Etiological Study of Neonatal Septicemia

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ABSTRACT

Introduction: *Neonatal sepsis is a clinical syndrome of bacteremia characterized by systemic signs and symptoms of infection in the first month of life*

Aims and objective

1. *To study the etiological profile of neonatal septicemia.*
2. *To determine the antibiotic sensitivity pattern of the bacterial isolates.*

Material and Method: *The study was undertaken for a period of 2 years in the diagnostic microbiology laboratory GMC Nagpur. A total 228 clinically suspected neonatal septicemia cases were studied and blood cultures were performed with help of BaCT-ALERT 3D system, isolates were identified with standard biochemical test and subjected for antimicrobial susceptibility test. CRP test of all suspected cases were also done.*

Results: *There were 139 (60.96%) male cases and 89 (39.04%) female cases with M: F ratio of 1.56:1. The 69.29% were of early onset septicemia and 30.71% were of late onset septicemia. The 108 (47.36%) blood cultures show growth out of which 35 (32.41%) gram positive , 70(64.81%) gram negative and 3 (2.78%) were candida albicans. time to positivity of blood culture 25% were positive within 24 hours, 82.41% by 1-2 days, 94.44% by 2-3 days and 99.07% by 3-4 days. Klebsiella pneumoniae (28.57%) were commonest organism in gram negative and Staphylococcus epidermidis (CONS) (45.71 %) were commonest organism in gram positive. The 25% of gram negative isolates as ESBL producers. 70% isolates of S. aureus were MRSA. No MBL and no VRSA detected. CRP test were positive (CRP ≥ 0.6 mg/dl) in 152 (66.67%) cases with a sensitivity of 69.08% and specificity of 96.05%. Overall mortality rate was 23.18%. Maternal fever and low birth weight were major risk factors.*

Conclusion: *Neonatal septicemia is a life threatening emergency. The study of etiological profile and their antibiotic sensitivity pattern plays a significant role.*

Key words: *Neonatal, Septicemia, Blood, Culture, BACT/ALERT*

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INTRODUCTION

Neonatal sepsis is a clinical syndrome of bacteremia characterized by systemic signs and symptoms of infection in the first month of life.[1] The term encompasses the diagnosis of septicemia, meningitis, pneumonia, arthritis, osteomyelitis and urinary tract infection in newborn. This excludes local infection of newborn such as omphalitis, pyoderma and conjunctivitis.[2] The immaturity of the immune system in the foetus and neonates makes them especially susceptible to infections during the perinatal and neonatal period.

Neonatal septicemia is by far the most important and often fatal sequelae of such infection.[3] Several authors categorize neonatal septicemia into early onset septicemia (presents within the first 72 hours of life) and late onset septicemia (usually presents after 72 hours of life) for epidemiological and therapeutic purposes.[4]

Neonatal sepsis is responsible for 30- 50 % of 5 million total neonatal deaths each year. The reported incidence of neonatal sepsis varies from 7.1 to 38 per 1000 live births in Asia.[5]

The gold standard for the diagnosis of neonatal septicemia is a positive blood culture.[4,6] Definitive culture results takes at least 48-72 hours resulting in treatment delays. It is now possible to detect bacterial growth within 12-24 hours by using improved bacteriological techniques such as BACTEC and BACT/ALERT blood culture system. These advanced techniques can detect bacteria at a concentration of 1-2 colony forming unit (cfu)/ml.[4,7]

Early diagnosis is a key to reduce morbidity and mortality of neonatal septicemia. Hence the study was conducted to know the etiological profile of neonatal septicemia cases and their antibiotic sensitivity pattern for planning strategy for the management of these cases.

AIMS AND OBJECTIVES

The present study was undertaken with the following aims and objectives.

1. To study the etiological profile of neonatal septicemia
2. To determine the antibiotic sensitivity pattern of the bacterial isolates

MATERIAL AND METHODS

The study was undertaken for a period between November 2010 to October 2012 in the diagnostic microbiology laboratory GMC Nagpur. Institutional ethical clearance was taken for the study.

SELECTION OF SUBJECTS

Neonates admitted to hospital with diagnosis of neonatal septicemia were included in study. Neonatal septicemia was diagnosed as per the clinical criteria given by **Vergnano et al (2005)[8].**

Blood sample(0.5 to 2 ml) was collected with all aseptic precaution and was inoculated into blood culture bottle BactT/Alert® PF (BIOMERIEUX, INC. Durhams, NC 27704) containing 20 ml of broth.

PROCEDURE

The blood and broth were mixed gently and bottles were transported to laboratory for incubation in BacT/Alert 3D system and further processing was done as per manufacturer's guideline.

INTERPRETATION

Those blood culture bottles which were indicated positive, query positive **(?+)** and query negative **(?-)** by Bact/ Alert 3D system were sub cultured on Sheep blood agar and MacConkey agar. The blood agar and MacConkey's medium were incubated at 35 ± 2^0 Celsius for 18 - 24 hours in aerobic atmosphere. Various organisms were identified on the basis of colony morphology and standard biochemical tests.

Those blood culture bottles which were indicated as negative by 5 days (as per setting of Bact / Alert 3D system) were reported as "no growth".

The isolates were subjected to antimicrobial susceptibility testing by Kirby Bauer disk diffusion method as per CLSI guidelines 2011[9].

OBSERVATIONS AND RESULTS

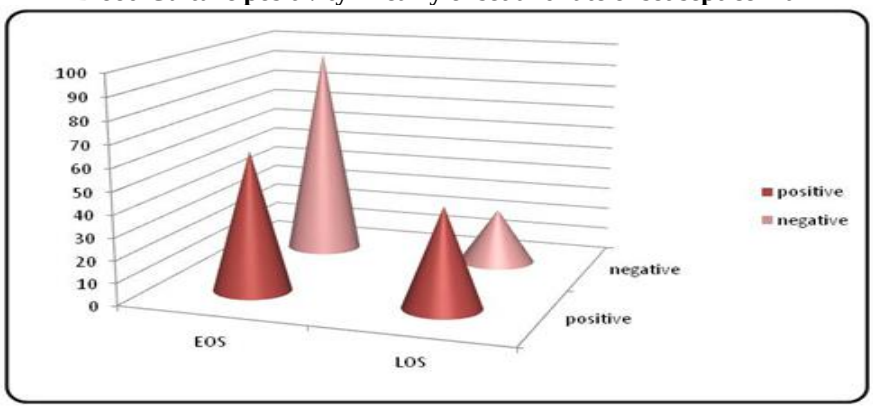
In the present study, a total of 228 clinically suspected neonatal septicemia cases were studied and blood culture was performed. Among these, 158 (69.29%) cases were of early onset septicemia and 70 (30.71%) cases were of late onset septicemia.

Age and sex distribution of cases

Age	Male	Female	Total (%)
0 – 72 hrs [EOS]	96	62	158 (69.29%)
72 hrs – 4 weeks [LOS]	43	27	70 (30.71%)
Total	**139 (60.96%)**	**89 (39.04%)**	**228**

Early onset septicemia was more common than late onset septicemia.
Male to female ratio was 1.56: 1.

Blood Culture positivity in early onset and late onset septicemia



Out of 158 early onset septicemia cases blood cultures were positive in 63 (39.87%) cases and out of 70 late onset neonatal septicemia cases, blood cultures were positive in 45 (64.28 %) cases.

Out of 228 clinically suspected neonatal septicemia cases blood culture was positive in 108 (47.36%) cases where as in 120 (52.63%) cases blood culture was negative.

Bac T / Alert 3D microbial detection system results (n=228)

Duration	Positive	Negative	? +	? -
≤ 24 hrs	27	-	-	-
1-2 days	61	48	1	-
2 -3 days	10	25	3	1
3-4 days	04	23	1	2
4-5 days	00	20	1	1
Total	**102**	**116**	**6**	**4**

Table 4 shows that, out of 102 positive samples, 27 blood culture samples were indicated positive within 24 hours by BacT/Alert 3 D system and among these 27 samples 10 were indicated within 4-5 hours. Sixty one samples were indicated in 1-2 days, 10 were between 2-3 days, 04 were between 3-4 days.

Out of 116 negative indicated samples, 48 were indicated by 1-2 days, 25 were between 2-3 days, 23 were indicated between 3-4 days and 20 samples were indicated 4-5 days.

Out of 6 query positive **(?+)** samples, 3 were indicated between 2-3 days, and 1 sample on each 1-2 days,3-4 days, and 4-5 days.

Out of 4 query negative **(?-)** samples 2 were indicated in 3-4 days, one on each 2-3 days and 4-5 days.

Maternal risk factors for neonatal septicemia like maternal fever (17.10 %) and prolonged rupture of membranes (20.17%) were found to be more common.

Low birth weight, prematurity, neonatal resuscitation, meconium aspiration were common risk factors for neonatal septicemia.

Most of the neonates presented with lethargy (69.74%), no sucking at all (68.42%) and temperature changes (42.98%).

Klebsiella pneumoniae (18.52%) was the commonest isolate in neonatal septicemia.

In early onset neonatal septicemia cases (63), gram negative bacilli (82.54%) were common etiological agents as compared to gram positive cocci (17.46%) while in late onset septicemia cases (45), gram positive cocci predominated (53.33%).

Only 30% isolates of *S. aureus* and 43.75% isolates of *S. epidermidis* were sensitive to penicillin and oxacillin. Methicillin resistance was seen in 70% isolates of *S. aureus.*

Gram positive cocci responded very well to vancomycin (100% sensitivity).

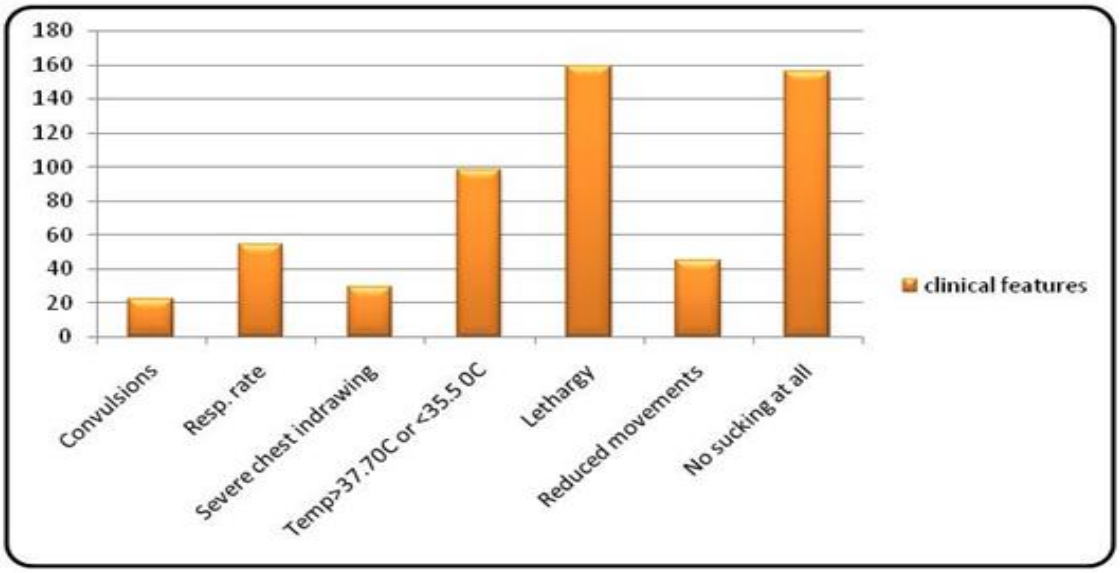
The enterobacteriaceae family showed high resistance to various antimicrobial agents like, ampicillin, gentamicin, aztreonam, cefazolin, cefoperazone, ceftazidime, cefotaxime, and ceftriaxone. Most of them had good sensitivity to amikacin (87.27%), tobramycin (78.18%) and ciprofloxacin (38.18%).

In present study, we obtained 25% (14/55) of gram negative isolates as ESBL producers. In these, maximum ESBL production was shown by *K. pneumoniae,* 55% (11/20) followed by *E.coli*, 16.67% (2/12) and *K.aerogenes*, 8.33% (1/12).

Pseudomonas aeruginosa and *Acinetobacter spp* showed showed high resistance to various antimicrobial agents like cefepime, cefoperazone, ceftazidime and cefotaxime. Most of them had good sensitivity to amikacin (86.67%) and piperacillin – tazobactam (73.33%).

We observed that imipenem was the best antibiotic for infections with multidrug resistant gram negative bacilli.

Out of 228 cases, CRP test was positive (CRP $\geq$ 0.6 mg/dl) in 152 (66.67%) cases and was negative (CRP < 0.6 mg/dl) in 76(33.33%) cases.



Clinical presentation of neonatal septicemia

Microbial profile of neonatal septicemia from blood culture

Organism	EOS	LOS	Total
Gram positive cocci	11(17.46%)	24(53.33%)	35(32.41%)
Staphylococcus aureus	4	6	10
Staphylococcus epidermidis (CONS)	3	13	16
Enterococcus faecalis	4	5	9
Gram negative organisms	52(82.54%)	18(40%)	70(64.81%)
Klebsiella pneumoniae	16	4	20
Klebsiella aerogenes	9	3	12
Klebsiella oxytoca	1	1	2
Escherichia coli	8	4	12
Salmonella typhi	0	1	1
Citrobacter freundii	3	0	3
Citrobacter koseri	0	1	1
Enterobacter aerogenes	0	2	2
Enterobacter cloacae	2	0	2
Acinetobacter baumannii	8	2	10
Acinetobacter lwoffii	2	0	2
Pseudomonas aeruginosa	3	0	3
Fungi		3(6.67%)	3 (2.78%)
Candida albicans		3	
Total	**63(58.33%)**	**45(41.67%)**	**108**

Correlation of CRP with blood culture in neonatal septicemia

Blood culture	CRP test		Total
	Positive (%)	Negative (%)	
Positive	105(69.08)	3(3.95)	108
Negative	47(30.92)	73(96.05)	120
Total	**152**	**76**	**228**

Mcnemar's chi^2 test =38.72 p=0.0000

CRP test was positive in 69.08% of blood culture positive cases (sensitivity) while it was negative in 96.05% of blood culture negative cases (specificity) with highly significant p value.

Overall mortality rate was 23.18%. Mortality in early onset septicemia was 27.84% while that in late onset septicemia it was 10%. Mortality in blood culture positive cases was more (40.74%) than blood culture negative cases (5.83%).

DISCUSSION

The microbiological pattern of neonatal septicemia varies and therefore there is need for an ongoing review of the causative organisms and their antibiotic sensitivity pattern. According to various reports the incidence of neonatal septicemia to vary between 36% to 55%.[10] The most common agents causing neonatal sepsis are bacteria but neonatal sepsis syndrome can also be caused by organisms other than bacteria like, rubellavirus, enterovirus, adenovirus, coxsackievirus, Toxoplasma species and Candida species[11]. Hence, only a proportion of the blood culture from cases with clinical sepsis will be positive for causative organisms. In addition, collection of blood samples after administration of antibiotics results in poor recovery of the bacterial pathogens in culture. In the present study the blood culture positivity rate was 47.36% which is similar with other studies .[10,12.13]

The etiological agents of neonatal sepsis vary between developed and developing countries[14,15]. *Klebsiella pneumoniae* and other Gram-negative organisms were the common causes of sepsis in the present study as well other studies from India and Nigeria[16,17].

The bacteriological profile of early-onset sepsis is different from that of late-onset sepsis as the mode of infection is different[18]. An ascending infection from cervix, passage of the baby through a colonized birth canal and transplacental infections is main route of infection in case of early onset neonatal sepsis[11]. In the present study, *Klebsiella pneumoniae* was the common agent implicated in early-onset sepsis.

Late-onset neonatal sepsis is often acquired from the care-giving environment and coagulase-negative staphylococci (CONS), *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella* species are the commonly involved[11]. In the present study, CONS was the common etiological agent of late-onset sepsis. In an epidemiological study performed to observe the long term trends in the agents causing neonatal sepsis, CONS were showing an increasing trend[19].

The bacteriological profile of neonatal septicemia in our hospital is comparable to that of National Neonatal Perinatal Network Database Report[20]. According to this report Group B *Streptococcus* is not common in our country and we also did not isolate group B *Streptococcus.*

In our study, no resistance was found to linezolid and vancomycin in case of gram positive isolates. So these drugs can be effectively used in resistant cases. In the present study, majority of the *Klebsiella pneumonia*e and E. coli isolates were resistant to all the antibiotics tested except imepenem, amikacin, tobramycin, piperacillin – tazobactam and 25% of them were ESBL producers. In our study, most of the *Klebsiella pneumoniae* isolates were resistant to commonly used antibiotics [17]. In another study from North India, 30–80% of the Gram negative isolates were resistant to third-generation cephalosporins[16]. As amikacin shows good activity against gram negative bacteria should preferably included in empirical regimen while third-generation cephalosporins should not be used alone. In our study one *Salmonella typhi* was isolated and that was sensitive to ampicillin, amoxicillin-clavulanic acid, ciprofloxacin and chloramphenicol. Sharma et al (2008)[21] found 9 isolates of *Salmonella spp*. which were sensitive to ciprofloxacin and resistant to ampicillin and amoxicillin-clavulanic acid.

The level of C-reactive protein (CRP) may increase more than 1000 fold during acute phase response. The half life of CRP is 19 hours so CRP levels can be expected to fall quickly after removal of microbial stimulus. Normalization of CRP levels has previously been proposed as a possible criterion for the discontinuation of antibiotic therapy.[22]In present study, CRP revealed sensitivity of 67.11% and specificity of 92.11%.

Squire et al (1979)[23] found CRP as the most efficient factor (sensitivity 86%) in distinguishing infected from non infected babies. Mondal SK et al (2012)[24] observed statistically significant association of C-reactive protein with neonatal septicemia with sensitivity of 84.2%. Our study showed that the measurement of CRP by latex agglutination method is a useful adjunctive tool in screening for neonatal septicemia. Overall case fatality rate in our study was 27.84% and maximum mortality was seen with *K. pneumoniae* (27.27%) followed by *E. coli* (15.91%). Similar observations were made by the some authors[25].

CONCLUSION

Neonatal septicemia is a life threatening emergency. The study of etiological profile and their antibiotic sensitivity pattern plays a significant role.

Definitive culture results takes at least 48-72 hours resulting in treatment delays. But with the use of improved bacteriological techniques such as BACTEC and BACT/ALERT blood culture system bacterial growth can be detected within 12-24 hours.

An appropriate use of antibiotic susceptibility surveillance programme along with good infection control practices and rational use of antibiotics will reduce infection rate, ensure better therapeutic success and prolong the efficacy of available antimicrobials.

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