Original Research Article

Bacteriological profile of neonatal septicaemia in tertiary care NICU in India

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Abstract

Background: Sepsis is the commonest cause of neonatal mortality; it is responsible for about 30-50% of the total neonatal deaths in developing countries (Bang et al). It is estimated that up to 20% of neonates develop sepsis and approximately 1% die of sepsis related causes. Sepsis related mortality is largely preventable with rational antimicrobial therapy and aggressive supportive care. Objective: The aim of this study was to identify the common bacteriological isolates of neonatal Septicemia and their antibiotic susceptibility patterns. Design: The present study was a prospective hospital based observational study. Duration: This study was conducted over a period of one year from January 2013 to December 2014. Setting: This study was organized and carried out at the Neonatal Intensive Care Unit of Malla Reddy Medical College for Women, Suraram, Hyderabad. Participants: This prospective study was conducted on 100 neonates presenting with signs and symptoms of septicemia admitted Neonatal Intensive Care Unit of Malla Reddy Medical College for Women Suraram, Hyderabad. Methods: Informed consent was obtained from the parents of neonates. Patients were selected after satisfying the inclusion criteria. Patient's details which included their name, age, sex, address date of admission, inpatient number were noted. All the patients underwent detailed clinical examination. Blood samples were collected using standard procedure under safety precautions and sent to laboratory for evaluation. 2 ml of blood sample was inoculated in blood culture bottle containing 10 ml of Brain heart infusion broth for the cultural isolation. Media used for sub culturing included Chocolate agar, Blood agar and Mc Conkey agar medium. The isolates were identified by colony morphology, Gram staining, motility, following biochemical reactions as per standard procedure. Data were presented in the form of statistical tables and charts. SPSS software version 20 was used for statistical analysis. Results: Blood culture positivity was 35 %. Early onset sepsis 28 (57%) was more common than late onset septicaemia 28 (43%). Out of total isolates, Gram positive organisms constituted 62.5% (20). Among gram positive organisms, coagulase negative Staphylococci and Staphylococcus aureus were commonest organisms 9 (45%). Gram negative organisms constituted 37.5% (15) out of 35 total bacterial isolates. Among the 15 gram negative isolates Klebsiella pneumonia was commonest isolate 8 (52%), followed by Escherichia coli 3 (20%), Pseudomonas aeuriginosa 3 (20%) and Acinetobacter 1 (8%). Conclusion: The present study suggests that there is an increased incidence of ESBL producers among Gram Negative pathogens i.e. Klebsiella pneumonia, Escherichia.coli, Pseudomonas, Acinetobacter in neonatal septicaemia of both early and late onset and many of them are resistant to commonly used third generation Cephalosporins i.e. Cefotaxim, Ceftriaxone, Ceftazidime.

Key Words: Neonatal Sepsis, Neonates, Bacteriological Profile, NICU, Antibiotic Susceptibility.

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INTRODUCTION

Neonatal septicaemia is a major cause of morbidity and mortality in newborns. The incidence of neonatal morbidity and mortality according to data from National perinatal database (NNPD 2002-03) is 30 per 100 live birth, contributing 19% of all neonatal deaths. World health organization (WHO) estimates that there are about 5 million neonatal deaths occurring globally per year. Ninety eight percent of them are in developing countries^{1,2,3}. The most common cause of death in neonatal period is infection (32%) followed by birth asphyxia (29%) and prematurity (30%). Neonatal septicaemia currently causes 1.6 million deaths in developing countries. The microbial etiology of neonatal

septicaemia is variable, often changes temporally, in developed countries. Group B streptococci (GBS) and Coagulase negative staphylococci (CONS) are most common etiological agents for early onset and late onset neonatal septicaemia respectively4. In developing countries Escherichia coli, klebsiella sp., Acinetobacter precede Group B streptococcus (GBS) and Coagulase negative staphylococci (CONS) in causing early onset neonatal septicaemia Klebsiella sp and Pseudomonas sp, Salmonella sp and Serratia precede Coagulase Negative Staphlococcus (CONS) and Staphylococcus aureus in causation of LOS⁵. The source of infection in early onset septicaemia (EOS) is usually the maternal genital tract. Infants with EOS, usually present with respiratory distress and pneumonia. The source of infection in late onset septicaemia (LOS) is either nosocomial or community acquired. The neonates usually present with pneumonia and meningitis in LOS6. Blood cultures are considered as the gold standard for diagnosis of neonatal septicaemia. Nevertheless their positivity varies widely (50 to 87 %) and the results are not available rapidly. For therauptic management, for this reason, other faster laboratory tests like white blood cell count(WBC) and other biological markers as Interleukin-8, C-reactive protein (CRP), Interleukin-6 (IL-6), Tumor necrosis factor alpha (αTNF) and procalcitonin are used⁶. Neonatal septicaemia is a life threatening emergency and its diagnosis is often difficult due to minimal signs and symptoms both in preterm and term infants5. Delay in diagnosis and judicious use of antibiotics can bring down and mortality neonatal morbidity substantially. Surveillance is needed to identify the pathogens of neonatal septicaemia as well as antibiotic resistance pattern and there newer mechanisms of resistance .Hence prospective study is conducted to evaluate the bacteriological profile, antibiotic resistance pattern and

extended spectrum beta lactamase (ESBL) mediated drug resistance among clinical isolates of aerobic gram negative bacteria isolated from neonatal blood of both EOS and LOS3.

MATERIALS AND METHODS

Place Of Study: The present study conducted at the Neonatal Intensive Care Unit of Malla Reddy Medical College for Women, Suraram, Hyderabad.

Type Of Study: The present study was a prospective hospital based observational study.

Sample Collection: Sample size: 100 patients. Blood samples were collected using standard procedure under safety precautions and sent to laboratory for evaluation (blood culture).

Sampling Methods: Consecutive sampling.

Inclusion Criteria:

- 1. Age less than 28 days.
- 2. Greater than 30 weeks of gestation and full term babies with signs of septicaemia like lethargy, poor feeding, irritability, fever, vomiting, abdominal distension, jaundice, respiratory distress, hypothermia cyanosis and convulsions.

Exclusion Criteria:

- 1. Extreme prematurity less than 30 weeks of gestation.
- 2. Gross congenital anamolies.
- 3. Undergone surgery.

Statistical Analysis: Data were presented in the form of statistical tables and charts. SPSS software version 20 was used for statistical analysis.

Ethical Approval: Approval was taken from the Institutional Ethics Committee prior to commencement of the study.

OBSERVATIONS AND RESULTS

Table 1: Culture Positivity In Neonatal Septicaemia

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	Culture positive	35(35%)
	Culture negative	65(65%)
	Total number	100 (100%)

Out of 100 blood samples from neonatal septicaemia 35(35%) were culture positive and 65(65%) were culture negative respectively.

Table 2: Age and Sex Distribution of Septicemic Neonates

Duration of septicaemia	Male	Female	Total
<7 days old (EOS)	30(50.84%)	29(49.15%)	59(59%)
7-28 Days old (LOS)	20(48.78%)	21(51.21%)	41(41%)
Total	50	50	100

Out of 35 culture positive isolates early onset septicaemia were 19 and 16 were late onset septicaemia accounting for 54% and 46% respectively. Out of total 100 septicemic neonates 50 were males and female were 50, showing equal sex distribution. Among 59 (EOS),i.e. early onset septicaemia neonates 30 were male and 29 were female accounting for 50.84% and 49.15% respectively. Out of 41 neonates (LOS) ,i.e. late onset septicaemia 20 are males and 21 are females accounting for 48.78% and 51.21% respectively.

Table 3: Comparison Of Gram Positive And Gram Negative Organisms In Culture Positive Isolates

Gram positive	20(62.5%)
Gram negative	15(37.5%)
Total	35 (100%)

Out of 35 culture positive isolates, 20(62.5%) are gram positive and 30(37.5%) are gram negative pathogens. In late onset septicaemia out of 16 culture positive isolates 5 are gram positive and 11 are gram negative.

Table 4: Organisms Among Gram Positive Pathogens And Gram Negative Organisms

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Gram positive	20	Gram negative	15
Coagulase negative staphylococcus 9(45%)		Klebsiella pneumoniae	8 (52%)
Staphylocococus aureus	9(45%)	Escherichia coli	3 (20%)
Streptococci sp	2(10%)	Pseudomonas aeruginosa	3 (20%)
		Acinetobacter baumanni	1 (8%)
Total	20 (100%)	Total	15 (100%)

Out of 20 gram positive isolates 9(45%) are coagulase negative staphylococcus followed by 9 (45%) staphylococcus aureus and 2(10%) streptococcus sp. Among gram negative pathogens, klebsiella pneumonia (52%) are followed by Pseudomonas 3(20%) and also Escherichia coli 3(20%) and 1(8%) is Acinetobacter sp.

Table 5: Antibiotic Sensitive To Gram Positive Organisms

	Ampicillin	Amoxy-clav	Ciprofloxacin	Ceftriaxone	Cefotaxim	Piperacillin	cefoperazone	Vancomycin
CONS	78%	75%	90%	87%	76%	80%	78%	100%
Staphylococcus aureus	59%	65%	85%	60%	75%	76%	76%	100%
Streptococuus species	54%	65%	87%	67%	76%	85%	75%	100%

Antibiotic sensitivities for various gram positive isolates in our study is as follows, coagulase negative staphylococcus was sensitive to 78% ampicillin, 75% amoxyclav, 90%ciprofloxacin, 87% ceftriaxone, 76% cefotaxim, 80% piperacillin, 100% vancomycin. Staphylococcus aureus was sensitive to 59% ampicillin, 85% amoxicillin, 60% ciprofloxacin, 75% ceftriaxone, 76% cefotaxim, 80% piperacillin, 100% vancomycin. Streptococci sp was sensitive to 54% ampicillin, 66% amoxyclav, 87% ciprofloxacin, 67% ceftriaxone, 76% cefotaxim, 85% piperacillin, 100% vancomycin.

Table 7: Anti Biotic Sensitivity To Gram Negative Bacteria

	Amoxy clav	Ciprofloxacin	Ceftriaxone	Cefotaxim	Cefperazone	Gentamicin	Piperacillin	Imipenem
Klebsiella	67%	64%	31%	33%	75%	14%	33%	100%
Escherichia coli	78%	66%	44%	44%	65%	35%	56%	100%
Psuedomonas	80 %	86%	72%	75%	80%	20%	80%	100%
Acinetobacter	-	-	100%	100%	100%	-	100%	100%

Antibiotic sensitivities for various gram negative isolates in our study is as follows,klebsiella sp was sensitive to 67% amoxyclav,,64%ciprofloxacin,31% ceftriaxone,33% cefotaxim,75% cefperazone,33% piperacillin, 100% imipenem,14% gentamycin. Escherichia coli was sensitive to78% amoxyclav, 66% ciprofloxacin, 44% ceftriaxon,44%cefotaxim, 65% cefperazone,35% gentamycin,56% piperacillin,100% imipenem. Pseudomonas was sensitive to 80%amoxyclav 86% ciprofloxacin, 72% ceftriaxon, 75% cefotaxim, 80% cefperazone,20% gentamycin,80% piperacillin, 100% imipenem. Acinetobacter sp was sensitive to, ceftriaxone, cefotaxim, cefperazone, piperacillin, imipenem.

DISCUSSION

Septicaemia is still a major cause of mortality and morbidity in neonates. Now a days, Gram negative microorganisms are increasingly been reported as the major cause of neonatal septicemia particularly in Asian countries. The emergence of multidrug resistant Gram-negative bacteria is mainly due to in adverent use of antibiotics^{1,2,3,4}. Present study was conducted in 100 clinically suspected septicemic neonates admitted in Neonatal Intensive care unit, MRMCW Hyderabad between January 2013 - December 2014, to know aerobic bacterial profile of neonatal septicaemia. The results were compared with other studies and discussed as follows.

Comparison of culture positive results

S.No	Author	Culture positive	Culture negative
1	Mahabatra et al (2002) ⁷	40%	60%
2	Ziba Mosoyebi et al (2003)	13%	87%
3	Momtaz Manesh et al(2004)2	30%	70%
4	Shanam Ghelbi et al (2008)8	11%	89%
5	Bhatacharjee et al (2008)9	48%	51%
6	A.K mane <i>et al</i> (2010) ²	29%	61%
7	Present study	35%	65%

The culture positivity in present study was 35 % and this coincides with Momtaz Mahesh *et al* (2004) (30%) and A.K.Mane *et al* (2010) 35% and Bhatacharjee *et al* (2008) 48%. The other studies culture positivity were Mahabatra *et al* (2002) 40%, Ziba Mosoyebi *et al* (2003) 13%, and shaman Ghelbi *et al* (2008) was 11%. This discerepency might have arisen due to the administration of antibiotics before blood collection either to the mother or to the baby or the possibility of infection with viruses, fungi or anaerobes.

	Comparison with time of onset of septicaemia (EOS/LOS)						
S.No	Author	EOS	LOS				
1	A.H .Movahedion et al (2006) Iran	77.5%	22.5%				
2	Choudry Habibur et al (2007)Bangladesh8	70%	30%				
3	vinod kumar etal (2008)India ³	55%	45%				
4	Ziba Mosaybi et al (2003)Iran	41%	59%				
5	A.K mane et al (2010)Nagpur India ²	81%	19%				
6	Present study (2012) Hyderabad, Andhra Pradesh, India	54%	46%				

In present study ,Early onset septicaemia was observed in 54% and late onset septicaemia in 46 neonates .Early onset septicaemia was more common than late onset septicaemia which is compatible with the reports from Choudry Habibur et al (2007) (70.7% EOS, 30% LOS), A.H.Movahedion et al (2006) (77.5%-EOS,22.5%-LOS) and vinod kumar et al (2008) (55%-EOS,47%-LOS).Contrasting results were published by Kuruvilla et al (1997) who reported that late onset septicaemia is more common than early onset septicaemia .Shaw et al and Ziba Mosaybi et al (2003) also reported that late onset septicaemia was more common than early onset septicaemia. Kuruvilla et al and Shaw et al study group comprised of babies delivered at their institution and treated with prophylactic antibiotic.

	Comparison of gram positive and gram negative isolates in study							
S.no	Author	Gram Positive	Gram Negative					
1	Nalini Agnihotri et al (2004) Chandigarh ³	41%	59%					
2	Reza <i>et al</i> (2007) Iran ¹¹	68%	32%					
3	Shahsanam et al (2008)	62%	38%					
4	A.K mane et al (2010)Nagpur India ²	39%	61%					
5	Present study (2012) Hyderabad, Andhra Pradesh, India	62.5%	37.5%					

In present study, Gram positive organisms constituted 62.5% of total bacterial isolates, causing neonatal septicaemi, and gram negative constituted 37.5%. This distribution pattern correlates with Shahsanam *et al* (2008) and Reza *et al* (2007) from Iran reported that Gram positive organisms constituted the major isolates (62.1%),68% respectively. In contrast Gram negative accounted more in Nalini Agnihotri *et al* (2004) Chandigarh reported that Gram negative organism accounted for 59%.

Comparison of total pathogens isolated							
S.No	Author	klebsiella	E.coli	Staphylococcus	CONS	Pseudomonas	Acinetobacter
1	Shaw etal (2007) Mumbai India ¹²	20%	10%	29%	15%	8%	5%
2	Vinod kumar et al (2008) ¹⁴	26.9%	10.5%	20%	13.4%	-	6.1%
3	A.K mane et al (2010)Nagpur India ²	17.1%	10%	14.3%	18.6%	-	14.3%
4	Present study (2013) Hyderabad, Andhra Pradesh, India	22.2%	8.5%	25%	24%	8.5%	6.1%

In present study, among Gram positive organisms Staphylococcus aureus constituted (25%) followed by CONS(24%) and Sterptococci (5%). Among Gram negative organisms Klebsiella pneumonia (22.2%) was most common organism followed by Escherichia coli and Pseudomonas (8.5%) and Acinetobacter (2%). This distribution correlates Shaw *et al* (2007) reported that Staphylococcus aureus was most common organism isolated from neonatal septicaemia, where as Vinod kumar *et al* (2008) reported that Klebsiella (26.9%) was most common infective organism followed by Staphylococcus aureus (20%), CONS(13.4%), E.coli (10.5%) and Acinetobacter sp (6.1%), In study conducted by A.K Mane *et al* (2010) at Nagpur India Klebsiella (17.1%). E.coli (10%), S.aeureus (14.3%), CONS (18.6%) and Acinetobacter (14.3%).

CONCLUSIONS

The present study suggests that there is an increased incidence of ESBL producers among Gram Negative pathogens i.e. Klebsiella pneumonia, Escherichia. coli, Pseudomonas, Acinetobacter in neonatal septicaemia of both early and late onset and many of them are resistant to commonly used third generation Cephalosporins i.e. Cefotaxim, Ceftriaxone, Ceftazidime.

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