

Original Research Article

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Bacteriological Study of Neonatal Septicemia and their Antibiotic Susceptibility Patterns at MGM Hospital, Warangal, India

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ABSTRACT

Neonatal septicemia is still a leading cause of mortality and morbidity in developing countries like India. This prospective study involved 382 clinically suspected cases of neonatal septicemia over a period of one year from April 2014 to March 2015. Of the total 382 cases studied 174 (45.54%) were blood culture positive. Among the culture positive cases, septicemia was more common in male neonates, seen in 55.23%, compared to 44.76% of female neonates. Of the 174 culture positive cases, early onset septicemia (EOS) was more common, seen in 75.86% compared to late onset septicemia (LOS) in 24.14% of the cases. Gram negative organisms were the predominant causative agents of septicemia in (61.49%), compared to Gram positive organisms in (38.50%) of the cases. *Klebsiella pneumoniae* was the commonest gram negative isolate in 39.65% of cases, followed by *Staphylococcus aureus* in 22.41% of cases. All the Gram positive isolates were 100% susceptible to Vancomycin followed by Amikacin 94.87% , linezolid 89.74% and 100% resistant to Penicillin . Majority of Gram negative isolates were susceptible to Meropenem followed by Amikacin and ciprofloxacin, while maximum resistance was noted with Ampicillin, followed by Cefuroxime, Cefotaxime, Ceftazidime and Gentamicin . To conclude, Blood culture is still the “Gold standard” for the diagnosis of septicemia in neonates and should be done in all cases of suspected septicemia prior to starting the antibiotics.

Keywords

Blood culture,
Neonatal
septicemia,
*Klebsiella
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*Staphylococcus
aureus*

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Introduction

Sepsis is the commonest cause of neonatal mortality; and is the four leading causes of neonatal mortality and morbidity in India¹. It is estimated that up to 20% of neonates develop sepsis and approximately 1% die of sepsis related causes². The risk factors for Neonatal septicemia include premature rupture of membranes, prolonged rupture of membranes, prematurity, UTI, poor maternal

nutrition, LBW, birth asphyxia and congenital anomalies³.

Mortality rate in neonatal sepsis differs according to the type of organism involved. Gram negative bacteria caused the highest mortality. Among the intramural births, *Klebsiella pneumoniae* was the most frequently isolated followed by *Staphylococcus aureus*.

Among the extramural neonates (referred from community) *Klebsiella pneumoniae* was again the commonest organism isolated followed by *Staphylococcus aureus* and *Pseudomonas*⁴. A study by Shrestha and co-workers observed that *Klebsiella* is the most common organism which is almost resistant to first line antibiotics⁵. Whereas, Gram negative and Gram positive isolates are resistant to first line antibiotics and even with Cefotaxim is emerging and is a major concern.

The successful treatment with a favorable outcome of the neonate depends on an ongoing review of the causative organisms and their antibiotic susceptibility pattern⁶. Bacterial organisms with increased antibiotic resistance have also emerged and have further complicated the management of neonatal sepsis⁷.

Several studies have shown the resistance pattern exhibited by the organisms isolated from sepsis patients, Gyawali and co-workers observed high incidence of neonatal septicemia and organisms isolated exhibited higher resistance towards commonly used antimicrobials⁸.

It is important that supportive and antimicrobial therapy of a neonate with sepsis is instituted quickly. Hence only a minimum of essential investigations should be undertaken. Blood culture is used as gold standard for diagnosis of septicemia and would be performed in all cases of suspected sepsis prior to starting antibiotics⁴. In the recent past, a study by Jyothi and co-workers concluded that, blood culture from all neonates suspected to have septicemia showed Gram negative organisms (*Klebsiella*, *Acinetobacter*), CONS and *S. aureus* are the leading cause of neonatal sepsis and also observed that most of them are resistant to multiple antibiotics⁹. The present study was aimed to study the Bacteriological profile and

antibiotic susceptibility pattern of neonatal septicemia at MGM Hospital, Warangal.

Materials and Methods

Source of the data: This prospective study was conducted in the Department of Microbiology, Mahatma Gandhi Memorial Hospital, Warangal a period of 1 year from April 2014 to March 2015. Blood samples from 382 clinically suspected neonatal septicemia cases are subjected to aerobic culture. The culture isolates obtained are tested for antibiotic susceptibility pattern.

Inclusion criteria: Neonates were included when at least three of the following risk factors were present⁹: 1) Febrile illness in the mother during or within two weeks of delivery (more than 38⁰ C, oral temperature). 2) More than 3 vaginal examinations during labor. 3) Prolonged rupture of membranes (more than 12 hours). 4) Foul smelling or meconium stained liquor. 5) Preterm baby or LBW baby. 6) Birth asphyxia and difficult resuscitation. 7) Pathological evidences of funisitis.

In addition, neonates who presented with symptoms of septicemia like refusal of feeds, decreased activity, lethargy, respiratory distress, fever, hypothermia, sclerema, abdominal distension, seizures and shock were all included.

Exclusion criteria: 1) Age > 28 days, 2) Neonates with lethal congenital anomalies.

Sample collection

An area of approximately 5cm over the vein puncture site was disinfected with 70% alcohol gently and allowed to dry. This was followed by application of Povidone Iodine in concentric circles over the site and allowed to dry for at least 1 minute. About 1ml of blood was drawn using a sterile syringe and

inoculated aseptically into a blood culture bottle (brain heart infusion broth).

Blood culture

About 1 ml of blood will be drawn aseptically and inoculated into a blood culture bottle containing 10 ml of Brain Heart Infusion broth, thus making a dilution of 1 in10 to nullify the natural bacteriostatic/bactericidal activity of blood. Brain Heart Infusion broth will be prepared using the commercially available ready to use powder, supplied by Hi-media Laboratories Pvt. Limited. The broth will be distributed into 10ml quantity in McCartney bottles and sterilized by autoclaving at 121° C for 15 minutes. After inoculation, the blood culture bottles will be incubated at 37° C under aerobic conditions in the incubator for 7 days. The first subculture will be done after 24 hours of incubation, the second on the third day and a final on the seventh day. Subcultures will be done onto 5% sheep blood agar and Mac Conkey agar plates. The inoculated plates will be incubated aerobically in the incubator at 37° C for 24hours, and the plates were observed for growth. [AIIMS –NICU protocol 2008]. The growth will be identified by colony characteristics, Gram's stain and standard biochemical tests described in Mackie and McCartney, Practical Medical Microbiology¹⁰, and Bailey and Scott's Diagnostic Microbiology¹¹. Cultures which did not yield any growth following three subcultures were reported negative at the end of 7 days.

Antibiotic susceptibility testing

Antibiotic susceptibility testing will be done for all the isolates on Muller Hinton agar using commercially available discs (Hi media), by Kirby- Bauer disc diffusion technique as per the CLSI guidelines. Two or three identical colonies were taken from the primary culture plates with sterile loop and suspended in BHI

broth, incubated at 37⁰C The turbidity was compared and adjusted to 0.5 Mc Farland tube turbidity standards.

A sterile swab was dipped into the inoculum. Excess inoculum was removed by pressing and rotating the swab firmly against the side of the tube, above the level of liquid. The swab was streaked all over the surface of a Muller Hinton Agar plate. Finally swab was passed round the edge of the agar surface. The inoculum was dried for a few minutes at room temperature with the lid closed. Commercially available antibiotic discs obtained from Hi-Media laboratories Ltd, were used. The antibiotic discs were placed on the inoculated plates using a pair of sterile forceps and gently pressed to ensure even contact. The plates were incubated at 37⁰ C After 16-18hrs incubation the diameter of each zone was measured with a scale, recorded in mm and interpreted as sensitive or resistant, in accordance to the indications of the disc manufacturer. The results were recorded and interpreted as per CLSI recommendations.

Results and Discussion

In the present study, out of the total 382 clinically suspected neonates,174 (44.54%) blood culture positive and 208 (54.45%) blood culture negative (Table 1). Out of 382 cases of clinical sepsis 211 (55.23%) were male neonates, 171 (44.76%) were female neonates. Male neonates with clinical sepsis were admitted more frequently than female neonates, which is statistically significant. (p-value0.04). Among 174 cases of proven sepsis 91 (52.29%) were male neonates and 83 (47.7%) were females neonates. There was no sex difference in blood culture positive sepsis (p-value 0.54) (Table 2).

In the cohort of neonates, with clinical sepsis term neonates were more than 215 (56.28%). pre term neonates167 (43.71%) which is

statistically significant (0.01). However, Blood culture positivity was comparable in both preterm 106(60.91%) and term 68 (39.08%) neonates (p-value 0.003) (Table 3).

Of the total 174 culture positive cases, Early onset septicemia was seen in 75.86% cases and 24.14 % cases showed late onset septicemia, indicating majority of culture positive cases were seen among early onset septicemia (Figure 1).

Out of 382 clinical sepsis 174 (45.54%) were blood culture positive. Out of 174 organisms isolated in blood culture, 67(38.5%) were gram positive and 107(61.49%) were gram negative (Table 4).

In the present study, *Klebsiella* species (39.65%) was the most common organism in our study followed by Coagulase positive *Staphylococcus* (22.41%) and CONS (16.09%). In gram positive organisms most common are coagulase positive *Staphylococcus* (22.41%), CONS (16.09%). In Gram negative organisms most common are *Klebsiella* species (39.65%), followed by

E. coli (14.94%) and *Pseudomonas* (6.89%) (Table 5).

In the present study majority of isolates were Gram negative accounting for 61.49% of the total cases, commonest organism was *Klebsiella pneumoniae* susceptible to Meropenem (91.3%), Amikacin (84.05%), Ciprofloxacin (56.52%), Ceftazidime (31.81%) and Gentamicin (31.81%). Most of the isolates were resistant to Ampicillin and Cefuroxime. In Gram positive organisms commonest was *S.aureus* (22.41%), 100% sensitive to Vancomycin followed by Amikacin (94.87%), Linezolid (89.74%) and Ciprofloxacin (87.17%). *E. coli* isolates were susceptible to Meropenem (88.46 %), followed by Amikacin (84.05%). Majority were resistant to Ampicillin, cefuroxime, Cefotaxime and Gentamicin. Other less commonly isolated Gram negative organisms were *P. aeruginosa* (6.89%), was sensitive to Meropenem 91.66% followed by Amikacin 83.33% and Ciprofloxacin 25%, and 100% resistant to Ampicillin and Cefuroxime (Table 6).

Table.1 Blood culture positive cases among total cases

Blood Culture	Number of Patients	Percentage
Positive	174	45.54
Negative	208	54.45
	382	100

Table.2 Sex distribution among clinical and blood culture positive sepsis

SEX	CLINICAL SEPSIS		BLOOD CULTURE POSITIVE SEPSIS	
Male	211 (55.23%)		91(52.29%)	
Female	171 (44.76%)		83 (47.7%)	
Total	382	p-value 0.04	174	p- value 0.54

Table.3 Gestational age distribution among clinical and blood culture positive sepsis

GESTATION AGE	CLINICAL SEPSIS		BLOOD CULTURE POSITIVE SEPSIS	
Preterm	167(43.71%)		106(60.91%)	
Term	215(56.28%)		68(39.08%)	
Total	382	p-value 0.01	174	p-value 0.003

Table.4 Bacterial profile in blood culture positive sepsis

	Gram positive cocci Blood culture sepsis	Gram negative bacilli Blood culture sepsis	Bacterial Sepsis (total)
No. of organisms	67(38.50%)	107(61.49%)	174

Table.5 Spectrum of isolates in blood culture positive sepsis

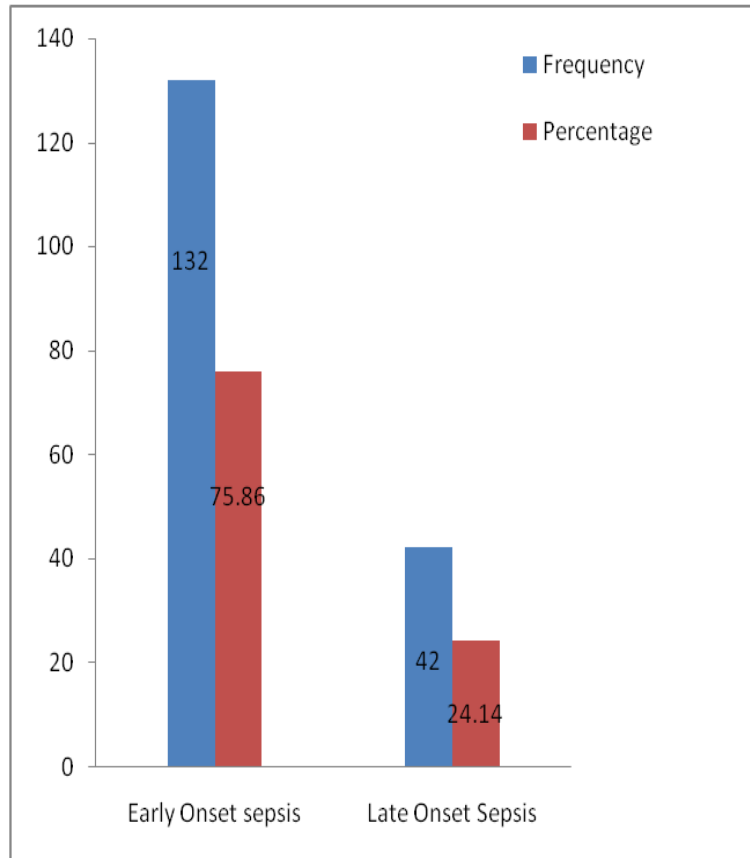
Organism	No of Patients
<i>Klebsiella</i> species	69(39.65%)
<i>E. coli</i>	26 (14.94)
<i>Pseudomonas</i>	12(6.89%)
Coagulase positive <i>Staphylococcus</i>	39(22.41%)
CONS	28(16.09%)
TOTAL	174

Table.6 Antibiotic susceptibility pattern of the isolates

ANTIBIOTICS	<i>K. pneu</i> (69)		<i>E. coli</i> (26)		<i>P. aerug</i> (12)		<i>S. aureus</i> (39)		CONS(28)	
	S%	R%	S%	R%	S%	R%	S%	R%	S%	R%
PENICILLIN (P)	-	-	-	-	-	-	0	100	7.14	92.85
ERYTHROMYCIN (E)	-	-	-	-	-	-	5.12	94.87	17.85	82.14
VANCOMYCIN (VA)	-	-	-	-	-	-	100	0	100	0
LINEZOLID (LZ)	-	-	-	-	-	-	89.74	10.25	92.85	7.14
AMPICILLIN (AMP)	5.79	94.20	19.23	80.79	0	100	2.56	97.43	14.28	85.71
GENTAMICIN (G)	31.88	68.11	53.84	46.15	8.33	91.66	82.05	17.94	89.28	10.71
AMIKACIN (AK)	84.05	15.94	84.61	15.38	83.33	16.66	94.87	5.12	96.42	3.57
CIPROFLOXACIN (CIP)	56.52	43.47	30.76	69.23	25	75	87.17	12.82	82.14	17.85
CEFOTAXIME(CTX)	23.18	76.81	26.92	73.07	8.33	91.66	69.23	30.76	75	25
CEFUROXIME (CXM)	13.04	86.95	23.07	76.92	0	100	12.82	87.17	39.28	60.71
CEFTAZIDIME(CA)	31.88	68.18	30.76	69.23	16.66	83.33	71.79	28.2	89.28	10.71
MEROPENEM(MRP)	91.30	8.69	88.46	11.53	91.66	8.33	-	-	-	-

K.pneu – *Klebsiella pneumoniae*; *E. coli* – *Escherichia coli*; *P. aerg* – *Pseudomonas aeruginosa*; *S. aureus* – *Staphylococcus aureus*; *CoNS* – *Coagulase Negative Staphylococci*

Figure.1 shows schematic distribution of culture positive cases according to onset of symptoms (%)



Bacterial septicemia is still a major cause of morbidity and mortality in the newborn period, particularly in the developing countries despite the considerable progress in early diagnosis and treatment measures ¹². The disease continues to pose a challenge to the pediatricians in making a definitive clinical diagnosis due to the subtle and nonspecific signs and symptoms; hence laboratory diagnosis plays a major role. Definitive diagnosis rests on a positive blood culture, to identify the pathogen and determine its antibiotic susceptibility pattern, but for better survival and outcome, simple and rapid diagnostics tests are required as adjuncts to the blood culture for early and effective initiation of treatment to the septicemic neonates.

In the present study an attempt has been made to know the various etiological agents responsible for neonatal septicemia and their antibiotic susceptibility patterns.

This prospective study involved 382 clinically suspected cases of neonatal septicemia. Of the total 382 cases studied 174 (45.54%) were blood culture positive. Among the culture positive cases, septicemia was more common in male neonates, compared to female neonates. Similar observations are seen by earlier studies ¹³. In the present study culture positive septicemia cases were higher among the preterm neonates. The results of our study were comparable to the studies conducted by Bhat and co-workers ¹⁴. Higher number of cases in preterm neonates may be due to

predisposing factors. Of the 174 culture positive cases, early onset septicemia was more common, compared to late onset septicemia. The higher number of EOS cases may be due to the immature immunological responses of the neonates in the first week of life, making them more susceptible to infections in this period^{15, 18}. Present study also shows that, culture positive septicemia cases were higher among males than the females, which is in accordance with earlier studies¹⁶.

Male preponderance in neonatal septicemia may be linked to the X-linked immunoregulatory gene factor resulting in the host's susceptibility to infections in males. In the present study, *Klebsiella pneumoniae* was the predominant isolate followed by *S. aureus*. Gram negative organisms were more compared to Gram positive organisms causing septicemia, present observations were in accordance with earlier studies¹⁷. In the present study, *Staphylococcus aureus* was the commonest isolate in Gram positive organisms, 100% sensitive to Vancomycin, 100% resistant to Penicillin. But study conducted by Neema Kayange and co-workers¹⁶ observed 86% of Vancomycin sensitive strains. It was also observed that, commonest Gram negative organism isolated was *Klebsiella pneumoniae* which was 91.3% susceptible to Meropenem, 84.05 % to Amikacin and 56.52% Ciprofloxacin. Most of other antibiotics were resistant. In view of the changing spectrum of the causative agents of neonatal septicemia and their antibiotic susceptibility patterns from time to time and from one hospital to another, a positive blood culture and the antibiotic susceptibility testing of the isolates are the best guide to the antimicrobial therapy. Blood culture is still the "Gold standard" for the diagnosis of septicemia in neonates and should be done in all cases of suspected septicemia prior to starting the antibiotics.

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Conflict of Interest: The authors declare that they have no conflict of interest.

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