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Original Research Article

Survey on the Etiological Profile of Bacterial Neonatal Sepsis in and Around Vellore

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ABSTRACT

Neonatal mortality is a major problem in the developing and under developed countries around the world. The commonest cause of mortality in neonates is sepsis, accounting for about 30-50% of neonatal deaths in developing countries. The clinical syndrome of sepsis is characterized by systemic signs of circulatory compromise caused by invasion of the blood stream by bacteria in the first four weeks of life. The present survey was carried out to determine the etiological profile of neonatal septicaemia and to find out the susceptibility pattern of pathogens causing neonatal sepsis so as to provide anti biogram appropriately. The blood samples were collected from the patients who come to OPD, Government Vellore Medical College and Hospital, Adukkamparai with suspected symptoms. The samples were inoculated onto various media to isolate the organisms from the blood samples. The isolated organisms were subjected to biochemical test for their identification and identified as CONS, *Klebsiella pneumonia*, *E. coli*, *Enterobacter* sps., *Pseudomonas aeruginosa* and *Acinetobacter* sps. The antibiogram of the isolates showed higher percentage of sensitivity to Cefazolin, Cotrimoxazole and Levofloxacin. This result suggests the specific drugs for appropriate treatment.

Keywords: Neonatal sepsis, antibiogram, blood samples, antibiotics

INTRODUCTION

Neonatal sepsis is one of the major and common causes for morbidity and mortality among neonates in India affecting 4% of the neonates. [1] The early signs and symptoms of infection are generally minimal, although the onset of the disease is often nonspecific; the clinical course may be fulminant, leading to septic shock, disseminated intravascular coagulation and death within hours of initial clinical symptoms. [2]

The standard treatment for the neonatal sepsis includes the use of antimicrobial agents. Antibiotics are continued, changed, or discontinued depending on the laboratory test results, extent of clinical suspicion, and cultures. [3]

Wide spectrums of organisms are involved in causing neonatal septicemia. The incidence of bacteremia in neonates varies widely. [4] Moreover, the organisms isolated are often resistant to multiple antimicrobials which make treatment difficult. [5] The varying microbiological pattern of septicemia in neonates warrants the need for an ongoing review of the causative organisms and their antimicrobial susceptibility pattern.

The aim of this present study is to determine the etiological profile of neonatal septicaemia in and around Vellore and to find out the antibiogram of pathogens causing neonatal sepsis so as to provide appropriate antibiotics.

MATERALS AND METHODS

Total of 70 neonates clinically suspected to have neonatal septicaemia reported to Hospital, were examined during a study period and the criteria were as outlined below.

Patients presented to Department of pediatrics were examined clinically by pediatrician and 70 cases suspected to have neonatal septicemia were identified on the basis of the signs and symptoms and were included for the study. The blood samples were collected for culture after obtaining getting written consent from the parent. The neonatal history including sex, gestational age, birth weight, term or preterm were noted. The data regarding maternal risk factor for neonatal sepsis including duration of labour, mode of delivery, maternal fever, chorio amnionitis (foul smelling liquor), maternal urinary tract infection, and duration of rupture of membrane were collected in a structured proforma and were classified and analysed.

2.1 Blood Specimen Collection

1-2 mL of blood was collected from the peripheral veins following all standard aseptic precautions as per Clinical Laboratory Standards Institute (CLSI) guidelines. The collected blood specimen was immediately inoculated onto 5mL (when 1mL was obtained) or 10mL (when 2mL was obtained) of liquid broth (BHI broth with SPS) culture medium and mixed gently immediately. After collection, the sample was sent to the Department of Microbiology for further processing.

2.2 Processing of blood samples and approach to identification

The culture media were incubated for 7 days at 37^{o}C under aerobic condition. Blind subculture were done on blood agar, chocolate agar and MacConkey agar media after 24 hours, 48hours, (when no growth was found upon first subculture) and on 7th day (when found sterile upon second subculture) of incubation. The cultures were declared negative only after 7th day of incubation. The colony characteristics including results of microscopic morphologic features of the colony such as Gram-staining and hanging drop preparation were noted. Finally the bacterial pathogen was identified by subjecting the growth for standard biochemical and other necessary tests. The culture media, chemical and oxidase discs for the study were procured from HiMedia, Mumbai, India.

2.3 Antibiotics susceptibility testing

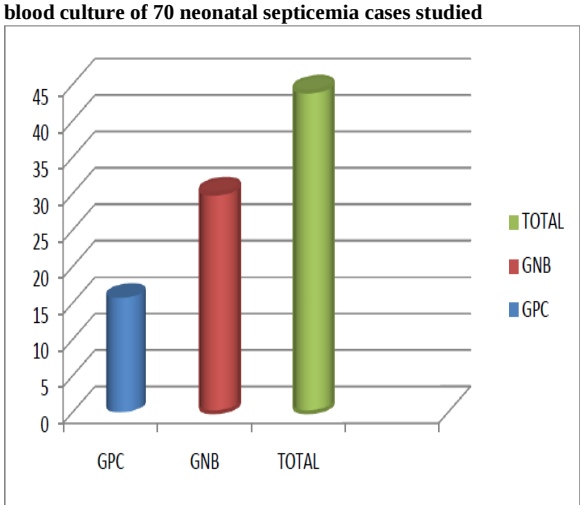
The isolates were tested for antibiotic susceptibly by Kirby-Bauer disk diffusion method on Muller Hinton agar as per (CLSI) guidelines. Antibiotics discs (HiMedia com, Ltd, India) used for Gram negative bacilli were ampicillin, amoxicillin, ceftazidime, amikacin, ciprofloxacin, gentamicin; and for Gram positive cocci levofloxacin, norfloxacin, oxacillin, penicillin, tetracycline, erythromycin.

RESULT

Out of 70 blood culture carried out, 44 (62.8%) yielded the growth. In our work, of these 44, GNBs which were isolated from 33 (30%) blood samples which were predominant cause of septicemia in neonates compared to GPCs were isolated from 11 (15.7%) blood samples (Graph 1). This result correlates with the report of Talur *et al.*, 2000, 156 (64.87%) blood samples positive for bacterial isolates of total 242 cases studied. [6]

Kaistha *et al.*, 2009 recorded 296 (13.17%) blood culture positivity among 2247 cases in their retrospective study and Agnihotri *et al.*, 2004 found 588 (19.2%) culture positive cases among 3064 cases studied which is less when compared to our study. The differences in the culture positivity rates in different studies could be due to the geographical distribution and also type of study for instance retrospective or prospective and also whether the patient were on antibiotics or not before obtaining blood sample for culture. [7,8]

Graph 1 Distribution of 44 cultures positive isolates from blood culture of 70 neonatal septicemia cases studied



GPB: Gram positive bacilli. GNB: Gram negative bacilli.

In this study, 30% organisms causing neonatal sepsis were Gram negative and 15.7 % were Gram positive. This is in agreement with the studies done by (Shrestha *et al.*, 2013) and (Kayange *et al.*, 2010) which also show that gram-negative organisms are more common causes of neonatal sepsis. [9,10]

Table 1: Biochemical identification of isolates

S.No	Triple sugar iron agar	Citrate	Utease	Organisms identified
1	K/NO	Negative	Negative	*Acinetobacter* sps.
2	A/A	Positive	Negative	*Enterobacter* sps.
3	K/NO	Negative	Negative	*Pseudomonas aeruginosa*
4	A/A	Negative	Positive	*Klebsiella pnemoniae*
5	A/A	Negative	Negative	*Escherichia coli*

Based on the biochemical test results the organisms isolated were identified as *Enterobacter* sps., *Klebsiella pneumoniae*, *Acinetobacter* sps., *Escherichia coli* and *Pseudomonas aeruginosa* (Table 1).

Table 2: Distribution of 44 bacterial isolates (organism wise) obtained

S.No	Organism	Percentage
1	CONS	11(15.7%)
2	*Klebsiella pnemoniae*	15(21.14%)
3	*Pseudomonas aeruginosa*	7(11.66%)
4	*Escherichia coli*	2(2.86%)
5	*Acinetobacter* species	4(8.51%)
6	*Enterobacter* species	5(7.14%)
	Total	**44**

Among 44 bacterial culture positive blood samples, CONS (Coagulase negative Staphylococci) were from 9 (12.85%) and *Enterobacter* sps., from 5 (7.14%). Of 33 Gram negative bacterial culture, *Klebsiella pneumoniae* from15 (21.14%) and *Enterobacter* sps., from 5 (7.14%) sample. 15 (21.14%) blood sample yielded, followed by isolation of *Acinetobacter* sps., in 4(8.51%), and *Escherichia coli*, from 2 (2.86%) *Pseudomonas aeruginosa*, were isolated from 5 (7.14%), blood sample each 11 cases of GPCs followed by GNBs in 33 cases (Table 2).

Klebsiella pneumonia was also the predominant organism for neonatal sepsis in the study done by Aletayeb *et al.*, 2011; Shrestha *et al.*, 2013; Jyothi *et al.*,2013. [11,9,12] *K. Pneumonia*, S.*aureus*, and Coagulase-negative *Staphylococci* were the predominant organisms for neonatal sepsis in another the study done by Shrestha *et al.*, 2013 and Jyothi *et al.*, 2013. [9,12] *P. aeruginosa* was the predominant organism for neonatal sepsis in the study done by Bhat *et al.*, 2011.[13] S .*aureus* was the predominant organism for neonatal sepsis in the study done by Mhada *et al.*, 2012; Shahian *et al.*, 2004-2007 reported Coagulase- negative *Staphylococci* as the major organisms for neonatal sepsis in their studies. [14,15]

Table 3: Culture sensitivity of Gram-Negative isolates

Drugs	Percentage of sensitivity				
	Klebsiella pneumoniae	***Escherichia coli***	***Acinetobacter* sps.**	***Enterobacter* sps.**	***Pseudomonas aeruginosa***
Ampicillin	0	0	0	0	0
Amoxicillin	0	0	1(25%)	0	1(11.1%)
Cefazolin	-	0	0	5(100%)	3(33.3%)
Ceftazidime	3(33.3%)	0	0	2(40%)	4(44.4%)
Cotrimoxazole	5(55.5%)	0	2(25%)	1(20%)	2(22.2%)
Doxycycline	1(11.1%)	-	-	-	-
Gentamicin	2(22.2%)	1(50%)	0	0	0

On observing the sensitivity pattern of Gram negative organisms, *Klebsiella pneumoniae* showed the highest sensitivity to Cotrimoxazole (55.5%), *Enterobacter* sps. towards Cefazolin (100%) and *Pseudomonas aeruginosa* showed the highest activity to Ceftazidime. There is resistance and less sensitivity of microorganisms was seen to the commonly used drugs such as ampicillin, amoxicillin and gentamicin (Table 3).

Table 4: Culture sensitivity of Gram-positive isolates

Drugs	CONS
Cephalexin	2(22.2%)
Erythromycin	4(44.4%)
Levofloxacin	5(55.5%)
Oxacillin	0
Penicillin	0
Tetracyline	1(11.1%)

CONS: Coagulase negative *Staphylococci*

For CONS, Levofloxacin (55.5%) was found to be the most effective drug followed by Erythromycin (44.4%), showed less sensitivity to Cephalexin (22.2%) and tetracycline (11.1%) and resistance to Oxacillin and Penicillin (Table 4). Similar results were also observed in the studies done by Shrestha *et al.*, 2013; Rahman *et al*., 2002. [9, 16]

Klebsiella were the most sensitive to ciprofloxacin in the studies done by Kayange *et al.*,2010; Aletayeb *et al.*, 2011. [10,11] Most of the strains showed a low sensitivity to amikacin (14.94%), gentamicin (14.29%), ampicillin+sulbactam (5.84%), piperacillin (5.84%), and cefotaxime (4.55%). There is a low sensitivity to cefotaxime when compared with the other studies. [9,16,10]

CONCLUSION

Sepsis remains a major problem in neonates all around the world. In this survey carried out in and around Vellore, *Klebsiella pneumonia* was the predominant Gram negative organisms and CONS among the Gram positive organism. The isolates were resistant to most of the commonly used antibiotics. Regular periodic testing of antibiotic susceptibility of the causative organisms of neonatal sepsis is needed for the choice of antibiotic prescription. Further study should be undertaken with more samples in different period of time and with different antibiotics.

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