Original Research Paper Microbiology

NEONATAL SEPSIS: BACTERIOLOGICAL AND DRUG RESISTANCE PROFILE.

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ABSTRACT Neonatal sepsis is an important cause of neonatal mortality and morbidity.

Aims: This study was conducted to know bacteriological profile and antimicrobial resistance of isolates from blood cultures received from neonates suspected to have septicemia.

Material and methods: In the present study 784 neonates clinically suspected to have suspected of sepsis admitted in NICU were included.

Results: Blood culture was positive in 128 (16.32%) cases. Gram positive bacteria 61(47.65%) were responsible for most cases of neonatal sepsis as compared to gram negative bacteria 55 (42.96%). Multidrug resistance was observed in 21(38.18%) Gram negative isolates.

Conclusions: Rational antimicrobial therapy can be started on time based local epidemiology and resistance pattern. Multidrug resistance in Gram negative isolates warrants great caution in selection of antibiotic therapy.

KEYWORDS : Neonatal Sepsis, Bacteriological Profile, Antimicrobial Drug Resistance

Introduction: Neonatal sepsis is a systemic infection occurring in infants at <28 days of life. It is an important cause of neonatal mortality and morbidity[1,2] It is arbitrarily divided as early onset infection(occurring before 1week of life usually within 72 hours of life) and late onset infection(occurring after 1 week of life).[1,2,3] Early onset infections are acquired before or during delivery(vertical mother to child transmission).Late onset infection develop after delivery from organisms acquired in the hospital or community.[1]

Commonest cause of neonatal mortality is sepsis. [4, 5] It contributes to 30-50% of the total neonatal deaths in developing countries.[6] 20% of the neonates develop sepsis and 1% die of sepsis.[6] Sepsis related mortality can be prevented with rational antimicrobial therapy and aggressive support care.[7]

Organisms causing sepsis vary from region to other and changes over time in same place. [2, 8]Treatment of neonatal sepsis with multidrug resistant strains is greatest challenge. [2] It is important to know common pathogens of neonatal sepsis, related antimicrobial susceptibility and drug resistance pattern at individual hospital level. At the same timeit is important to monitor the local epidemiology of neonatal sepsis to detect any changes in infection pattern and drug sensitivity. [3]

As there are no specific signs and symptoms of neonatal sepsis, diagnosis is difficult. Blood culture is the gold standard for diagnosis of septicaemia.[9,10] So, present study was conducted to study the incidence of neonatal sepsis, microbiological profile of neonatal septicemia cases and their antimicrobial susceptibility pattern. This would help choose rational antimicrobial drug based on local epidemiology.

Material and methods: The present prospective descriptive type study was conducted at a tertiary care hospital in Mumbai. A total of 784 neonates clinically suspected to have suspected of sepsis admitted in NICU were included in study. Consecutive samples received during study period of 12 months were included. Fully informed consent was obtained from parents. Detailed history, clinical examination, findings of conventional laboratory investigations and imaging study were recorded. Neonates were classified into EOS (occurring before 1week of life) and LOS (occurring after 1 week of life) groups. [1,3]

1-2 ml of blood was obtained by venepuncture from neonates prior to initiation of antibiotic therapy[9] .Specimen was collected taking all aseptic precautions. Blood was inoculated into two Bact/ALERT PF blood culture bottles. (Biomerieux India Pvt .Ltd) which were continuously monitored in the automated blood culture system. Blood from Bact/ALERT bottle was subcultured on 5% sheep blood agar, Mc Conkey agar and chocolate agar with (NAD).as soon as machine flagged positive for positive bottle. [9, 11] These plates were incubated overnight. The negative result was followed by incubating bottles for 7 days. Positive growth was identified by Gram staining, colony characteristics and standard conventional biochemical tests. [9, 11] Blood cultures were interpreted on the basis of result of single blood culture result as repeat specimen collection was not possible in all the cases. Antibiotic susceptibility testing including detection of methicillin resistance in staphylococci and screening test for ESBL among gram negative bacilli were performed according to CLSI (2015) guidelines by modified Kirby Bauer disc diffusion method. [12] In staphylococcus and Coagulase negative staphylococci (CONS) susceptibility to vancomycin was detected by Vancomycin E strips (Hi Media). [12] Growth of single potentially pathogenic organism (bacterium or fungus) from blood in neonates with clinical and laboratory findings consistent with infection was considered as neonatal sepsis. [3]

Results: In 784 suspected cases of neonatal sepsis73 (57.02%) were classified as EOS and 55 (42.96%) as LOS. Blood culture was positive in 128 (16.32%) cases. Male neonates were found to be more commonly affected.

The type and frequency of isolated pathogen in relation to the type of sepsis is shown table 1. Gram positive bacteria 61(47.65%) were responsible for most cases of neonatal sepsis as compared to gram negative bacteria 55 (42.96%). Coagulase negative staphylococci (CONS) were the most frequent isolated pathogens in EOS and LOS followed by *Klebsiella pneumoniae.*Other common isolates in EOS were *staphylococcus aureus, Pseudomonas aeruginosa* and *Candida*. In case of LOS *Pseudomonas aeruginosa, Acinetobacter baumannii* and *Candida spp.* were common isolates.

Table1: Microbiological profile of positive blood cultures from neonatal sepsis

Sr. No	Isolated microorganism	Total (%)	EOS No (%)	LOS No (%)
A	**Gram-positive bacteria**	61(47.65)	38(52.77)	23(41.81)
1	*Staphylococcus aureus*	11(8.59)	7(18.42)	4(17.39)
2	Coagulase negative staphylococci	43(33.59)	28(73.68)	15(65.21)

3	*Enterococcus faecalis*	7(5.46)	3(7.89)	4(17.39)
B	**Gram-negative bacteria**	55(42.96)	28(38.88)	27(49.09)
	Enterobacteriaceae:			
1	*Escherichia coli*	4(3.1)	3(10.71)	1(3.7)
2	*Klebsiellapneumoniae*	16(12.5)	8(28.57)	8(29.62)
3	*Enterobacteraerogens*	7(5.46)	3(10.71)	4(14.81)
4	*Citrobacterkoseri*	7(5.46)	3(10.71)	4(14.81)
5	*Salmonella Typhi*	1(0.78)	1(3.57)	0(0)
	Non fermenters:			
1	*Pseudomonas aeruginosa*	12(9.37)	7(25)	5(18.51)
2	*Acinetobacterbaumannii*	8(6.25)	3(10.71)	5(18.51)
C	**Fungi**			
	Candida spp.	12(9.37)	7(9.7)	5(9.09)
D	TOTAL	128	73(57.03)	55(42.96)

Table 2: Antimicrobial resistance pattern of Gram-positive bacteria.

Antibiotics	Gram positive bacteria No.(%)		
	Staphylococcus aureus 11(18.03%)	CONS 43 (70.49%)	*Enterococcus faecalis* 7(11.47%)
Penicillin	11(100)	43(100)	6(85.71)
Ampicillin	-	-	4(57.14)
Ciprofloxacin	10(90.90)	21(48.33)	-
Ofloxacin	7(63.63)	21(48.33)	-
Gentamicin	6(54.54)	14(32.55)	6(85.71)
Amikacin	2(18.18)	14(32.55)	-
Teicoplanin	2(18.18)	0(0)	3(42.85)
Linezolid	0(0)	0(0)	0(0)
Vancomycin	0(0)	0(0)	3(42.85)
Methicillin resistance	11(100)	43(100)	-

Table3: Antimicrobial resistance pattern of Gram-negative bacteria.

Antibiotics	Gram negative bacteria No(%)						
	E.coli (4)	K.pneumoniae (16)	E.aerogens (7)	C.koseri (7)	S.Typhi (1)	P.aeruginosa (12)	A.baumannii (8)
ESBL production	4(100)	16(100)	-	-	-	-	-
Ampicillin	-	-	-	-	0	-	-
Ceftazidime	4(100)	16(100)	7(100)	7(100)		10(83)	7(87)
Ceftriaxone	4(100)	16(100)	5(72)	7(100)	0	10(83)	7(87)
Piperacillin	4(100)	16(100)	4(57)	7(100)	-	3(25)	6(75)
Aztreonam	4(100)	16(100)	4(57)	7(100)	-	6(50)	6(75)
Cefepime	3(75)	16(100)	4(57)	7(100)	0	6(50)	6(75)
Amoxicillin+ Clavulinic acid	3(75)	16(100)	5(72)	6(86)	-	-	7(87)
Piperacillin+ Tazobactum	1(25)	14(87)	2(29)	4(57)	-	1(8)	6(75)
Ciprofloxacin	2(50)	6(31)	3(43)	1(14)	1	0(0)	4(50)
Ofloxacin	1(25)	5(31)	2(29)	2(29)	-	-	5(62)
Gentamicin	2(50)	12(75)	3(43)	3(43)	-	2(17)	4(50)
Amikacin	1(25)	11(69)	2(29)	3(43)	-	3(25)	5(62)
Imipenem,	1(25)	8(50)	2(29)	3(43)	-	2(17)	4(50)
Meropenem	1(25)	5(31)	2(29)	2(29)	-	2(17)	4(50)
Cotrimoxazole	-	-	-	-	0	-	-
Tetracycline	-	-	-	-	0	-	-
Azithromycin	-	-	-	-	0	-	-
chloramphenicol		-	-	-		-	-

Antimicrobial resistance pattern of Gram positive isolates is shown in table 2. All isolates of *Staphylococcus aureus* and CONS were methicillin resistant but all were sensitive to vancomycin and linezolid. *Enterococci* also showed no resistance to linezolid however vancomycin and teicoplanin resistance (42.85%) was observed in present study.

Amongst Gram-negative isolates all strains of *Escherichia coli* and *Klebsiella pneumonia*e showed production of extended spectrum β lactamase enzyme production (ESBL). Best sensitivity was observed to carbapenems and ciprofloxacin, fluroquinolones and aminoglycosides.

Discussion: Neonatal sepsis is one of the important causes of neonatal morbidity and mortality. Signs and symptoms of neonatal sepsis are nonspecific, making its early diagnosis difficult. Treatment of neonatal sepsis with multidrug resistant strains is great challenge. they carry higher risk of mortality.[3] Blood is the still gold standard for diagnosis of neonatal sepsis.[9,11] In our study incidence of neonatal sepsis during study period was 16.32%.this is lower when compared with KJ Desai et al.[13] This may be because of early institution of empirical antibiotic therapy as clinical features are nonspecific. Verylow rates are reported by developed countries. In contrast African countries have highest rates. This variation can be explained by difference in standards of life, health care and health services.[2] Out o784cases 128(16.32%)were culture positive. Early onset neonatal sepsis was found to be more frequent in present study.similar finding was observed in study conducted by R Basu et al. [14] although contrary observation has been noticed in other studies. [2]

Over the last few decades etiology of neonatal sepsis has changed with the extensive use of antibiotics.in 1970 s Gr B Streptococci were leading cause earlier .in the past decade some studies have revealed E.coli as a major cause. [3] However in our study gram positive microorganisms (CONS) found to be the commonest cause of both EOS and LOS followed by *Klebsiella pnemoniae*. *Staphylococcus epidermidis* was the commonest species amongst CONS. Although in absence of repeat cultures, it is difficult to establish etiological role of CONS in causation of neonatal sepsis. All isolates of CONS were resistant to methicillin but all were sensitive to vancomycin and linezolid. The extensive use of invasive devices for caring of immunologically immature neonates is the main cause of bacteraemia in NICU.Except for *S.Typhi*,a very high resistance to B lactam antibiotics was observed in all Gram negative isolates. However most Gram negative isolates were fairly sensitive to Carbapenems, fluroquinolones and aminoglycosides tested. Multidrug resistance was observed in 21(38.18%) Gram negative isolates which warrants great caution in selection of antibiotic therapy.

Conclusion: Bacteriological profile and antimicrobial resistance pattern of neonatal sepsis will help to start rational empirical therapy on time. Multidrug resistance in case of Gram negative isolates warrants great caution in selection of antibiotic therapy.This will help to reduce neonatal mortality and indiscriminate use of antibiotics which would reduce bacterial drug resistance and cost of treatment.

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