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BACTERIOLOGICAL PROFILE IN NEONATAL SEPTICEMIA

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Abstract: Neonatal sepsis or sepsis neonatorum refers to systemic infection of the newborn characterized by a constellation of a nonspecific symptomatology in association with bacteremia Neonatal septicemia remains one of the most important causes of mortality despite considerable progress in hygiene, introduction of new anti-microbial agents, and advanced measures for early diagnosis and treatment This study on neonatal septicemia comprised of 100 neonates who were clinically suspected as septicemia, from the pediatric NICU ward, Government General Hospital, Guntur was conducted in the department of Microbiology, Guntur Medical College, Guntur. Out of the 100 clinically suspected cases 40 were culture positive. Among the 40 isolates, 25 were Gram negative bacilli (62.5%) and 15 were Gram positive cocci (37.5%). Out of 40 proven cases, males were 29 (72.5%) and females were 11(27.5%). Early onset sepsis (65.5%) was more common than late onset sepsis Neonatal sepsis or sepsis neonatorum refers to systemic infection of the newborn characterized by a constellation of a nonspecific symptomatology in association with bacteremia. In the pre-antibiotic era neonatal sepsis was usually fatal. Case fatality rates in antibiotic treated infants now range between 5% to 60% with the highest rates reported from the lowest-income countries [1]. It is estimated that almost 20 per cent of all neonates develop infection during the neonatal period with the mortality rate reaching as high as 50% for infants who are not treated timely. Not surprisingly, sepsis is the commonest admitting diagnosis among neonates at referral facilities. Even though neonatal care has dramatically improved over the last decade, the overall as well as gestation specific mortality due to sepsis has not changed much due to more and more babies weighing less than 1.5 kg surviving in the intensive care units. (34.5 %.)The mainstay for therapy for Neonatal Septicemia being appropriate Supportive care, antibiotics used based on susceptibility testing of organism isolated.

Keywords: Neonatal septicemia, CRP, Blood



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INTRODUCTION

Neonatal septicemia remains one of the most important causes of mortality despite considerable progress in hygiene, introduction of new antimicrobial agents, and advanced measures for early diagnosis and treatment. The World Health Organization (WHO) estimated that 1 million deaths per year are due to neonatal sepsis, 42% of these deaths occur in the first week of life. 40% of all neonatal deaths due to sepsis occur in developing countries. (Lawn, Cousens, and Zupan 2005).

Although globally only 16 percent of newborns have low birth weight, 60 to 80 percent of neonatal deaths occur in LBW infants (Lawn, Cousens, and Wilczynska forthcoming). As per National Neonatal Perinatal Database (NNPD) 2002-2003, the incidence of neonatal sepsis in India was 30 per 1000 live births. Twenty percent of symptomatic neonates in India suspected to have EOS(early onset sepsis) were blood culture positive. Overall, 30% neonates clinically suspected to have LOS(late onset sepsis) in an NICU setting have positive blood culture results. The fourth Millennium Development Goal (MDG) aspires to a global target, by 2015, of reducing the under-five mortality rate by two-thirds, which implies approximately 30 deaths per 1,000 live births for children under five. Currently, there are an estimated 30 deaths per 1,000 live births in the neonatal period alone

MATERIALS AND METHODS

This study on neonatal septicemia comprised of 100 neonates who were clinically suspected as septicemia, from the pediatric NICU ward, Government General Hospital, Guntur was conducted in the department of Microbiology, Guntur Medical College, Guntur spread over for a period of one year from August 2012 to August 2013.

Patient selection

Inclusion criteria:

Neonates who were clinically suspected of septicemia , Age < 28 days > 22 weeks of gestation and full term babies. Presence of three (3) or more clinical symptoms like refusal of feeds, lethargy, irritability, hypothermia, respiratory distress, jaundice, vomiting, apnea, abdominal distension, hyperthermia, pustular skin lesions, seizures, sclerema, cyanosis, conjunctival discharge, bulging of anterior fontanellae, diarrhea.

Exclusion criteria: Extreme prematurity <22 weeks of gestation Gross congenital anomalies Undergone surgery

Specimen collected were Blood and CSF in case of suspected meningitis having seizures.

Blood was Processed by 1. Serological tests 2.Culture and sensitivity

The processing of CSF sample:With a sterile inoculating wireloop,CSF was inoculated on blood agar ,chocolate agar and MacConkey agar. The inoculated plates were incubated at 37°C for 18-24 hours. The remaining part was centrifuged at 1500-2000 rpm for 10-15 minutes. After centrifugation, the deposit was used for gram stain as per the standard procedures. -

Isolation of bacteria from blood:

Brain heart infusion broth with inoculated blood was incubated aerobically at 37°C for 18 hours. Subculture was done in Biosafety cabinet on 5% sheep blood agar, chocolate agar and MacConkey agar were incubated at 37°C for 24 hours, and then at 7 days in between these time points, sub culturing was done only if there was visible turbidity.

Identification of the isolates:

The isolates from blood and CSF were identified by colony morphology, gram staining, motility and the following biochemical reactions as per the standard procedures

1. Catalase test.
2. Oxidase test.
3. Coagulase test.
4. Indole production test.
5. Methyl red test
6. Voges-proskauer test.
7. Urease test
8. Citrate utilization test.
9. Triple sugar iron agar test
10. Sugar fermentation test

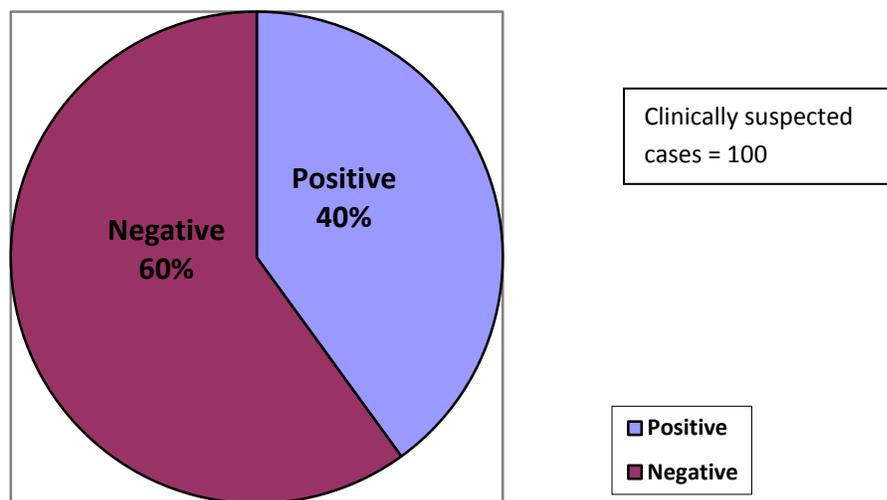
Antibiotic sensitivity testing: Antimicrobial susceptibility testing was performed by KirbyBauer disk diffusion method on Muller Hinton agar according to clinical laboratory standard institute (CLSI) recommendations.

Results:

Table 1: Out of 100 clinically suspected cases 40 were culture positive

Total cases	Culture positive	Culture negative
100	40	60
%	40%	60%

Blood culture positives



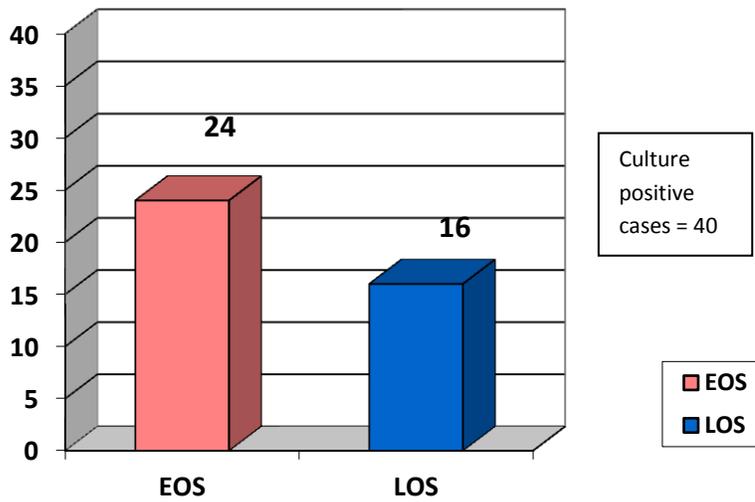
Age of onset and sex distribution

Out of 40 proven cases, males were 29 (72.5%) and females were 11(27.5%). Thus incidence of neonatal septicemia was more in the males. Those neonates, where the onset of septicemia was 0 to 3 days were diagnosed as early onset neonatal septicemia and those with onset between 3 to 28 days were diagnosed as late onset neonatal septicemia. Out of the 40 proven septicemia cases, males categorized as EOS were 19 (65.5%) and LOS 10 (34.4%) of total 29 cases. Females categorized as EOS were 5 (45.4%) and LOS 6 (54.5%) out of total 11.

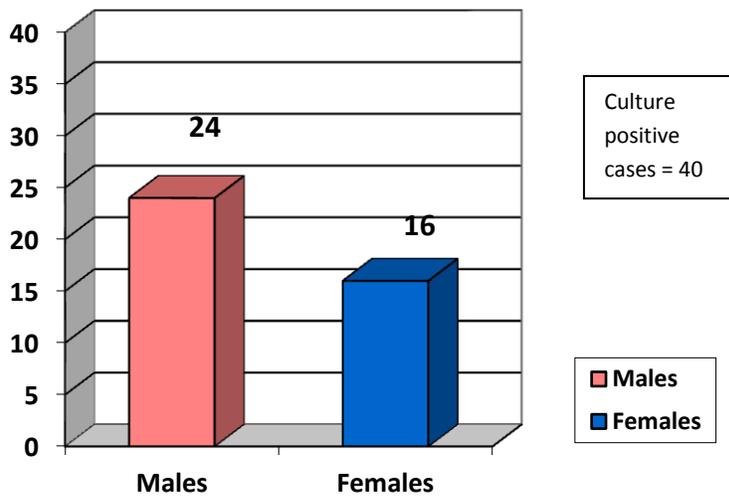
Table 2: Age and sex wise distribution of septicemic neonates

Characteristic	Male	Female	Total
EOS < 3 days	14(58.3%)	10 (62.5%)	24
LOS (4-28 days)	10 (41.6%)	6 (37.5%)	16
Total	24 (60%)	16 (40%)	40

Time of onset of septicemia



Distribution of cases according to sex



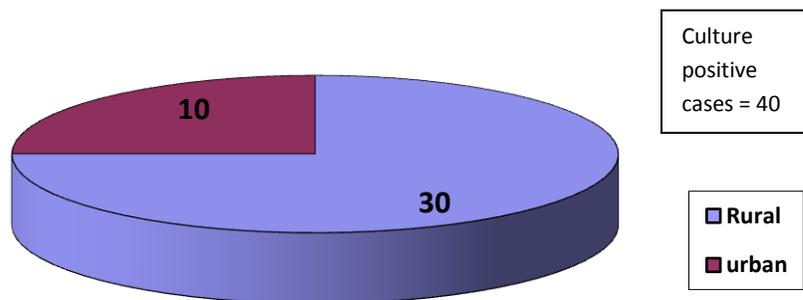
Rural –urban distribution

Among 40 cases of neonatal septicemia 30 were from rural area and 10 were from urban area. Thus more babies from rural area were affected with neonatal septicemia.

Table 3: Table showing rural and urban distribution of cases

Area	Total	%
Rural	30	75%
Urban	10	25%

Rural and urban distribution of cases



Distribution of organisms among culture positive cases:

Among the 40 isolates, 25 were Gram negative bacilli (62.5%) and 15 were Gram positive cocci (37.5%).

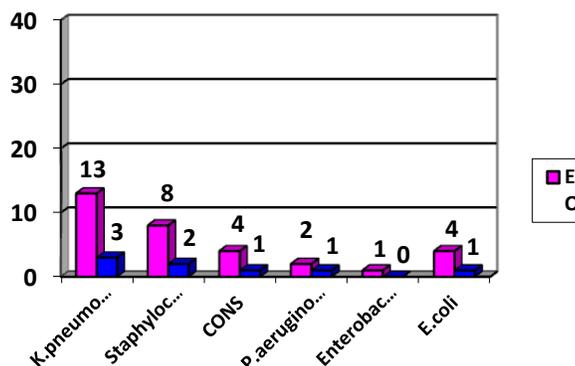
Out of the 25 Gram negative bacilli 16 were Klebsiella (64%) 5 were E-coli (20%) 3 were Ps. aeruginosa (12%) and 1 was Enterobacter (4%).

Out of the 15 Gram positive cocci (37.5%) 10 were coagulase positive staphylococci (66.6%) and 5 were CONS. (Coagulase negative staphylococci)

Table 4 Percentage of blood culture positive isolates in neonatal sepsis.

Sno	Name of the bacteria	EOS	LOS	Total	Percentage
1	Klebsiella pneumonia	13	3	16	40%
2	Staphylococcus aureus	8	2	10	25%
3	CONS	4	1	5	12.5%
4	Pseudomonas aeruginosa	2	1	3	7.5%
5	Enterobacter	1	0	1	2.5%
6	Escherichia coli	4	1	5	12.5%

Bacterial pattern in blood culture



Antibiotic sensitivity pattern :

All the Gram negative bacilli were sensitive to amikacin, gentamicin and piperacillin and Imipenem. Out of 25 GNB, 6 (24%) were ESBL producers. Out of 6 ESBLs Klebsiella were 5 (83.3%) and E-coli was 1 (16.6%).

Pseudomonas aeruginosa isolated in one case was found to be sensitive to piperacillin, gentamicin, amikacin and cefotaxime.

All the coagulase positive and negative staphylococci isolated were found to be sensitive to vancomycin, linezolid and piperacillin. Most of the coagulase positive staphylococci sensitive to penicillin, ceftriaxone, cefotaxamine, gentamicin and amikacin.

Table 5: Antibiotic sensitivity pattern of organisms isolated from neonatal septicemia

Antibiotics	Klebsiella		E .coli		Enterobacter		Pseudomonas		Staphylococcus		CONS	
	16	5	1	3	10	5	n	%	n	%	n	%
Gentamycin	14	87.5%	5	100%	1	100%	2	66.6%	10	100%	3	60%
Amicacin	16	100%	5	100%	1	100%	3	100%	10	100%	5	100%
Cefotaxime	11	68.75%	4	80%	1	100%	3	100%	7	70%	4	80%
Ceftriaxone	11	68.75%	4	80%	1	100%	3	100%	8	80%	4	80%
Ciprofloxacin	12	75%	4	80%	1	100%	2	66.6%	7	70%	2	40%
Imipenem	16	100%	5	100%	1	100%	3	100%	-	-	-	-
Pip / + Sulb	16	100%	5	100%	1	100%	3	100%	-	-	5	100%
Ceftazidime	10	62.5%	4	80%	1	100%	3	100%	8	80%	4	80%
Erythromycin	-	-	-	-	-	-	-	-	7	70%	3	60%
Vancomycin	-	-	-	-	-	-	-	-	10	100%	5	100%
Ampicilin	-	-	-	-	-	-	-	-	6	60%	3	60%
Linezolid	-	-	-	-	-	-	-	-	10	100%	5	100%

Distribution of ESBLs among Gram negative bacilli.

Among 25 Gram negative bacilli, 6 were ESBLs (24%). Out of 6, Klebsiella pneumoniae 5 cases (83.3%) and E.coli 1 case (16.7%).

Table 6

Gram negative bacteria	ESBL	Klebsiella	E.coli
25 (62.5%)	6 (24%)	5 (83.3%)	1 (16.7%)

DISCUSSION

For the effective management of neonatal septicemia cases, study of the bacteriological profile with their antibiotic pattern plays a significant role.

In the present study, out of the 100 clinically suspected neonates, 40 (40%) were culture positive which correlated with Zakariya BP et al (41.6%) and Rakhee Agarwal (42.7%). Out of the 40 culture positive cases males were 26 (60%), females were 16 (40%). It was found that the incidence of neonatal septicemia was higher in males than females, ratio was 1.5 : 1 which correlated with Rakhee Agarwal (42.7%) where the ratio was 1.4:1 and nearly correlated with Nawshad uddin ASM et al, where the male and female ratio was 1.7 :1. NNPD also showed higher percent of males. **(Table No. 2)**

Though the exact reason for this male preponderance was not known with certainty, it was probably due to the fact that the factors regulating the synthesis of gamma globulins were situated on the x-chromosome. Since the male had only one X-chromosome he was less immunologically protected than the female. Out of the 40 culture positive septicemia cases EOS were 24 cases (60%), LOS 14 cases (40%) which correlated with B.Subitha where EOS was 60% and LOS 40%, and also nearer to NNPD where EOS was 56.1% LOS was 45.3%. The high proportion of cases in early onset sepsis may be due to lack of adequate medical facilities for antenatal and neonatal care. **(Table No. 2)**

Out of the 40 cases of neonatal septicemia, 30 (75%) were from rural areas and 10 (25%) were from urban area which correlated with Bhutta ZA with 77% of cases from rural area and 23% from urban area. And also near to T.Sirisha, Tirupathi, where 82% of cases from rural area and 18% from urban area. **(Table No. 3)**

Out of the 40 isolates Gram negative organisms constituted 25 (62.56%) and gram positive organisms were 15 (37.5%). This finding correlated with that of T.Sirisha, Tirupathi, where GNB

were 64.5% and gram positive organisms 35.5% and little higher to Rekha Sriram where 56.9% GNB and 43.1% Gram positive organisms. **(Table No. 4)**

Klebsiella pneumoniae was found to be the predominant organism causing neonatal septicemia in this study. *Klebsiella pneumoniae* was isolated in 16 cases constituting 40% of the total blood culture isolates which correlated with B.Subitha, 40% and nearer to Rakhee Agarwal, who isolated *Klebsiella* in 44.6% of cases. Chugh et.al has noticed that most of the publications have reported high incidence of *Klebsiella* septicemia of this organism emerging as the predominant causative organism, although this was not a universal phenomenon.

Monga et al had isolated *Klebsiella* in 41.2% of their cases followed by *Staphylococcus aureus*, *E.coli* and *Pseudomonas aeruginosa*. M.Singh et al in their study found *Klebsiella pneumoniae* to be the commonest organism constituting 47.61% of the positive blood cultures.

Escherichia coli were isolated in 5 cases (12.5%) which correlated with Rakhee Agarwal of 12.3%. *Pseudomonas aeruginosa* was isolated in 3 cases (7.5%) correlated with Mane AK et al, 2010 of Nagpur of 8.5%. *Enterobacter cloacae* were isolated in one case (2.5%) which was near to Waseem R et al, 2005 at Lahore.

Coagulase positive staphylococci was isolated in 10 cases (25%) of total blood culture isolates which was higher to Mane AK et al, 2010 of Nagpur of 14.2%. Coagulase negative staphylococci isolated in 5 cases (12.5%) correlates with Zakariya BP et al, of 12%.

NNPD also showed *Klebsiella pneumoniae* as the most frequently isolated pathogen (32.5%), followed by *Staphylococcus aureus* (13.6%) and *Escherichia coli* (10.6%).

In this study *Klebsiella* showed a high degree of sensitivity of (100%) to imipenem, piperacillin with tazobactam correlating with the study of Rakhee Agarwal (2012) where *Klebsiella* shown 100% sensitivity to imipenem and 82.8% to Pip/Taz except 6 isolates, which were ESBL producers.

In this present study the sensitivity rate of *Klebsiella pneumoniae* to ceftazidime 62.5%, cefotaxime and ceftriaxone 68.7% , *Escherichia coli* showed 100% sensitivity to imipenem and piperacillin, *Enterobacter cloacae* showed 100% sensitivity to amikacin, cefotaxime, Ceftriaxone, ceftazidime, imipenem and piperacillin. *Pseudomonas aeruginosa* showed 100% sensitivity to cefotaxime, Ceftriaxone, imipenem and piperacillin which correlates with B.Subitha et al.

Coagulase positive staphylococci and CONS isolates were 100% sensitive to vancomycin, piperacillin, amikacin and linezolid which correlate with Rakhee Agarwal, 2012, Hyderabad. **(Table No. 5)**

In the present study 6 (24%) out of 25 Gram Negative isolates were ESBL producers out of 6 ESBLs, out the 16 Klebsiella 5(31.25%) were ESBL producers showed 68.75% sensitivity, and E.coli showed 80% sensitivity to 3rd generation cephalosporins. The prevalence of ESBL among clinical isolates varies greatly worldwide and within geographic areas, and is rapidly changing over time. This increased prevalence of Enterobacteriaceae producing ESBLs creates a great need for laboratory testing methods that will accurately identify the presence of these enzymes in clinical isolates.⁶ The DDST is the most widely used test due to its simplicity and ease of interpretation.⁶ It is a reliable method for the detection of ESBL₆. **(Table No. 6)**

CONCLUSION:

The number of lives lost in the perinatal and neonatal period exceeds that of any other period in life of a similar duration. In order to sustain gains in child survival made in recent decades, attention must be focused on reduction of morbidity and mortality in the newborn period. The evaluation of tests for neonatal sepsis is important because the infection may present a very serious threat to the baby. The mainstay for therapy for Neonatal Septicemia being appropriate supportive care, antibiotics used based on susceptibility testing of organism isolated.

Rapid microbe-specific diagnostic tests would assist in the early detection of neonatal sepsis and in safely withholding antibiotics for patients in whom sepsis is unlikely. The high percentage of ESBL producing Klebsiella spp may be due to the selective pressure imposed by extensive use of antimicrobials in Intensive care unit, in which antibiotic use is heaviest and the potential for patient to patient transmission of organisms is greatest.

Continuing surveillance of Neonatal infections, Local patterns and antibiotic sensitivity of pathogens is vital to determine trends in the infection improve reliability of the data and guide empiric antibiotic therapy & preventive measures.

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