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Original Research Article

Clinico-bacteriological profile of neonatal sepsis

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ABSTRACT

Background: Neonatal sepsis is the commonest cause of neonatal mortality responsible for about 30-50% of total neonatal deaths in developing countries. Surveillance of causative organisms and their antibiotic sensitivity pattern promotes rational use of antibiotics and antibiotic stewardship.
Methods: A retrospective study, relevant data regarding the neonates diagnosed with culture positive sepsis was obtained from the case records during the period from July 2014 to June 2017. Culture positive sepsis was defined as isolation of bacterial pathogen from blood in neonates with clinical suspicion of sepsis.
Results: Of the 414 neonates with clinical suspicion of sepsis, 110 neonates had blood culture positive sepsis. Sepsis was predominant in males (64.5%). Low birth weight (47.2%) and prematurity (40.9 %) were important neonatal risk factors for sepsis. Early onset sepsis occurred in 58.1% of the cases and late onset sepsis in 41.9% of the neonates. Gram-positive cocci constituted 67.52% of all isolates and gram negative 30.76%. The most frequently isolated organism in blood was methicillin resistant coagulase negative staphylococcus(MRCONS) (32.47%). Gram positive organisms included MRCONS, methicillin resistant *Staphylococci aureus* (MRSA), group B Streptococci (GBS), *Staphylococcus aureus* and Enterococci. Among Gram-negative organisms, Acinetobacter was most frequently isolated followed by Klebsiella, *Escherichia coli*, Pseudomonas, Citrobacter and Burkholderia species. The mortality in the study group was 13.5%. Gram negative organisms were most resistant to ampicillin and cephalosporins. Gram positive isolates were least resistant to vancomycin and linezolid.
Conclusions: Gram positive sepsis was the most common type of sepsis among the neonates, although mortality was more in gram negative sepsis.

Keywords: Antibiotic stewardship, Blood culture, Neonatal sepsis

INTRODUCTION

Sepsis remains as one of the most important causes of mortality in neonates; especially in very low birth weight preterm infants and its incidence increases in the presence of maternal and neonatal risk factors. However, sepsis is a preventable cause of death unlike other causes like congenital anomalies cardiac anomalies, indicating that mortality rate can be reduced if appropriate measures are implemented. The clinical signs and symptoms of neonatal sepsis are indistinct and nonspecific, making its early diagnosis difficult.[1] The difficulties in diagnosing sepsis have ushered the erratic use of antibiotics which has given rise to the advent of multidrug resistance pathogens (MDR).[2] A remarkable percentage of the deaths are due to MDR pathogens, further complicating sepsis management.[3,4] Hence, understanding the risk factors, clinical features, organisms involved, their antibiotic sensitivity pattern becomes crucial and guides management and promotes antibiotic steward-ship. Neonatal infections cause about 26% of neonatal deaths according to World health organisation (WHO) estimates,

2006.[5] According to national neonatal- perinatal database (NNPD) 2002-2003, the neonatal mortality rate of 44 per 1000 live births accounts for two thirds of the infant mortality in India. Neonatal septecimia accounted for 18.6% and 37.6% of the intramural and extramural deaths respectively. The most frequently isolated organism was *Klebseilla pneumonia*.[6]

The main objective of present study was to find out the common organisms causing neonatal sepsis, change in the trends of organisms causing sepsis, and the antibiotic sensitivity of these organisms. It also determines risk factors and clinical features associated with neonatal sepsis. This will aid in the recognition and management of sepsis in appropriate way.

METHODS

A retrospective study was conducted at a tertiary care hospital in southern India. The case files of the neonates diagnosed with culture positive sepsis from July 2014 to June 2017 were retrieved from medical records department. These case files were studied for demographic details of the neonates, clinical features, risk factors for sepsis, laboratory data. The blood culture reports were obtained from the records in microbiology department.

The data regarding the sensitivity and resistance pattern of organisms was collected from the computer-based records. Blood culture was done by BACTEC method and antimicrobial susceptibility test was performed using Kirbey Bauer disc diffusion method.

Culture positive sepsis was defined as isolation of bacterial pathogen from blood in neonates with clinical suspicion of sepsis. Cases of sepsis were divided into early onset sepsis (EOS) and late onset sepsis (LOS). Early onset sepsis was defined as onset of sepsis within 72 hours of life and late onset as after 72 hours of life.

Poor feeding, temperature instability, cyanosis, tachypnoea, apnoea, grunting, chest retraction, jaundice, pus draining from umbilicus, pustules on the skin, vomiting, abdominal distension, bleeding, diarrhoea, abnormal movements (including seizures), hypertonia/ hypotonia, lethargy, depressed or bulged fontenallae, altered cry were considered as clinical features of sepsis. The risk factors in the mother and the neonates were also evaluated. Data was collected for lab parameters-total count, neutrophil count, platelet count, C-reactive protein (CRP). Cerebrospinal fluid (CSF) analysis, its culture sensitivity and information on cultures from other sites was also gathered.

Statistical analysis

Collected data was analysed statistically by frequency, percentage and chi square test, p values <0.05 was considered statistically significant.

RESULTS

Of the 414 neonates with clinical suspicion of sepsis, 110 neonates had blood culture positive sepsis and majority of them were males (64.5%). The demographic details of the neonates are shown in Table 1.

Table 1: Characteristics of the culture positive cases.

Characteristics	Categories	N=110	Percentage
Sex	Male	71	64.5
	Female	39	35.5
Place of birth	Inborn	65	59.1
	Out-born	45	40.9
Gestation (weeks)	<28	3	2.7
	28-33	20	18.2
	34-37	22	20.0
	>37	65	59.1
Birth weight (grams)	<1000	7	6.4
	1001-1500	12	10.9
	1501-2500	33	30.0
	2501-4000	54	49.1
	>4000	4	3.6
Mode of delivery	Vaginal	67	60.9
	C-section	43	39.1

Early onset sepsis occurred in 58.1% of the cases and LOS in 41.9%. Out of 110 neonates, 67 neonates were born by vaginal delivery, of which 42 developed EOS and 25 developed LOS whereas in neonates extracted by caesarean section (n=43), EOS (n=22) and LOS (n=21) occurred almost in equal numbers (Table 1 and 2).

Table 2: Characteristics of neonates associated with EOS and LOS.

Characteristics	Early n=64 (%)	Late n=46 (%)
Sex		
Male	38 (59.4)	33 (71.7)
Female	26 (40.6)	13 (28.3)
Gestation (weeks)		
<28	1 (1.6)	2 (4.3)
28-33	12 (18.8)	8 (17.4)
34-37	13 (20.3)	9 (19.6)
>37	38 (59.4)	27 (58.7)
Birth weight (grams)		
<1000	3 (4.7)	4 (8.7)
1001-1500	6 (9.4)	6 (13.0)
1501-2500	17 (26.6)	16 (34.8)
2501-4000	34 (53.1)	20 (43.5)
>4000	4 (6.3)	0
Mode of delivery		
Normal	42 (65.6)	25 (54.3)
C- section	22 (34.4)	21 (45.7)
Prematurity	26 (40.6)	19 (41.3)

In present study, the most common maternal risk factor identified for neonatal sepsis was meconium stained

amniotic fluid (MSAF) (11.8%), followed by urinary tract infection and leaking per vaginum (10.9% each). Among the neonates exposed to MSAF, 10 of them developed EOS and only 3 developed LOS.

Table 3: Clinical features and risks factors for neonatal sepsis.

Features	Cases (n=110)	Percentage
Maternal risk factors		
MSAF	13	11.8
Leaking per vagina	12	10.9
UTI	12	10.9
Febrile illness	3	2.7
Foul smelling liquor	2	1.8
Neonatal risk factors		
Low birth weight	52	47.2
Prematurity	45	40.9
Perinatal asphyxia	17	15.5
No /weak/excessive cry	15	13.6

UTI- Urinary tract infection
MSAF- Meconium stained amniotic fluid

Low birth weight was the most common neonatal risk factor (47.2%) for sepsis followed by prematurity (40.9%), however it was not statistically significant. Of the neonates who developed LOS, 56.5% were low birth weight babies.

Neonates had one or more clinical features of sepsis. More than 50 % of them had tachypnoea (58.2%) and chest retractions (51%).

Table 4: Clinical features.

Clinical features	Cases (n=110)	Percentage
Tachypnoea	64	58.2
Chest retractions	56	51.0
Jaundice	26	23.6
Grunting	18	16.4
Poor feeding	17	15.5
Abdominal distension	14	12.7
Abnormal movements	11	10.0
Cyanosis	11	10.0
Pus from umbilicus	10	9.1
Vomiting	10	9.1
Apnoea	9	8.2
Temperature instability	7	6.4
Lethargy	7	6.4
Shock	6	5.5
Altered cry	5	4.5
Hypotonia/ Hypertonia	5	4.5
Bleeding	2	1.8
Skin pustules	2	1.8

Grunt was present in only 16.4%. The neonates presented with jaundice in 23.6% of the cases, which was second common clinical symptom following the respiratory symptoms (Table 3 and 4).

Table 5: Procedures and interventions.

Procedures and interventions	EOS n (%)	LOS n (%)
UAC (n=15)	**n=9**	**n=6**
< 24 hours	1 (11.11)	0
24-72 hours	7 (77.77)	1 (16.66)
3-7 days	0	1 (16.66)
>7 days	1 (11.11)	4 (66.66)
UVC (n=44)	**n=25**	**n=19**
< 24 hours	5 (20)	3 (15.78)
24-72 hours	6 (24)	2 (10.5)
3-7 days	6 (24)	6 (31.57)
>7 days	8 (32)	8 (42.1)
Mechanical ventilation (n=39)	**n=21**	**n=18**
<24 hours	8 (38.1)	2 (11.11)
24-72 hours	6 (28.57)	4 (22.22)
3-7 days	2 (9.52)	6 (33.33)
>7 days	5 (23.80)	6 (33.33)

UVC - Umbilical venous catheter
UAC - Umbilical arterial catheter

Of the 5 neonates who had umbilical arterial line (UAC) for >7 days, 4 developed LOS and among the 16 neonates who had umbilical vein catheter (UVC) for < 3 days, 11 had EOS. In neonates (n=20) who were ventilated for < 3 days, majority had EOS (n=14) and among the 19 infants who were ventilated for >3 days, 12 had LOS (Table 5). C- reactive protein was positive in 70 % of the cases with positive blood culture but was not statistically significant. Abnormal low platelet count <150000/cumm3 was observed in 34.5% of neonate. Cerebrospinal fluid analysis and culture was done in 32 neonates and culture was positive only in 2 cases; one grew Candida albicans and the other MRCONS (Table 6).

Table 6: Laboratory findings.

Laboratory findings	EOS n=64 (%)	LOS n=46 (%)
Leucocytosis (> 20000/mm^3)	14 (21.9)	10 (21.7)
Leukopenia (<4000/mm^3)	4 (6.3)	0
Platelets (<150000/mm^3)	16 (25.1)	22 (47.8)
CRP (>6mg/dL)	40 (62.5)	37 (80.5)

CRP- C reactive protein

A total of 117 organisms were isolated from 110 blood cultures, of which 78 were gram positive organisms which constituted 66.66%, 37 were gram negative organisms which constituted 31.63% and 2 fungal isolates constituted 1.71%. Gram positive organisms included methicillin resistant coagulase negative Staphylococci (MRCONS), methicillin resistant *Staphylococci aureus* (MRSA), group B Streptococci (GBS), *Staphylococcus aureus* and *Enterococcus feacalis* in the decreasing order.

Among the gram-negative organisms, Acinetobacter was the commonest organism isolated, followed by

Klebsiella, *Escherichia coli*, Pseudomonas, Citrobacter and Burkholderia species. Six blood cultures had polymicrobial growth.

Table 7: Organisms isolated.

Organisms	Numbers (n=117)	Percentage
MRCONS	38	32.47
MRSA	16	13.70
GBS	9	7.70
Acinetobacter spp	9	7.70
Staphylococcus aureus	8	6.83
Klebsiella pneumonia	8	6.83
E. coli	5	4.30
Pseudomonas spp	4	3.42
Citrobacter spp	3	2.56
Burkolderia spp	3	2.56
Enterococcus feacalis	3	2.56
α-haemolytic Streptococci	3	2.56
Candida spp	2	1.71
Staphylococci sciuri	1	0.85
Enterobacter cloacae	1	0.85
Listeria spp	1	0.85
Moraxella spp	1	0.85
Acromobacter spp	1	0.85
Aeromonas spp	1	0.85

Spp- Species; MRCONS- Methicillin resistant coagulase negative Staphylococci; MRSA- Methicillin resistant *Staphylococci aureus;* GBS- Group B Streptococci; *E. coli*- Escherichia coli

The most frequently isolated organism in the blood was MRCONS (32.47%) followed by MRSA (13.70%), GBS (7.7%) and Acinetobacter (7.7%). Methicillin resistant coagulase negative staphylococcus was the most common pathogen isolated in EOS as well as LOS, however 14 of them were commensals. Three isolates of MRSA were also commensals (Table 7).

Among the Gram-positive organisms, only GBS showed good sensitivity to amoxicillin (55.55%), ampicillin (88.88%) and ceftriaxone (55.55%). It also showed good sensitivity to fluoroquinolones. *Enterococcus feacalis* was 100% sensitive to ampicillin. *Enterococcus fecalis*, GBS, *Stahpylococcus aureus* and MRSA showed 100% sensitivity to vancomycin, linezolid and tiecoplanin.

High sensitivity pattern was observed for amikacin in isolates of MRCONS (81.57%), *Staphylococcus aureus* (75%) and MRSA (75%). Methicillin resistant coagulase negative staphylococci and MRSA were resistant to most of the antibiotics tested and were highly resistant to amoxiciilin, ampicillin and cephalosporins.

All isolates of *Stalylococcus aureus* were resistant to amoxiciilin and ampicillin but showed good sensistivity to ceftriaxone and levofloxacin (62.2% each), 100% to vancomycin, linezolid and tiecoplanin. Gram negative organisms were highly resistant to ceftriaxone, amoxicillin, ampicillin. Among them, Citrobacter showed 100% sensitivity to most of the antibiotics tested.

Klebsiella and Pseudomonas showed good sensitivity to aminoglycosides and fluoroquinolones. Most of the gram-negative isolates showed 100% sensitivity to colistin except Burkholderia and Pseudomonas. Although Acinetobacter was highly resistant to most of the antibiotics, all isolates were sensitive to colistin (Table 8 and 9).

Table 8: Antibiotic sensitivity of gram-positive organisms.

Organisms → Antibiotics	MRSA n=16 (%)	MRCONS n=38 (%)	GBS n=9 (%)	*Staphylococcus aureus* n=8 (%)	*Enterococcus faecalis* n=3 (%)
Amoxicillin	3 (18.75)	1 (2.6)	5 (55.55)	0	2 (66.66)
Ampicillin	1 (6.25)	2 (5.2)	8 (88.88)	0	3 (100)
Ceftriaxone	3 (18.75)	2 (5.2)	5 (55.55)	5 (62.5)	NT
Amikacin	12 (75)	31 (81.57)	0	6 (75)	0
Gentamycin	4 (25)	19 (50)	0	3 (37.5)	NT
Ciprofloxacin	3 (18.75)	5 (13.15)	6 (66.66)	4 (50)	1 (33.33)
Levofloxacin	6 (37.5)	11 (28.94)	8 (88.88)	5 (62.5)	1 (33.33)
Azithromycin	2 (12.5)	7 (18.42)	7 (77.77)	3 (37.5)	1 (33.33)
Clindamycin	9 (56.25)	12 (31.57)	6 (66.66)	5 (62.5)	2 (66.66)
Linezolid	16 (100)	38 (100)	9 (100)	8 (100)	3 (100)
Vancomycin	16 (100)	38 (100)	9 (100)	8 (100)	3 (100)
Teicoplanin	16 (100)	32 (84.21)	9 (100)	8 (100)	3 (100)

NT- Not tested; MRCONS- Methicillin resistant coagulase negative Staphylococci; MRSA- Methicillin resistant Staphylococci aureus; GBS- Group B Streptococci

Table 9: Antibiotic sensitivity of gram-negative organisms.

Organisms → Antibiotics	*E. coli* n=5 (%)	Klebsiella n=8 (%)	Acinetobacter n=9 (%)	Citrobacter n=3 (%)	Pseudomonas n=4 (%)	Burkholderia n=3 (%)
Amoxicillin	0	0	0	0	0	1 (33.33%)
Ampicillin	0	1 (12.5)	NT	0	0	0
Ceftriaxone	0	1 (12.5)	0	2 (66.66)	0	0
Amikacin	4 (80)	5 (62.5)	2 (22.22)	3 (100)	3 (75)	0
Gentamycin	2 (40)	6 (75)	2 (22.22)	2 (66.66)	3 (75)	0
Ciprofloxacin	1(20)	6 (75)	2 (22.22)	3 (100)	3 (75)	1 (33.33)
Levofloxacin	3 (60)	7 (87.5)	2 (22.22)	3 (100)	3 (75)	1 (33.33)
Colistin	5 (100)	8 (100)	9 (100)	3 (100)	3 (75)	0
Piperacillin-Tazobactam	5 (100)	7 (87.5)	1 (11.11)	3 (100)	1 (25)	0
Meropenem	5 (100)	7 (87.5)	1 (11.11)	3 (100)	0	0
Cefaperazone-Sulbactum	5 (100)	5 (62.5)	2 (22.22)	2 (66.66)	1 (25)	2 (66.66)
Ceftazidime	NT	NT	NT	NT	1 (25)	3 (100)
Tigecycline	4 (80)	5 (62.5)	1 (11.11)	NT	2 (50)	3 (100)

NT- Not tested; *E. coli- Escherichia coli*

Mortality in the study group was 13.5%, of which 73.33% occurred in EOS. Gram negative sepsis was responsible for 73.33% of the total neonatal deaths, of which Acinetobacter species was a major contributor (45.45%). Mortality was least with gram positive organisms GBS, MRCONS, MRSA and staphylococcus aureus (6.6% each).

DISCUSSION

The selection of empirical antibiotics for neonatal sepsis should cover most of the common organisms and should be started immediately after obtaining cultures as neonatal sepsis is an important cause for mortality.[7] Although blood culture is gold standard for diagnosis of neonatal sepsis, the use of intra-partum antibiotics and empirical antibiotics prior to collecting blood for culture decreases yield of culture.[2,8-10] For choosing the appropriate empirical therapy, one should be aware of the common organisms causing EOS and LOS, so that the antibiotic resistance and emergence of MDR organisms can be reduced. The present study aims to find the common organisms causing neonatal sepsis and their antibiotic sensitivity pattern. The blood culture positivity in neonates with clinical suspicion of sepsis was 26.57% during the given study period which was similar to study done by Roy et al.[11] It was only 18% in Bhat et al study 4 and was higher (42.8%) in a study done in Egypt by Moshen et al.[12] Half of the neonates in present study, presented with respiratory symptoms, identical to studies done by Jain et al and Galhotra et al.[13,14] Contrary to this, 72% presented with poor activity / poor cry in Reddy K V et al, study.[15] The most common type of sepsis in present study was EOS which is in parallel to studies by Galhotra et al, and Madavi et al.[14,16] Opposite to this, studies done in India by Goyal et al, and his associates and by Ozkal et al, in Turkey showed LOS as common sepsis type.[17,18] Late onset sepsis occurs usually in neonates with prolonged hospital stay, especially in low birth weight and preterm neonates. In present study it was found that, LOS was more common in neonates with birth weight < 2500g which was similar to the study done by Ozkal et al, where very low birth weight was main risk factor for LOS and was statistically significant.[18]

The major organism causing EOS and LOS was MRCONS in the current study which was in line with Ozkal's et al, study whereas in a study carried out by Sethi et al, Klebsiella was relatively more common in LOS while Enterococcus was more frequent in EOS.[18,19] All the 9 GBS organisms isolated in this study caused EOS which suggests possible association of maternal genital tract infection with EOS in neonates. Worldwide, gram negative organisms are more common causes for neonatal sepsis and main organisms are Klebsiella spp, *E. coli*, Pseudomonas and Salmonella. Staphylococcus aureus, CONS, Streptococcus pneumoniae and Streptococcus pyogenes are most commonly isolated gram-positive organisms. In developing countries, *E. coli*, GBS, Enterobacter, Enterococcus, and Listeria are mostly associated with EOS. Klebsiella, Acinetobacter, CONS and Staphylococcus aureus are associated with both EOS and LOS. Pseudomonas, Salmonella, and Serratia are more often associated with LOS disease.[20] In developing countries GBS is reported to be rare, but this study shows 7.7% of culture positivity.[20]

Present results indicate that, gram positive organisms are predominant over the gram-negative organisms corresponding to other studies done in Ghana and China.[21,22] In a recent cohort study involving three different tertiary care hospital NICUs in Delhi, the predominant gram positive pathogens were CONS (15%), *Staphylococcus aureus* (12%), Enterococcus (6%), GBS (1%) and the gram negative included Acinetobacter (22%), Klebsiella (17%), E.coli (14%), Pseudomonas (7%) and Enterobacter (4%); the mortality was highest with Acinetobacter (59%).[23] In this study major gram-

positive organism was MRCONS and gram negative was Acinetobacter and mortality were highest with Acinetobacter (45.5%) comparable to the cohort study (59%).

Many studies have documented high antimicrobial resistance of the organisms causing neonatal sepsis. In present study, methicillin resistance was seen in 97.36% of CONS and 66.66% of *Staphylococcus aureus*. Staphylococcus aureus showed 100% resistance to amoxicillin-clavulanate and ampicillin, 62.5% to azithromycin, levofloxacin and gentamycin and 50% to ciprofloxacin. A study done by Iregbu K. C, showed decreases in susceptibility of *Staphylococcus aureus* to various antibiotics observed in two time periods 2002-2004 and 2013-2015.[24] A decrease in sensitivity of amoxicillin-clavulanate (85% to 76%), cefuroxime (45% to 0%), ciprofloxacin (71% to 67%), erythromycin (64% to 30%), gentamicin (40% to 29%) and ceftriaxone (36% to 27%) between the 2 study periods was observed. Non-fermenting gram-negative bacilli (NFGNB) are emerging organisms causing neonatal sepsis. They exhibit multi-drug resistance. Commonly isolated NFGNBs include Pseudomonas, Acinetobacter, and Burkholderia. In the current study, gram negative organisms, mainly Acinetobacter and Burkholderia were highly resistant to many antimicrobials. Six out of 9 Acinetobacter isolates were sensitive only to colistin and resistant to all other antimicrobials tested. Burkholderia isolates were 100% sensitive to only to ceftazidime and cefaperazone-sulbactum. These results are similar to study done by Vishwanatan et al.[3] Even though sepsis rate was more among gram positive organisms, the mortality was high in gram negative sepsis, in comparison to results of study done by Upadhay et al.[25]

CONCLUSION

In conclusion, gram positive sepsis was found to be common in present study, although mortality was high in gram negative sepsis. Emergence of NFGNB complicated by multidrug resistance associated with high mortality is increasing in numbers. Careful measures have to be taken to overcome the change in trend of organisms causing sepsis, and selection of antibiotics should be prudent. Every NICU should develop their antibiogram in order to have appropriate antibiotic stewardship and decrease incidence of MDR.

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