycin 80 (96.39%) and linzolid 72 (86.74%). Among Gram-negative organism, the most sensitive antibiotic was polymyxin B (87.71%) and chloramphenicol, tigecycline, and meropenem (27.36%).

Conclusion: *S. aureus* is the most common Gram-positive bacterium, and *Klebsiella* is the most common Gram-negative bacterium causing neonatal sepsis. There is an increasing trend of antibiotic resistance against the commonly used first-line drugs. Continuous surveillance of antibiotic susceptibility is needed to ensure proper empirical therapy.

Key words: Early onset sepsis, Late onset sepsis, Neonatal intensive care unit

INTRODUCTION

Neonatal sepsis is defined as a clinical syndrome characterized by systemic signs of infection and bacteremia during the first 28 days of life. It is a frequent cause of mortality and morbidity.[1] In India, 11-24.5/1000 live births were varied by the incidence of neonatal sepsis.[2] In the developing world, the major cause of morbidity and mortality among the newborns is neonatal sepsis.[3]

According to the age of onset, neonatal septicemia is divided into two types: Early-onset sepsis (EOS) (<72 h) and late onset sepsis (LOS) (≥72 h-28 days). EOS is acquired during delivery, fetal life, or at the nursery. Gram-positive as well as Gram-negative bacteria cause neonatal sepsis.[4] There has been constantly changing the pattern

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of the causative organism and frequent emergence of resistant bacteria.[5]

At birth, the neonate may be symptomatic in severe cases. Usually in EOS, neonates are present with respiratory distress and pneumonia. In general, the maternal genital tract is the source of infection.[6] In LOS, the source of infection is either nosocomial or community acquired and usually presented with septicemia, pneumonia, or meningitis in infants.[7] To select appropriate antimicrobial treatment, the knowledge of common pathogens causing neonatal septicemia and their antimicrobial susceptibility pattern is essential.

In neonates, the most common infection is blood stream infection. In the developing world, the most common reported organisms among LOS are *Escherichia coli*, *Klebsiella* species, and *Staphylococcus aureus,* whereas the most common pathogens among EOS, are *S. aureus, Streptococcus pneumoniae,* and *Streptococcus pyogenes. Klebsiella pneumoniae*, *S. aureus,* and *E. coli* are the three most common organisms, causing neonatal septicemia both in hospital and community, according to the National Neonatal Perinatal Database of India. In the developing countries, especially in the hospital setting, the causative agents of EOS and LOS sepsis are moreover similar. Geographically, antibiotic susceptibility pattern of pathogens are vary and are temporarily dependent on local pathogens and patterns of antibiotics use. Due to their weak immunity neonates are more prone to infection.[8]

There have been resistance bacteria of frequent emergency, and it has been changing constantly of causative organism pattern.[5] The comparison of developed countries with developing countries like India there is a quite different organism's spectrum causing neonatal septicemia.[9] The organism pattern differs from place to place, and it can change over a period of time in the same place.[10]

Moreover, a number of risk factors for the emergence and spread of antibiotic resistance are also home in developing countries. The reasons behind the emergence and spread of antibiotic resistance are over-the-counter and parallel market access, misuse of antibiotics, and counterfeit or poor quality drugs, combined with substandard hygiene and living conditions.[11,12]

The isolation of bacterial agent from blood culture is a gold standard for diagnosis of septicemia.[13]

During the neonatal and pediatric age group, many infections can only be established on the basis of etiological agent recovered from blood,[14] the possibility of neonatal sepsis does not rule by a negative blood culture.[15]

The prevalence of bacterial profile of blood cultures and their susceptibility patterns in an area, provide guidance to start empirical treatment which is the cornerstone in the management of sepsis.

Therefore, this study was aimed to determine the bacteriological profile and their antimicrobial susceptibility patterns in neonatal septicemia cases.

MATERIALS AND METHODS

The study is a 1-year, non-interventional prospective study of 388 patients suspected of septicemia visiting the neonatal intensive care unit in Teerthanker Mahaveer Medical College and Research Centre, Moradabad.

Collection of Blood Specimen

Blood samples were collected from the patients. First, 2-3 ml blood was drawn from the anti-cubital vein of each neonate into a sterile disposable syringe and then it is inoculated directly into BactAlert culture bottle by a trained study technician with all safety measures and then incubated at 37°C overnight for visible growth.

Subcultures were done on blood agar and MacConkey's agar. All positive blood cultures were identified by their characteristic appearance on their respective media, Gram-staining and confirmed by the pattern of biochemical reactions using the standard method. Members of Enterobacteriaceae were identified by indole production, citrate utilization, motility test, urease test, triple sugar iron test, and other relevant tests. For Gram-positive bacteria coagulase, catalase, and other tests were done.

Antimicrobial susceptibility test was performed by using modified Kirby-Bauer Disk diffusion method using Muller-Hinton agar and results were incorporated according to the CLSI, 2009. From pure culture 3-5, selected colonies of bacteria were taken with a sterile cotton swab and transferred to a tube containing peptone water, mixed well and incubated at room temperature for 30 min. Then swab was taken and the excess suspension removed by gentle rotation of the swab against the surface of the test tube. Then swab was used to evenly distribute the bacteria over the entire surface of Muller-Hinton agar. The inoculated plates were then left at room temperature to dry for 3-5 min and antibiotic discs were placed on the surface of Muller-Hinton agar plate.

For determining sensitivity, following antimicrobial disks were used such as ampicillin (10 μg), ampicillin/sulbactam (10/10 μg), cephalexin (30 μg), norfloxacin (10 μg), co-trimoxazole (25 μg), erythromycin (15 μg), gentamycin

(120 μg), ciprofloxacin (5 μg), levofloxacin (5 μg), linezolid (15 μg), tobramycin (10 μg), vancomycin (30 μg), amikacin (30 μg), pristinamycin (15 μg), teicoplanin (30 μg), chloramphenicol (30 μg), cefoperazone (75 μg), ceftazidime (10 μg), imipenem (10 μg), meropenem (10 μg), polymyxin B (300 units), tigecycline (15 μg), ceftriaxone (30 μg), ofloxacine (5 μg), tetracycline (30 μg), cefixime (5 μg), piperacillin/tazobactam (100/10 μg), and gatifloxacin (5 μg). The plates were then incubated at 37°C for overnight. Diameters of the zone of inhibition around the disc were measured to the nearest millimeter, and the isolates were classified as sensitive, intermediate, and resistant according to the standard table supplied by the CLSI guidelines.

RESULTS

In our study, the total number of culture positive cases was found to be 140 giving the culture positive rate of 35% (Table 1). Among culture, positive males were 92 (65.71%) and were females were 48 (34.29%), giving the male to female ratio was 1.91:1 (Table 1 and Figure 1).

The age of culture positive neonates ranged from 12 h to 28 days. Among culture positive neonates, 97 (69.29%) were having EOS and 43 (30.71%) were having LOS (Table 2 and Figure 2).

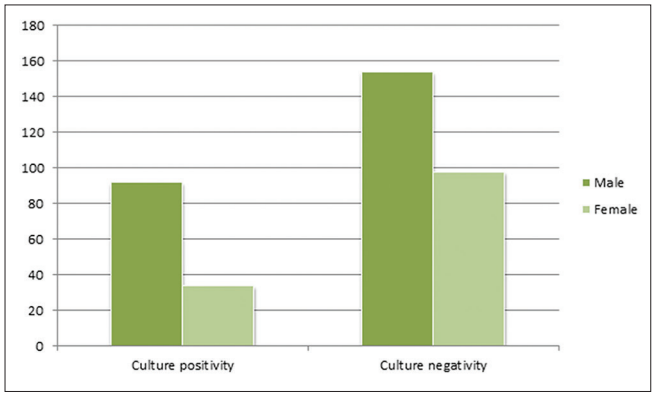

Figure 1: Blood culture positivity

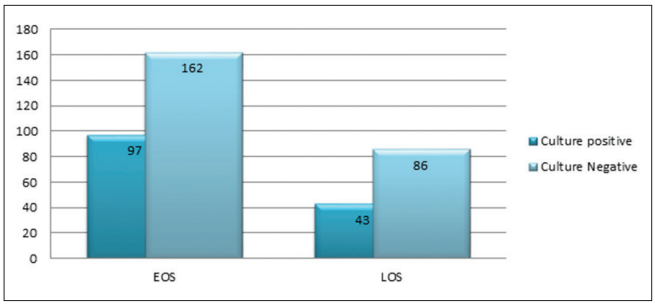

Figure 2: The distribution of cases according to the age of onset and culture positivity

Gram-positive organisms were more than Gram-negative organism, constituting about 60% of total isolates.

Most common pathogen identified was *S. aureus* 60 (42.8%) followed by *K. pneumoniae* 23 (16.43%), *Acinetobacter* 19 (13.57%), coagulase-negative *Staphylococci* 16 (11.43%), *Pseudomonas* 9 (6.43%), *Enterococcus faecalis* 5 (3.57%), *E. coli* 4 (2.86%), *S. pyogenes* 2 (1.43%) and *Enterobacter aerogenes* 2 (1.43%) (Table 3 and Figure 3). In this study, among *S. aureus,* methicillin-resistant *S. aureus* (MRSA) 34 (56.67%) were isolated.

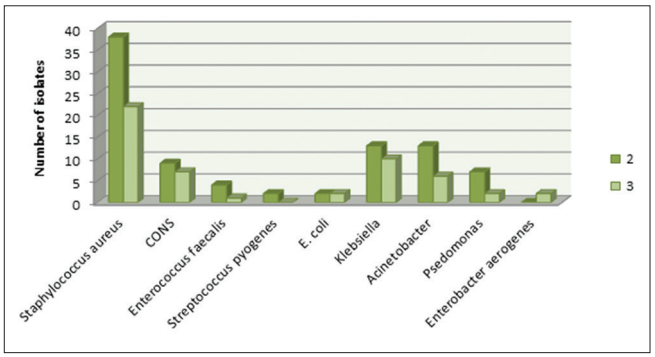

Figure 3: Bacterial isolates in blood culture

Table 1: Blood culture positivity

Blood culture	Male (%)	Female (%)	Total (%)
Culture positive	92 (65.71)	48 (34.29)	140 (36.08)
Culture negative	154 (60.10)	98 (37.90)	260 (63.92)
Total	254	146	400

Table 2: Distribution of cases according to age of onset and culture positivity

Age of onset	Culture positive (%)	Culture negative (%)	Total (%)
EOS	97 (69.29)	162 (65.32)	259 (66.75)
LOS	43 (30.71)	86 (34.68)	129 (33.25)
Total	140	248	388

EOS: Early onset sepsis, LOS: Late onset sepsis

Table 3: Bacterial isolates in blood culture

Organism	EOS (%)	LOS (%)	Total *N* (%)
Staphylococcus aureus	38 (63.33)	22 (36.67)	60 (42.85)
CONS	9 (56.25)	7 (43.75)	16 (11.43)
E. faecalis	4 (80)	1 (20)	5 (3.57)
S. pyogenes	2 (100)	0	2 (1.43)
E. coli	2 (50)	2 (50)	4 (2.86)
Klebsiella	13 (56.52)	10 (43.48)	23 (16.43)
Acinetobacter	13 (68.42)	6 (31.58)	19 (13.57)
Pseudomonas	7 (77.78)	2 (22.22)	9 (6.43)
E. aerogenes	0	2 (100)	2 (1.43)
Total	88	52	140

S. aureus: Staphylococcus aureus, CONS: Coagulase-negative *Staphylococci, E. faecalis: Enterococcus faecalis, S. pyogenes: Streptococcus pyogenes, E. aerogenes: Enterobacter aerogenes*, EOS: Early onset sepsis, LOS: Late onset sepsis

Among Gram-positive organism were found to be highly sensitive to vancomycin (96.39%) and linezolid (86.74%) followed by chloramphenicol (63.86%) and teicoplanin (67.47%) (Table 4 and Figure 4).

In our study, among *S. aureus* (60), 26 (43.33%) were methicillin sensitive *S. aureus* and 34 (56.67%) were MRSA, which is very high; it may be due to prolonged hospitalization, intake of broad spectrum antibiotics and nasal carriage.

Among Gram-negative organism were found to be highly sensitive to polymyxin B (87.71%) followed by meropenem (47.37%), chloramphenicol (47.37%), and tigecycline (47.37%) (Table 5 and Figure 5).

DISCUSSION

Throughout the world, developing countries share 99% of the estimated 4 million neonatal deaths, and the major contributor to it are sepsis, pneumonia, diarrhea, and tetanus infections[16] being responsible for about 34/1000 live births compared to developed countries and among them the neonatal mortality due to sepsis is approximately 5/1000 live births.[17]

One of the major causes of neonatal morbidity and mortality is sepsis. The myriads of neonatal sepsis are risk factors and the clinical presentation, with differences in the usually responsible organism for the EOS and LOS.[18] The gold standard for the confirmation of sepsis is blood culture.[19]

In our study, blood culture positivity rate in neonatal septicemia cases was 35% whereas in 65% of cases there

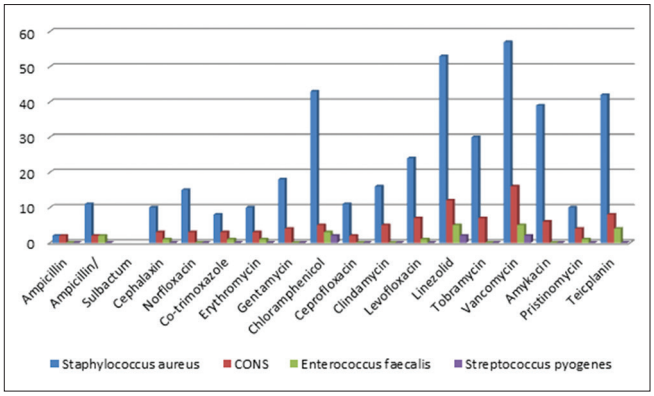

Figure 4: Antibiotic sensitivity of Gram-positive bacterial isolates

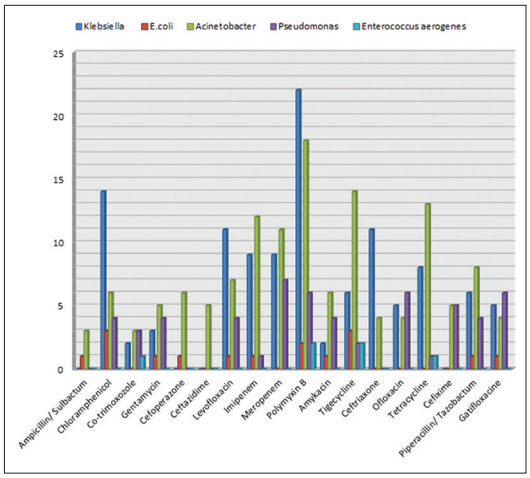

Figure 5: Sensitivity pattern of Gram-negative bacterial isolates

Table 4: Antibiotic sensitivity pattern of Gram-positive bacterial isolates

Antibiotics	*N* (%)				
	S. aureus (60)	CONS (16)	*E. faecalis* (5)	*S. pyogenes* (2)	Total (83)
Ampicillin	2 (3.33)	2 (12.5)	0	0	4 (4.82)
Ampicillin/Sulbactam	11 (18.33)	2 (12.5)	2 (40)	0	15 (18.07)
Cephalexin	10 (16.67)	3 (18.75)	1 (20)	0	14 (16.86)
Norfloxacin	15 (25)	3 (18.75)	0	0	18 (21.69)
Co-trimoxazole	8 (13.33)	3 (18.75)	1 (20)	0	12 (14.46)
Erythromycin	10 (16.67)	3 (18.75)	1 (20)	0	14 (16.86)
Gentamycin	18 (30)	4 (25)	0	0	22 (26.50)
Chloramphenicol	43 (71.67)	5 (31.25)	3 (60)	2 (100)	53 (63.86)
Ciprofloxacin	11 (18.33)	2 (12.5)	0	0	13 (15.67)
Clindamycin	16 (26.67)	5 (31.25)	0	0	21 (25.30)
Levofloxacin	24 (40)	7 (43.75)	1 (20)	0	32 (38.55)
Linezolid	53 (88.33)	12 (75)	5 (100)	2 (100)	72 (86.74)
Tobramycin	30 (50)	7 (43.75)	0	0	37 (44.58)
Vancomycin	57 (95)	16 (100)	5 (100)	2 (100)	80 (96.39)
Amikacin	39 (65)	6 (37.5)	0	0	45 (54.21)
Pristinomycin	10 (16.67)	4 (25)	1 (20)	0	15 (18.07)
Teicoplanin	42 (70)	8 (50)	4 (80)	2 (100)	56 (67.47)
Total	399	92	24	8	523

S. aureus: Staphylococcus aureus, CONS: Coagulase-negative *Staphylococci*, *E. faecalis: Enterococcus faecalis, S. pyogenes: Streptococcus pyogenes, E. aerogenes: Enterobacter aerogenes*

Table 5: Antibiotic sensitivity of Gram-negative bacterial isolates

Antibiotics	*N* (%)					
	Klebsiella (23)	*E. coli* (4)	*Acinetobacter* (19)	*Pseudomonas* (9)	*Enterococcus aerogenes* (2)	Total (57)
Ampicillin	0	0	0	0	0	0
Ampicillin/Sulbactam	0	1 (25)	3 (15.79)	0	0	4 (7.01)
Chloramphenicol	14 (60.87)	3 (75)	6 (31.58)	4 (44.44)	0	27 (47.37)
Co-trimoxazole	2 (8.70)	0	3 (15.79)	3 (33.33)	1 (50)	9 (15.79)
Gentamycin	3 (13.04)	1 (25)	5 (26.31)	4 (44.44)	0	13 (22.80)
Norfloxacin	0	0	0	0	0	0
Cefoperazone	0	1 (25)	6 (31.58)	0	0	7 (12.28)
Ceftazidime	0	0	5 (26.31)	0	0	5 (8.78)
Levofloxacin	11 (47.82)	1 (25)	7 (36.84)	4 (44.44)	0	23 (40.34)
Imipenem	9 (39.13)	1 (25)	12 (63.15)	1 (11.11)	0	23 (40.34)
Meropenem	9 (39.13)	0	11 (57.90)	7 (77.77)	0	27 (47.37)
Polymyxin B	22 (95.65)	2 (50)	18 (94.73)	6 (66.66)	2 (100)	50 (87.71)
Amikacin	2 (8.70)	1 (25)	6 (31.58)	4 (44.44)	0	13 (22.80)
Tigecycline	6 (26.08)	3 (75)	14 (73.69)	2 (22.22)	2 (100)	27 (47.37)
Ceftriaxone	1 (4.34)	0	4 (21.05)	0	0	5 (8.78)
Ofloxacine	5 (21.73)	0	4 (21.05)	6 (66.66)	0	15 (26.31)
Tetracycline	8 (34.78)	0	13 (68.42)	1 (11.11)	1 (50)	23 (40.34)
Cefixime	0	0	5 (26.31)	5 (55.55)	0	10 (17.54)
Piperacillin/tazobactam	6 (26.09)	1 (25)	8 (42.10)	4 (44.44)	0	19 (33.33)
Gatifloxacin	5 (21.73)	1 (25)	4 (21.05)	6 (66.66)	0	16 (28.07)
Total	103	16	134	57	6	316

E. coli: Escherichia coli

was no growth while that in Batt Sima *et al*.,[20] study was 56.67%, K. J. Desai *et al*.,[21] study was 46.20%, Premlatha *et al*.,[22] study was 82.35%. Sharma *et al*.,[23] study was 37.63%.

The incidence of neonatal septicemia is variable and differs from place to place because it depends on various factors such as fetal birth weight, gestational age, perinatal care and hygienic conditions, child health care facilities, and maternal nutrition.

In our study, males were more affected than females and male to female ratio was 1.91:1. This is comparable to other studies by Sharma *et al*.[23] The actual reason for the predominance of a male is not clear but its reason may be sex dependent factors.[24] X-linked immunoregulatory genes are probably regulate the synthesis of gamma globulins and as males contain one X-chromosome, hence, they are more susceptible to neonatal septicemia than females.[25]

In our study, early onset septicemia (66.75%) was more than late onset septicemia (33.25%) which is comparable with the studies of Sharma *et al*.[23] in which 205 (56.32%) were aged <72 h (early onset) and 159 (43.68%) were aged >72 h (late onset).

Gram-positive bacterial isolates (59.29%) were more than Gram-negative bacterial isolates (40.71%) in our study and it is very similar to the study of Sodani and Mutha.[26]

In our study, the most frequent isolate was *S. aureus* 66 (42.85%) in both EOS and LOS. This is very similar to the study of Shaw *et al*.,[27] *K. pneumoniae* was the second most common organism followed by other organisms.

In our study, the Gram-positive organism was highly sensitive to vancomycin (96.39%) and linzolid (86.74%). This was comparable to the study of Bhurle and Solabannavar.[28]

Among Gram-negative organism *K. pneumoniae* 23 (16.43%) was the predominant and second most causative organism of neonatal septicemia, which is followed by *Acinetobacter* 19 (13.57%), *Pseudomonas* 9 (6.43%), *E. coli* 4 (2.86%), and *E. aerogenes* 2 (1.43%) which is similar to the study of Sharma *et al*.,[24] in which also *Klebsiella* (27.01%) was the second predominant organism.

CONCLUSION

Our study showed that the neonatal septicemia causes life-threatening emergency in the world, and rapid treatment with antibiotics is essential for the favorable outcome. From this study, it is showed that Gram-positive bacteria were more commonly the cause of neonatal septicemia.

Our study showed that bacterial spectrum for sepsis could be different in different regions, and sensitivity pattern also differs accordingly. In our study, vancomycin and polymyxin B are the drugs that are susceptible among the Gram-positive and Gram-negative organisms, respectively. Neonatal septicemia is more prevalent among the EOS.

The positive blood culture with antibiotic sensitivity is the best guide to antimicrobial therapy. Hence, early diagnosis and judicious use of antibiotic therapy are a good solution to this problem.

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