

Original Research Article

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Study of Bacterial Profile in Neonatal Sepsis and their Antibiotic Sensitivity Pattern in a Tertiary Care Hospital

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ABSTRACT

Septicemia remains a significant cause of morbidity and mortality in the newborns. The main objective of the study was to identify the common bacterial pathogens associated with neonatal sepsis and their antibiotic sensitivity pattern. During July 2015 and August 2015, 232 Blood samples were collected aseptically from newborns with sepsis in Govt. Kilpauk Medical College, Chennai and processed by standard conventional method. Antibiotic susceptibility pattern of isolates was studied by Kirby Bauer Disc diffusion technique as per the CLSI recommendations. 83 out of 232 cases were culture positive, giving a culture positivity rate of around 35.77%. Males were more affected than their female counterparts. 85.5% of cases are reported in the early days of life and Gram negative bacilli were found to be the commonest cause of neonatal septicemia (56.5%). *Klebsiella pneumonia* (39.75%) was the most frequent isolate which showed 90.9% sensitivity against Amikacin. All the Gram negative bacilli were found to be highly sensitive to third generation cephalosporins especially Cefperazone sulbactam. All the gram positive isolates showed 100% sensitivity to Vancomycin. The study concludes that it is important to continue the tracking of neonatal septicemia in order to closely monitor the changes in trends, to obtain information for empiric antibiotic therapy and to act rapidly in case of emergencies. Since Govt. Kilpauk Medical College being a tertiary health care centre, most of its sepsis cases are referred ones. Hence awareness should be created about institutional deliveries along with antenatal and postnatal care.

Keywords

Neonatal sepsis,
Multidrug
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Introduction

Neonatal sepsis is a clinical syndrome characterized by systemic signs of infection and accompanied by bacteremia in the 1st month of life.

It is an important cause of morbidity and mortality among neonates. World Health Organization has estimated that 1.6 million deaths occur globally every year due to

neonatal infections and 40% of all neonatal deaths occur in developing countries (WHO report 2006). In India, the incidence of blood culture proven sepsis was reported as 8.5 per 1,000 live births for the year 2002–2003 by the National Neonatal Perinatal Database (NNPD report 2002-03). Most of the neonatal sepsis related deaths are preventable if suspected early and treated with appropriate antibiotics. Early-onset neonatal sepsis (EONS) occurs within first 72 h of life, while the late-onset neonatal sepsis (LONS) occurs between 72 h to 90 days of life (Sundaram *et al.*, 2009). The varying microbiological pattern of septicemia in children warrants the need for an ongoing review of the causative organisms and their antimicrobial susceptibility pattern.

The bacterial agents implicated in early-onset sepsis include group B *Streptococcus* (GBS), *Escherichia coli*, coagulase-negative *Staphylococcus*, *Haemophilus influenzae* and *Listeria monocytogenes* (Anderson-Belly *et al.*, 2010; Maayan-Metzger *et al.*, 2009). The organisms commonly associated with late-onset sepsis include coagulase-negative *Staphylococci* (CONS), *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherichia coli*, *Enterobacter spp.*, *Pseudomonas aeruginosa* and *Acinetobacter* species (Kaistha *et al.*, 2009). The bacteriological profile for causative organisms of neonatal sepsis differs significantly between developed and developing countries (Sanghvi and Tudehope, 1996; Stoll *et al.*, 2002) a pneumoniae is the most common bacterial agent causing neonatal sepsis in developing countries, while group B Streptococcus and coagulase-negative staphylococci (CONS) are the common agents in developed countries (Iregbu *et al.*, 2006). Even among developing countries, regional variation in prevalence of the bacterial agents causing

neonatal sepsis exists (Kuruvilla *et al.*, 1998; Chacko and Sohi, 2005). Therefore, it is essential to establish the bacteriological profile of organism associated with septicemia. Prompt diagnosis and effective treatment is necessary to prevent deaths and complications due to septicemia.

Physical signs and symptoms are useful in identifying infants and children with septicemia. These clinical characteristics can be good predictors for positive blood culture but they have limited specificity and sensitivity (Tumbarello *et al.*, 2007; Weber *et al.*, The uncertainty surrounding the clinical approach to treatment of neonatal septicemia can be minimized by periodic epidemiological surveys of etiological agents and their antibiotic susceptibility patterns leading to recognition of the most frequently encountered pathogens in a particular neonatal setting.

The rational and correct use of antibiotics requires understanding of common pathogens and their drug sensitivity pattern in the regions. Due to constantly evolving antimicrobial resistant patterns there is the need for constant antimicrobial sensitivity surveillance. This will help clinicians provide safe and effective empirical therapies, develop rational prescription programs and make policy decisions and finally assess the effectiveness of all (Karki *et al.*, 2010). As antibiotic sensitivity pattern to common pathogen has been changing day by day, so it has been necessary to study about bacteriological analysis and antibiotic sensitivity pattern. Determination of antibiotic sensitivity patterns in periodic intervals is mandatory in each region for choosing appropriate antibiotic therapy.

The present study was undertaken to study the bacteriological profile of neonatal septicemia cases and their antibiotic

susceptibility pattern for planning strategy for the management of these cases.

Materials and Methods

This prospective observational study was conducted in the department of Microbiology, Govt. Kilpauk Medical College, Chennai, over a period of Two months (July 2015 and August 2015). This study was approved by the Research and Ethical committees of our institute and informed consent was obtained from each patient's next of kin. The samples were collected with proper aseptic precautions. All neonates in whom sepsis was suspected and had at least two positive screening tests were included in the study.

Data Collecting Procedure

Many infections in the neonatal age group can only be established on the basis of etiological agent removed from blood but a negative blood culture does not rule out the possibility of Neonatal Sepsis. Gold standard for diagnosis of Neonatal Sepsis is isolation of bacterial agent from blood culture. With all aseptic precautions about 1-2 ml of blood is drawn from each neonate. About 1 ml of blood is inoculated into 10 ml of brain heart infusion (BHI) broth and processed as per the protocol and incubated for one week at 37°C and is checked daily for evidence of bacterial growth. For positive broth cultures, subcultures are done next day on blood agar, MacConkey's agar and chocolate agar and are incubated at 37°C for 24 hours. If no growth occurred on plates after 24 hours, subsequent cultures are done on 2nd, 5th and 7th day. The grown bacteria are identified by colony morphology, Gram stain and standard biochemical tests. The antibiotic susceptibility testing is performed by Kirby-Bauer disc diffusion method for the bacterial

isolates, as per Clinical and Laboratory Standards Institute guideline (CLSI). ATCC control strains are used accordingly as per standard procedures. Observation made in the Microbiology Department will be documented and compiled using computer software.

Plan of Analysis

The following variables will be analyzed:

1. Age
2. Sex
3. Microbial species
4. Drug resistance as in antibiogram forms

The pathogens are accessed for resistance against the most commonly used eight anti microbials.

The antimicrobials under the study were:

Fluroquinolones	: Ciprofloxacin
Penicillin	: Ampicillin
Aminoglycosides	: Gentamicin
Glycopeptides antibiotics	: Vancomycin
Macrolide antibiotics	: Erythromycin
Tetracyclines	: Doxycycline
Cephalosporins	: Ceftazidime, Cefmetazole, Cephalexin

Results and Discussion

83 suspected clinical cases of sepsis was included in the study and analysed for antibiotic sensitivity. Among culture positive there was 51 male (61.5%) neonates and 32 female (38.5%) The male: female ratio stands at 1.6:1.Fig:1.

Early On Set Neonatal Sepsis cases were found higher than Late on Set Neonatal Sepsis. EONS were seen in 71 cases (85.5%) and 12 cases recorded LONS (14.5%).fig:2.

Detailed etiology of 83 isolates proves that Gram negative bacilli 47/83 (56.6%) dominate the picture when compared to Gram positive cocci 36/83 (43.4%). Fig: 3

Antibiotic Sensitivity of *Klebsiella*

90% of the *Klebsiella* show sensitivity to Amikacin. None of them are found to be sensitive towards Ampicillin and Cephalexin. Sensitivity to Gentamicin is as low as 27% (given in Table: 1)

Antibiotic Sensitivity of CONS

CONS showed 100% sensitivity to Vancomycin, Doxycycline and Amikacin. Low sensitivity pattern is recorded in case of Ampicillin (0%) and Ciprofloxacin (8.6%) given in Table 2.

Antibiotic Sensitivity of *Staphylococcus aureus*

Staphylococcus aureus was found to show 100 % sensitivity to Vancomycin, Erythromycin, Doxycycline and Amikacin. It showed 0% sensitivity to Ciprofloxacin. given in Table:3.

Antibiotic Sensitivity of *Acinetobacter*

Third and Second generation Cephalosporins were found to be 100% effective against *Acinetobacter*. First generation cephalosporin, Cephalexin as well as Ciprofloxacin could not resist the growth of *Acinetobacter* given in Table:4.

Antibiotic Sensitivity of *Enterobacter*

Second and third generation Cephalosporins yet again showed 100% efficiency in restricting the growth of *Enterobacter*. Ampicillin, Gentamicin, Ciprofloxacin and Cephalexin failed to

restrict the growth of *Enterobacter* (0% sensitivity) given in Table:5.

Antibiotic Sensitivity of *Pseudomonas*

Pseudomonas showed high sensitivity (100%) to second and third generation cephalosporins, Amikacin and Ciprofloxacin. *Pseudomonas* showed 0% sensitivity to Ampicillin, Gentamicin. Given in Table:6.

Septicemia is still a major cause of mortality and morbidity in the first month of life. New treatment approaches are being explored throughout the world because of its rapidly changing pathogen spectrum and high mortality rate. Area based knowledge of the bacteriological spectrum is essential because the first antibiotic administered will not wait for the culture results. Keeping in mind the high mortality associated with neonatal septicemia, a right choice for such empiric therapy is of utmost importance. Periodic revision of this knowledge is equally important since the spectrum of causative pathogens varies in same place from time to time over a period of time.

In our study, 83 out of 232 cases were culture positive, giving a culture positivity rate of around 35.77%. This is in accordance with the study conducted by Uddin Ahmed *et al.*, (35%), Edwin Dias *et al.*, (35%). This is in contrast to study conducted by Roy *et al.*, (47.50%), R.S.Jaswal *et al.*, (42%) and Tallur *et al.*, (64.87%). In the present study, males were more affected than their female counterparts, males contributing 61% of culture positive cases and the Male: Female ratio being 1.6:1. This is comparable to the study conducted by Tallur *et al.*, Uddin Ahmed *et al.*, and P.Jyothi *et al.*, Khatua *et al.*, postulated that the factors regulating the production of gamma globulins are most likely situated on the X chromosome.

Presence of single X chromosome in male infants thus confers less immunological protection compared to their female counterparts.

In our study, 85.5% of cases are reported in the early days of life, Early Onset Neonatal Sepsis which goes hand in hand with other studies conducted by Tallur *et al.*, (83.47%), Roy *et al.*, (71.30%).

The proportion of Early Onset Neonatal Sepsis cases may be due to immature immunological responses of neonates in the first week of life, making them more susceptible to infections in this period. Gram negative bacilli were found to be the

commonest cause of neonatal septicemia (56.5%). The foetus is frequently exposed to enteric bacteria during the course of maternal peripartal infections. In those instances in which the same agent was recovered from mother and infant, gram negative bacteria were predominant. Post natively the infant may get exposed to Gram negative organisms through humidification apparatus, resuscitation equipment or articles used in daily care. Since the neonates lack normal flora at birth he/she may become heavily colonized with Gram-negative bacteria in sites where these agents are not normally found. The newborn infant has been shown to have a lack of serum bactericidins against Gram-negative bacilli.

Table.1 Antibiotic sensitivity of *Klebsiella*

Antibiotic	No. of samples showing sensitivity	% of sensitivity
Ceftazidime	14	42.42%
Cefmetazole	33	100%
Cephalexin	0	0%
Ciprofloxacin	25	75.75%
Gentamicin	9	27.2%
Amikacin	30	90.9%
Ampicillin	0	0%

Table.2 Antibiotic sensitivity of CONS

Antibiotic	No. of samples showing sensitivity	% of samples showing sensitivity
Vancomycin	23	100%
Doxycycline	23	100%
Erythromycin	7	30.4%
Ciprofloxacin	2	8.6%
Gentamicin	9	39.1%
Amikacin	23	100%
Ampicillin	0	0%

Table.3 Antibiotic Sensitivity of *Staphylococcus aureus*

Antibiotic	No. of samples showing sensitivity	% of sensitivity
Vancomycin	13	100%
Doxycycline	13	100%
Erythromycin	13	100%
Ciprofloxacin	0	0%
Gentamicin	3	23.07%
Amikacin	13	100%
Ampicillin	4	30.7%

Table.4 Antibiotic Sensitivity of *Acinetobacter*

Antibiotic	No. of samples showing sensitivity	% of sensitivity
Ceftazidime	7	100%
Cefmetazole	7	100%
Cephalexin	0	0%
Ciprofloxacin	0	0%
Gentamicin	3	23.07%
Amikacin	7	100%
Ampicillin	2	28.5%

Table.5 Antibiotic Sensitivity of *Enterobacter*

Antibiotic	No. of samples showing	% of sensitivity
Ceftazidime	5	100%
Cefmetazole	5	100%
Cephalexin	0	0%
Ciprofloxacin	0	0%
Gentamicin	0	0%
Amikacin	1	20%
Ampicillin	0	0%

Table.6 Antibiotic Sensitivity of *Enterobacter*

Antibiotic	No. of samples showing sensitivity	% of sensitivity
Ceftazidime	2	100%
Cefmetazole	2	100%
Cephalexin	1	50%
Ciprofloxacin	2	100%
Gentamicin	0	0%
Amikacin	2	100%
Ampicillin	0	0%

Fig.1 Sex Distribution in Patients

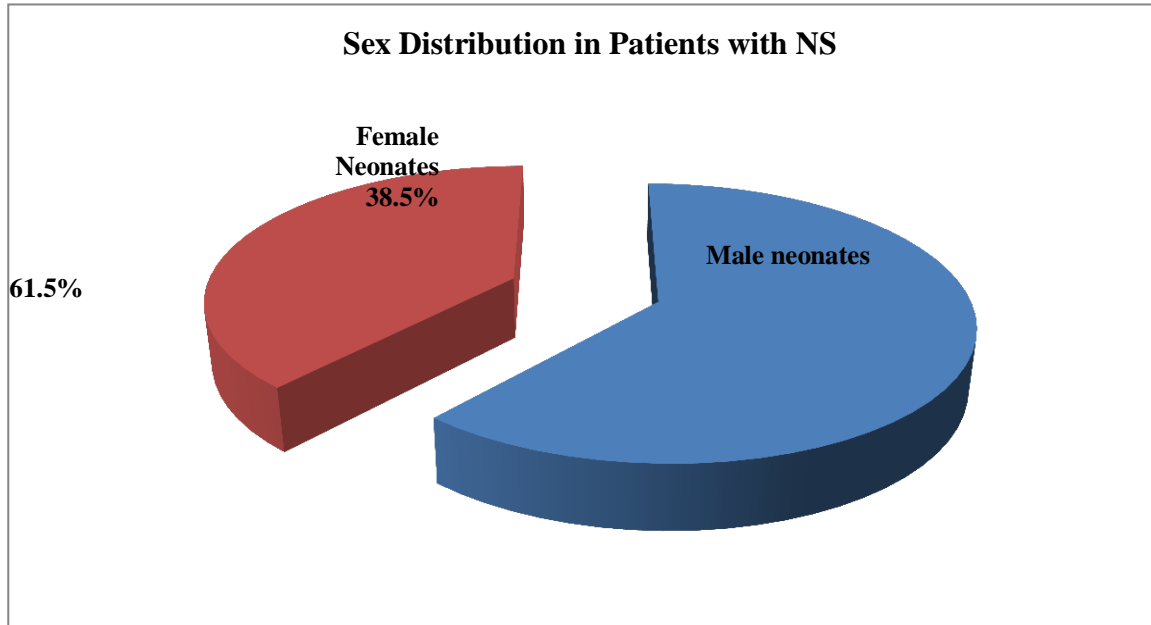


Fig.2 Based on Onset of Neonatal Sepsis

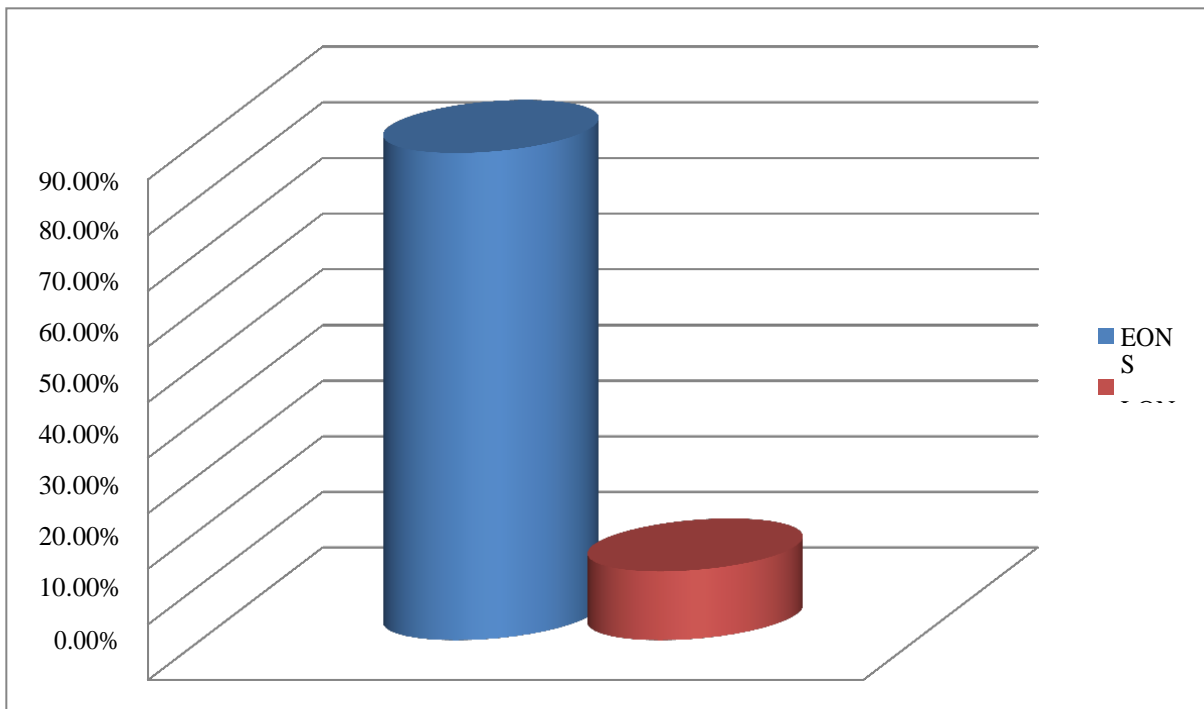
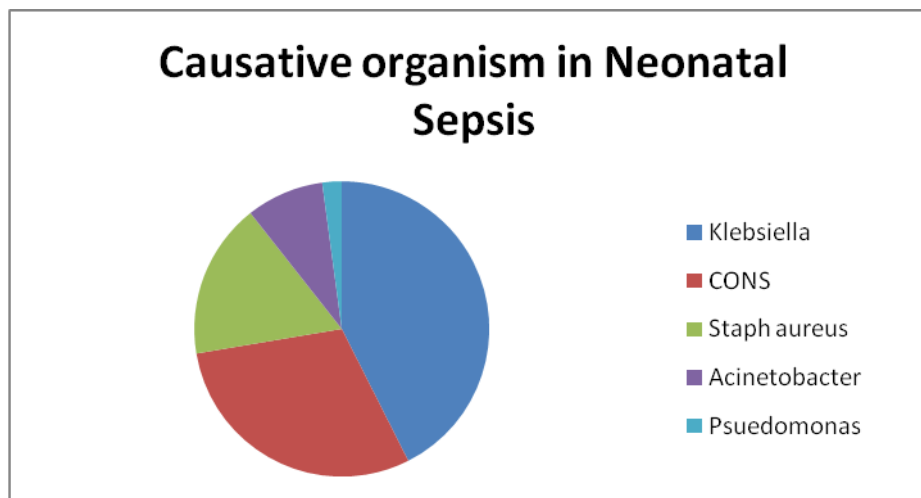


Fig.3 Causative organism in Neonatal Sepsis



These antibodies against somatic or "O" antigens in Gram negative bacteria are in the gamma-M fraction which is not passed transplacentally from mother to foetus.

Yet another contributing factor might be the recent rampant use of antibiotics both in the mother and the infant, which would tend to select out the relatively more resistant Gram-negative organisms. Hence; Gram-negative organisms are commonest cause of neonatal septicemia. In this study, the most frequent isolate was *Klebsiella pneumoniae* 33/83 (39.75%). This was in accordance with other Indian studies NNPD 2003, Roy *et al* and second most common isolate is *CONS* 23/83 (27.7%). Initially Ampicillin is frequently used as first choice drug for initial treatments of acute infections without bacteriological data. But now many organisms are found to have developed resistance against this group casting doubts about their efficacy. In this study *Klebsiella*, *CONS*, *Enterobacter* and *Pseudomonas* (totally 33+23+5+2 = 63/83 around 75.9%) showed 0 % sensitivity. Thus including Ampicillin as part of empirical treatment for Neonatal Sepsis is not justified and can cause treatment failure. *Klebsiella*

showed 90.9% sensitivity against Amikacin which is in accordance with the study conducted by Bambala *et al.*, in 2011. All the Gram negative bacilli were found to be highly sensitive to third generation cephalosporins especially Cefperazone sulbactam. All the gram positive isolates showed 100% sensitivity to Vancomycin. Tallur *et al.*, showed that all the Gram positive isolates were 100% susceptible to Vancomycin. Ciprofloxacin showed less efficiency in case of Gram positive isolates which is in contrast to the study conducted by Dias *et al* in 2010. Cephalosporins still remain the most effective drugs for the treatment of Neonatal Sepsis

In conclusion, from our study, it was concluded that Gram negative bacilli take a major share in the spectrum of pathogens causing Neonatal Sepsis. *Klebsiella* was the most common isolate and it is highly sensitive to third generation Cephalosporins and Amikacin. In case of Gram positive isolates, Vancomycin is found to be highly effective. An antibiotic policy should be formulated in the hospital and depending on the antibiotic sensitivity pattern of the

isolated pathogens, antibiotics should be used. It will be important to continue the tracking of neonatal septicemia in order to closely monitor the changes in trends, to obtain information for empiric antibiotic therapy and to act rapidly in case of emergencies. Since Govt. Kilpauk Medical College being a tertiary health care centre, most of its sepsis cases are referred ones. Hence awareness should be created about institutional deliveries along with antenatal and postnatal care.

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