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Research Article

NEONATAL SEPSIS: CULTURE AND SENSITIVITY PATTERN AT TERTIARY LEVEL NEONATAL INTENSIVE CARE UNIT

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ABSTRACT
Neonatal sepsis is one of the most common causes of neonatal mortality and morbidity, particularly in the developing countries. Its causative bacteria and their respective sensitivity patterns are different in each hospital and region. To know organism responsible for neonatal sepsis and to study their culture and sensitivity pattern at our NICU. A Prospective observational study was conducted at well equipped tertiary level neonatal intensive care unit (NICU) at Dinanath Mangeshkar Hospital, Pune. All neonates admitted in the NICU with suspected sepsis and culture positive for bacteria were included in the study. They were divided into early onset sepsis and Late Onset Sepsis as well as Extramural and Intramural groups and evaluation was done for common infecting organism in each group along with culture positivity. In both late onset sepsis and early onset neonatal sepsis most common organism encountered were gram negative 20 (66.67%) and 4(13.33%) respectively with Enterobacter being most common 5 (25%) and 2(6.67%) respectively. Extended spectrum β lactamase inhibitor (ESBL) producing gram negative organisms were major problem. All gram negative organisms were sensitive to Meropenam along with fluroquinolone group of antibiotics. In gram positive organisms, methicillin resistant Coagulase negative streptococci were seen. Chloramphenicol showed good sensitivity pattern especially to ESBL producing gram negative organisms. Gram negative organisms were common cause of sepsis in both late onset 20 (66.67%) and early onset of sepsis 4(13,33%). High level of resistance noted to 1st line drug used in our hospital. Continuous surveillance for antibiotic susceptibility is needed to ensure proper empirical therapy.

Keywords: *Neonatal Sepsis, Culture Sensitivity, Early Onset Sepsis, Late Onset Sepsis*

INTRODUCTION
Neonatal Sepsis is one of the commonest causes of neonatal morbidity and mortality particularly in the developing countries. According to Report of National Neonatal Perinatal Database (NNPD) 2002-03 in India, sepsis is probably responsible for 30% to 50% of total neonatal deaths each year (Klein, 2001; NNPD, 2002-03).
India contributes to around one-quarter of all neonatal deaths in the World and more than half (52%) of these are estimated to occur due to infections. Neonatal septicaemia is a clinical syndrome characterized by systemic signs of infection and accompanied by bacteraemia in the first 28 days of life (Klein, 2001).
Neonatal sepsis is of two types; early onset sepsis and late onset sepsis. Early Onset Sepsis (EOS) presents within first 72 hours of life. In severe cases, the neonate may be symptomatic at birth. Infants with EOS usually present with respiratory distress and pneumonia. The source of infection is generally the maternal genital tract (Singh *et al.,* 1994). Late onset sepsis usually presents after 72 hours of age. The source of infection is either nosocomial or community acquired or neonates usually presented with septicemia, pneumonia or meningitis (NNPD, 2002-03).
NNS is a preventable cause of neonatal mortality by early detection of sepsis and judicious use of right antibiotics as per local culture and sensitivity. With respect to emerging problem of gram negative infections and increasing antibiotic resistance pattern, present study was conducted with the objectives to know organisms responsible for neonatal sepsis and their culture and sensitivity pattern in patient with

suspected neonatal sepsis in our neonatal intensive care unit (NICU), department of paediatrics at tertiary care hospital.

MATERIALS AND METHODS

This observational prospective study was done in well equipped tertiary level NICU of Dinanath Mangeshkar Hospital, Pune, which is a multispeciality hospital. Total duration of study was 14 months from April 2007 to June 2008.

Any newborn with suspected sepsis (as per criteria in Table 1, 2 & 3) and positive blood culture were included in this study. Newborn with culture positive for fungi and/or growth suggestive of contamination like Cornybacterium, Propionibacterium acnes and Bacillus species, multiple organisms in series, late appearing growth species and/or negative blood and other culture were excluded. During this period, 452 babies were admitted in NICU including both extramural (EM) and intramural (IM), out of this, 122 fulfilled the criteria of neonatal sepsis with 150 suspected sepsis events ,of which 44(29.33%.) were culture positive. Culture positivity for early onset of sepsis was 14.10 % (11/78) and for late onset sepsis it was 45.83% (33/72). Out of 44 blood culture positive samples, 8 were false positive samples due to contamination and 6 showed growth of fungi so were excluded from the study. Thus, thirty culture positive for bacterial organisms were included in study.

Blood culture was done by standard microbiological techniques (BACTEC 9050 Method) in all the cases and if required other cultures (urine, cerebrospinal fluid, endotracheal tube, central venous catheter tip) was done in all included neonates.

They were divided into early onset group (EOS) and late onset (LOS) depending on whether the signs were seen before or after seventy two hours of births respectively (NNPD, 2002-03). Similarly they were classified into EM and IM groups depending on whether delivered in outside hospital or delivered in same hospital respectively (NNPD, 2002-03).

Cultured isolates were identified by standard biochemical tests. Antibiograms were studied by disk diffusion method according to National Committee for Clinical Laboratory Standards (NCCLS, 2002). If no growth was detected in five days culture reported as negative.

Approval from Institutional Ethical Committee was obtained before the start of study. Data was analyzed by using MINITAB 15th version. Chi square test of significance was used and p-value < 0.05 was considered as statistically significant.

Inclusion Criteria

Any patient with suspected sepsis (as per criteria in Table 1, 2 & 3) and positive blood culture.

Table 1: Perinatal risk factor for early onset sepsis

Sr. no	A. MAJOR (any one of three)
1	Febrile illness in mother within two weeks prior to delivery (fever more than 38 degree Celsius.)
2	Foul smelling liquor (MSL).
3	Prolonged rupture of membrane.(PROM) >18 hours in preterm and 24 hours in full term.
	B. MINOR (any two of six.)
1	Low birth weight (LBW) and/ or Preterm (PT)
2	More than 3 vaginal examinations during delivery.
3	Prolonged and difficult delivery with instrumentation.
4	Perinatal asphyxia, APGAR score less than 4 at 1 minute or/and less than 7 at 5 minute.
5	Maternal leukocytosis, total leukocyte count > 18000.
6	Symptomatic bacteriuria.
7	More than 3 vaginal examination during delivery.

Table 2: Clinical feature for suspected case of sepsis (any two of nine)

Sr. No	Clinical Feature
1	Looking unwell, sick looking newborn.
2	Lethargy, poor cry, refusal to feed.
3	Respiratory distress, apnea, gasping respiration.
4	Hypothermia or fever.
5	Poor perfusion, prolonged capillary refill time, more than 3 second.(CRT)
6	Hypotonia, absent neonatal reflexes.
7	Bradycardia (heart rate less than 100) or tachycardia (heart rate more than 160).
8	Sclerema.
9	Acidotic breathing and/or lab show metabolic acidosis.

Table 3: Septic screen positive (any 2 of 3 positive)

1	Total leukocyte count < 5000. / Absolute neutrophil count less than 1500
2	I/T Ratio more than 0.2.
3	CRP more than 6 mg/dl,Micro-ESR more than 15 in one hour, organism in Buffy coat examination.

Exclusion Criteria

1) Blood culture negative sepsis
2) Blood culture positive for fungal growth
3) False positive blood culture due to contamination

RESULTS AND DISCUSSION

Results

Out of 122 cases of suspected sepsis with 150 suspected sepsis events, thirty were culture positive. From this thirty culture positive isolates, 22(73.33%) were gram negative organisms and the rest 8(26.67%) were gram positive bacteria (Table-1).

Most common organisms in early onset sepsis were gram negative 4 (13.33 %) with common being Enterobacter species in 2 (6.67%), while gram positive organisms seen were Coagulase negative streptococci (CONS) in 2 (6.67%) events. In late onset sepsis most common organism encountered were gram negative 20 (66.67%) with Enterobacter being most common25% (5/20), Klebsiella pneumoniae (5/20,25%) followed by Pseudomonas species 20%(4/20), Gram positive organisms were seen in only 3 (15 %) events, with two Coagulase negative staphylococcus (CONS) and one Enterococcus.

As shown in Table 2, percentage of gram negative organism showing ESBL production were 14 (63.37%) while Non-ESBL producing organism were 8 (36.36%). The difference observed was statistically significant (p= 0.001).

Table 3 shows Antibiotic sensitivity of different organisms. Majority of organisms like Enterobacter, K. pneumoniae, E. coli, were ESBL producing showing sensitivity mainly to meropenem (100%). They also showed sensitivity to fluroquinolone group of drugs (95%). Pseudomonas isolated was non-ESBL producing and showed good sensitivity to piperacillin (100%), most of fluroquinolones (100%) and also to cefotaxime and cefoperazome (100%), but sensitivity to aminoglycoside was very low (20%). In gram positive organisms most were sensitive to penicillin group of drug specially amoxycillin and clavulanic acid combination along with aminoglycoside mainly amikacin.

Sensitivity to fluroquinolone was also good (75%) and to some extent they showed sensitivity to cotrimoxazole and chloramphenicol, but poor sensitivity to cephalosporin group. While methicillin resistance coagulase negative staphylococci (MR CONS) showed sensitivity to only vancomycin (100 %) and Linezolid. Chloramphenicol showed overall good sensitivity to all organisms (47%) especially to gram negative ESBL organisms (65%) while Cephalosporin group showed poor sensitivity pattern (10-30%).

Discussion

In our study overall culture positivity rate was 29.33% which was lower than other studies like (Sharma *et al.*, 2013), (Shah *et al.*, 2012) where it was 37.63% and, 31.75% respectively.

Table 4: Bacterial organisms in Suspected sepsis events

Bacterial organism	IM EOS	IM LOS	EM EOS	EMLOS	Total
Gram positive organism n=8 (8 CONS cases were contaminated)					
B-Hemolytic streptococci	0	0	0	2	2
Coagulase negative staphylococcus	1(1)	1	0(3)	1(4)	3(8)
Staphylococcus aureus	1	0	0	0	1
Enterococcus (gr.D streptoccous)	1	0	0	1	2
Gram negative organism (n=22)					
Enterobacter	1	3	1	2	7
K. Pneumoniae	0	3	1	2	6
Pseudomonas	0	2	1	2	5
E. coli	0	1	0	2	3
Serratia maracescens	0	1	0	0	1
Total	4(1)	11	3(3)	12(4)	30(8)

Table 5: Classification of organism depending on ESBL production

Organism	*ESBL*	*Non-ESBL*
Enterobacter spe.	6	1
K. Pneumoniae	5	1
Pseudomonas	0	5
E.coli	3	0
Serratia maracescens	0	1
Total	14	8

The commonest organisms causing neonatal sepsis in our study were gram negative 73.33%, while 26.67%. were gram positive. In late onset sepsis most common organism were gram negative 60% with Klebsiella pneumoniae and Enterobacter species both being 16.67%, and Pseudomonas species being 14.81%. This is comparable with data from (NNPD, 2002-03), (Ojukwu *et al.*, 2005), (Bell *et al.*, 2005) which showed most common isolates as gram negative organisms, Klebsiella pneumoniae as most common 30%, 37.4%,28% respectively followed by staphylococcus aureus and Escherichia coli. Our findings are consistent with the other studies (Stoll *et al.*, 2002), (Misallati, 2000).

While gram positive organism were only seen in 20 %, most common being Coagulase negative staphylococcus (CONS), B-Hemolytic streptococci, and occasional S. aureus and Enterococcus species. This would be an indicator that coagulase negative streptococci (CONS) may be one of emerging pathogen in NICU. Study conducted by (Bizzarro *et al.*, 2005) also showed most common gram positive pathogen identified was CONS (29%), followed by E.coli (12%), group B streptococcus (10%) and S. aureus (8%). Study conducted by (Bindayana *et al.*, 2006) showed main agents isolated were coagulase negative staphylococcus (41%), Staphylococcus aureus (8%) but show decreasing trend of gram negative organisms which is contradictory to our findings.

Table 6: Antibiotic sensitivity of different organisms

Antibiotics	ORGANISMS									
	Entero baccter (n=7)	K. pneum oniae (n=6)	E. coli (n=3)	S. mara cescens (n=1)	Pseudo monas (n=5)	B-hemolytic streptoco cci (n=2)	Enteroco ccus (n=2)	CONS (n=3)	S. aureus (n=1)	Total (n=30)
Ampicillin	0	0	0	1	-	2*	2	-	-	3(2*)
Amox + Clav	0	0	1	1	0	2	2	0	1	7
Penicillin	-	-	-	-	-	2*	2	-	1	3(2*)
Piperacillin (P)	-	-	-	-	5	-	-	-	-	5
P+ Tazobactam	-	0	1	-	5	-	-	-	-	6
Gentamicin	0	2	2	1	0	2*	1	-	1	7(2*)
Amikacin	5	3	2	1	1	1	1	1*	1	15(1*)
Fluroquinolone	4	3	2	1	5	2	2	1	1	21
Ciprofloxacin	1	0	2	1	5	2*	2	1	1	13(2*)
Levofloxacin	4	3	1	-	5	2	2	1	1	19
Gatifloxacin	3	0	1	-	5	2	2	1	1	15
Cefotaxime	-	0	1	1	5	1	0	-	1	9
Cefoperazone	-	0	1	1	5	-	-	1*	0	7(1*)
Ceftriaxone	0	0	1	1	1	2	0	-	1	6
Ceftazidime	-	0	-	1	2	-	-	-	-	3
Cefepime	-	0	1	1	1	-	-	-	-	3
Aztreonam	-	0	-	-	2	-	-	-	-	2
Meropenem	7	6	3	-	5	-	-	1	-	22
Imipenem	7	6	3	-	4	-	1	1	-	22
Vancomycin	1	-	-	-	-	2	2	3	-	7
Linezolid	-	-	-	-	-	-	2	3	-	5
Nitrofurantion	-	-	1	-	-	-	-	-	-	1
Doxycycline	1	-	-	-	-	2	2	3	1	8
Chloramphenicol	4	4	2	1	-	2*	2	1	-	14(2*)
Cotrimoxazole	-	1	1	1*	-	1	0	1	1	5 (1*)

Drug resistance in causative organisms of sepsis is a rapidly emerging issue. Our study revealed a very high degree of resistance of gram negative organisms (GNO) not only to commonly used antibiotics, but also to broad spectrum cephalosporins. Our findings are consistent with the other studies (Joshi *et al.*, 2000; Koksal *et al.*, 2001; Aftab *et al.*, 2006; Muhammad, 2006; Misallati *et al.*, 2000). Similar to our study, study conducted by (Levine *et al.*, 1999) showed 100% sensitivity to meropenem along with good sensitivity pattern to fluroquinolones, but high level resistance to conventional antibiotics. Contradictory to our study, in this study, all the GNO were susceptible to gentamicin and third generation cephalosporins.

Fortunately our study did not show problem of methicillin resistant staphylococci, but showed emergence of methicillin resistant coagulase negative organisms, which showed poor sensitivity to penicillin but good sensitivity to vancomycin.

Conclusion

In both late onset sepsis and early onset neonatal sepsis most common organism encountered were gram negative 20 (66.67%) and 4(13.33%) respectively. There was a high incidence of resistance noted with ampicillin, gentamicin amongst most gram negative organism's where-in meropenem and fluroquinolones were effective in most cases. There is an increasing trend of antibiotic resistance to the commonly used first line drugs. Continuous surveillance for antibiotic susceptibility is needed to ensure proper empirical therapy.

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