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ORIGINAL ARTICLE

Neonatal Sepsis in a Tertiary Care Hospital in South India: Bacteriological Profile and Antibiotic Sensitivity Pattern

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Abstract
Objective To identify the common bacterial pathogens associated with neonatal sepsis and their antibiotic susceptibility pattern.
Methods This prospective observational cohort study was done in a tertiary care teaching hospital located in South India for a period of 2 years. All the admitted inborn and out born neonates during this study period were screened for sepsis based on the clinical features and septic screening. All infants satisfying the criteria for sepsis were subjected to blood culture. Growths, if any, were noted and standard antibiotic sensitivity testing was done by Kirby-Bauer disk diffusion method. The *Chi-square* test or Fisher's exact test was used to compare two groups.
Results Out of the 120 clinically suspected and positive screening test cases of neonatal sepsis, 41.6% (50 of 120) were culture-proven cases of neonatal sepsis. *Klebsiella pneumoniae* was isolated from 66% of culture positive cases followed by Coagulase-negative staphylococci in 12% of cases. *Klebsiella pneumoniae* was resistant to most of the antibiotics tested except amikacin and meropenem. Of the total 33 *Klebsiella pneumoniae* isolates, 16 (32.0%) were ESBL producers. The prevalence of ESBL producing *Klebsiella pneumoniae* during two month outbreak and rest of the study period was 83.3% (15 of 18) and 20% (3 of 15) respectively (*P* value 0.0010).
Conclusions *Klebsiella pneumoniae* was the most common agent causing both early-onset and late-onset sepsis and significantly associated with sepsis in inborn babies. Amikacin should be used along with the third-generation cephalosporins for empirical treatment of gram-negative neonatal sepsis.

Keywords Neonatal sepsis · Bacteriological profile · *Klebsiella pneumoniae* · Antibiotic sensitivity

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Introduction

Neonatal sepsis is a significant cause of morbidity and mortality among neonates worldwide [1, 2]. 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 [3]. 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 [4]. Most of the neonatal sepsis related deaths are preventable if suspected early and treated with appropriate antibiotics.

Neonatal sepsis is broadly categorized into early and late onset sepsis depending upon the postnatal day of presentation. 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 [3–5]. The bacterial agents implicated in early-onset sepsis include group B Streptococcus (GBS), *Escherichia coli*, coagulase-negative Staphylococcus, *Haemophilus influenzae* and *Listeria monocytogenes* [5–7]. 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 [2, 5].

The bacteriological profile for causative organisms of neonatal sepsis differs significantly between developed and developing countries [8, 9]. *Klebsiella 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 [2, 5, 10]. Even among developing countries, regional variation in prevalence of the bacterial agents causing neonatal sepsis exists [11, 12]. The overall improvement in the neonatal survival due to newer drugs, better neonatal care and advanced life support facilities has led to a change in the spectrum of agents causing neonatal sepsis in developed countries [5]. However, there is a paucity of data on the recent trends of organisms causing neonatal sepsis in developing countries [13].

As delay in the treatment of neonatal sepsis is associated with increased mortality, empirical therapy is the cornerstone in the management of neonatal sepsis. A combination of ampicillin or third generation cephalosporins with an aminoglycoside (gentamicin) is the commonly used empirical regimen [2, 7]. However, the appropriateness of this empirical therapy is being challenged in the present era of changing bacteriological profile and increasing antimicrobial resistance. Knowledge of common organisms causing neonatal sepsis in a particular area and their antibiotic sensitivity pattern should be borne in mind before setting guidelines for empirical therapy.

Hence, there is a need for surveillance to understand the trends in pathogens causing neonatal sepsis and the antibiotic susceptibility profile of those pathogens in a particular area. This study was therefore undertaken to determine the common bacterial agents associated with neonatal sepsis and their antibiotic susceptibility pattern in a tertiary care hospital in India.

Material and Methods

This prospective observational cohort study was conducted in the division of neonatology, of our tertiary care hospital over a period of 2 years from August 2004 through July 2006. 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.

During the study period, all the inborn babies were screened for sepsis. Sepsis was clinically suspected if the neonate had symptoms and signs suggestive of sepsis such as poor feeding, poor activity, respiratory distress, apnea, seizure, lethargy, bulging anterior fontanel, fever, hypothermia, jaundice, vomiting, loose stools, abdominal distension, cyanosis, bleeding, mottling, tachycardia, weak pulse, grunting, retractions, nasal flaring etc. Septic screening tests like band cell count, C- reactive protein, and micro erythrocyte sedimentation rate were done in all these cases. All neonates in whom sepsis was suspected and had at least two positive screening tests were included in the study. A detailed antenatal, natal and postnatal history was taken. The birth weight, sex and day of onset of sepsis were noted. Details regarding risk factors such as ventilator support, CPAP, central line and exchange transfusion prior to the onset of sepsis were also noted. Blood culture was done for all the neonates. Blood was collected with aseptic precautions before starting antibiotics and 2 ml of blood was added to each of two bottles containing 25 ml of Brain heart infusion broth (HiMedia, Mumbai, India). Both the bottles were incubated aerobically at 37°C for 7 days. Subculture was done on sheep blood agar and MacConkey agar (HiMedia, Mumbai, India) routinely after 48 h and 7 days. Subculture was also done in between if visible turbidity appeared. The isolates were identified based on standard bacteriological techniques [14]. The growth of an organism was considered pathogenic if the same organism was isolated from both broths and contaminated if either the growth was obtained in only one bottle or a mixed growth was obtained. If coagulase negative staphylococci (CONS) were isolated from neonates with sepsis, a repeat blood culture was performed to confirm the infection.

The susceptibility of the clinical isolates to some routinely used antibiotics was determined by the Kirby-Bauer disk diffusion method according to Clinical Laboratory Standards Institute guidelines [15]. All the antibiotic disks were obtained from HiMedia, Mumbai, India. Ampicillin, gentamicin, ceftriaxone, ciprofloxacin, ceftazidime, amikacin, chloramphenicol and meropenem were tested for gram-negative bacteria. Penicillin, oxacillin, gentamicin, ciprofloxacin, erythromycin and vancomycin were tested for *Staphylococcus* spp. Oxacillin, erythromycin and vancomycin were tested for *Streptococci pneumoniae*. Double-disk test using both cefotaxime and ceftazidime, alone and in combination with clavulanic acid, was performed for detection of extended spectrum β-lactamase (ESBL) in *Klebsiella pneumoniae*, according to CLSI guidelines [16]. In this test, an overnight culture suspension of the test isolate adjusted to 0.5 McFarland standard was inoculated using a sterile cotton swab on the surface of a Mueller Hinton Agar. The Cefotaxime (30 μg) and cefotaxime-clavulanic acid (30 μg/10 μg) disks were placed 20 mm apart on the agar. Similarly, the ceftazidime (30 μg) and ceftazidime-clavulanic acid (30 μg/10 μg) disks were placed 20 mm apart. After incubating overnight at 37°C, a ≥5 mm increase in the zone diameter for either antimicrobial agent tested in combination with clavulanic acid vs. its zone when tested alone was interpreted as

positive for ESBL production. *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923 and *Streptococcus pneumoniae* ATCC 49619, obtained from Christian Medical College, Vellore, were the QC strains used for quality control of Kirby-Bauer disk diffusion method. *Klebsiella pneumoniae* ATCC 700603 and *Escherichia coli* ATCC 25922 were used for quality control of ESBL testing.

Proven sepsis was defined as the presence of clinical features of sepsis along with the isolation of an organism in blood culture. Early-onset neonatal sepsis was defined as sepsis occurring within the first 3 days of life, while late-onset sepsis was defined as sepsis occurring after 3 days of life [3, 5, 7].

Data entry and analysis were done using SPSS for Windows Version SPSS 16.0 (SPSS Inc, Chicago, IL, USA). Means and standard deviations (SD) were calculated as required for numerical variables. The Chi-square test or Fisher's exact test was used to compare two groups. P value$<$ 0.05 was considered statistically significant.

Results

During the study period, there were a total of 120 clinically suspected cases of neonatal sepsis. The demographic data of these 120 cases are summarized in Table 1. Of these 120 neonates, 60 (50%) were inborn, while the other 60 (50%) were outborn. Of the 120 clinically suspected cases of neonatal sepsis, 69 (57.5%) were early-onset and 51 (42.5%) were late-onset sepsis. In 39 (56.5%) early-onset cases the blood-culture was positive, while in only 11 (21.6%) late-onset sepsis cases a pathogen was isolated from blood culture (P value 0.0003). In total, 41.6% (50 of 120) of the clinically suspected cases were culture-proven cases of neonatal sepsis.

Table 1 Demographic details of the 120 neonates with clinical sepsis

Parameter	Value
Neonates' characteristics	
No. of Preterm neonates (%)	45 (37.5)
Birth weight (g), mean ± SD (range)	2.25±0.66 (1.2–3.9)
Age (days), mean ± SD (range)	5.85±7.15 (1, 30)
Sex	
No. of males (%)	70 (58.3)
No. of females (%)	50 (41.7)
Maternal data	
No. of cases with PROM > 24 h (%)	13 (10.8)
No. of cases with meconium stained liquor (%)	19 (15.8%)
Type of delivery	
Spontaneous vaginal delivery (%)	89 (74.2)
Caesarean section (%)	15 (12.5)
Instrumental delivery (%)	14 (11.7)
Assisted breech delivery (%)	2 (1.7)

Gram-negative organisms were isolated from 41 (82%) out of 50 culture proven cases. *Klebsiella pneumoniae* (66%) was the most common causative agent of neonatal sepsis. Coagulase-negative staphylococci (12%) were the most common gram-positive agent. The etiological agents of early-onset and late-onset neonatal sepsis are shown in Table 2. The etiological agents of sepsis in inborn and outborn neonates are compared in Table 3.

The 33 *Klebsiella pneumoniae* isolates were susceptible to meropenem (100%) and amikacin (82%) and varying susceptibility to chloramphenicol (24%), ciprofloxacin (18%), ceftriaxone (3%) and ceftazidime (3%), but none were susceptible to gentamicin. Of the total 33 *Klebsiella pneumoniae* isolates, 16 (32.0%) were ESBL producers. The prevalence of ESBL producing *Klebsiella pneumoniae* among inborn and outborn neonates was 64% (16 out of 25) and 25% (2 out of 6), respectively (P value 0.1015). The prevalence of ESBL producing *Klebsiella pneumoniae* during May–June 2006 and rest of the study period was 83.3% (15 of 18) and 20% (3 of 15) respectively (P value 0.0010).

All the three *Enterobacter* spp. were susceptible to meropenem, while only one was susceptible to amikacin and none were susceptible to the other antibiotics tested. The one *Escherichia coli* isolated was susceptible to ciprofloxacin, amikacin, chloramphenicol and meropenem, but resistant to other antibiotics tested. All the three *Acinetobacter* spp. were susceptible to meropenem, while two of them were susceptible to gentamicin, ceftriaxone, ciprofloxacin, ceftazidime and amikacin. The *Pseudomonas stutzeri* was susceptible to gentamicin, ciprofloxacin, ceftazidime, amikacin and meropenem. All the six coagulase-negative staphylococci were susceptible to gentamicin, ciprofloxacin and vancomycin, but only one was susceptible to penicillin, three to oxacillin and four to erythromycin. Both the *Streptococcus pneumoniae* isolates were susceptible to oxacillin (indicative of susceptibility to penicillin, ampicillin and cephalosporins), erythromycin and vancomycin. The Group B streptococci was susceptible to ampicillin, penicillin, erythromycin and vancomycin.

Discussion

Neonatal sepsis is a clinical syndrome characterized by signs and symptoms of infection with or without accompanying bacteremia in the first month of life [17]. Although bacteria are the most common agents implicated in neonatal sepsis, neonatal sepsis syndrome can also be caused by organisms other than bacteria like adenovirus, enterovirus,

Table 2 Etiological agents of early-onset and late-onset neonatal sepsis

Organism	No. of isolates (%)		*P* value
	Early-onset sepsis	Late-onset sepsis	
Klebsiella pneumoniae	29 (74.4)	4 (36.4)	0.0304
Escherichia coli	0	1 (9.1)	0.2200
Enterobacter spp.	2 (5.1)	1 (9.1)	0.5337
Acinetobacter spp.	2 (5.1)	1 (9.1)	0.5337
Pseudomonas stutzeri	1 (2.6)	0	1.0000
CONS	3 (7.7)	3 (27.3)	0.1114
Streptococcus pneumoniae	2 (5.1)	0	1.0000
Group B *Streptococcus*	0	1 (9.1)	0.2200
Total	39	11	

coxsackievirus, rubellavirus, *Toxoplasma* species and *Candida* species [5]. Therefore, only a proportion of the blood culture from cases with clinical sepsis will be positive for pathogenic organisms. In addition, collection of blood samples after administration of empirical antibiotics can also result in poor recovery of the bacterial pathogens in culture. In a study done in neonatal intensive care units of Georgia, 63% of the clinically suspected cases were blood culture positive. In the present study the blood culture positivity rate was 41.6%, which is lower than the above study. However, in other studies from India, the culture positivity rate was 13–22% [2, 10].

The etiological agents of neonatal sepsis vary between developed and developing countries [8, 9]. *Klebsiella pneumoniae* and other Gram-negative organisms were the common causes of sepsis in the present study as well other studies from India and Nigeria [2, 10]. However, in the developed countries Group B Streptococcus and coagulase-negative staphylococci (CONS) are the predominant causes of sepsis [5]. The bacteriological profile of early-onset sepsis differs from that of late-onset sepsis as the mode of infection is different [18]. Early-onset neonatal sepsis is acquired transplacentally or as an ascending infection from cervix or during passage of the baby through a colonized birth canal [5]. In the present study, *Klebsiella pneumoniae* was the common agent implicated in early-onset sepsis, while Group B *Streptococcus* was isolated from none of these neonates. Although Group B *Streptococcus* was considered as an important agent associated with early-onset sepsis, the recent studies are showing a decreasing trend in the incidence of this pathogen [19]. Late-onset neonatal sepsis is usually acquired from the care-giving environment and coagulase-negative staphylococci (CONS), *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella* species are the common agents involved [5]. In the present study, *Klebsiella pneumoniae* and CONS were the common etiological agents of late-onset sepsis. In an epidemiological study performed to observe the long term trends in the agents causing neonatal sepsis, CONS were showing an increasing trend [19].

Table 3 Etiological agents of sepsis in inborn and outborn neonates

Organism	No. of isolates (%)		*P* value
	Inborn	Outborn	
Klebsiella pneumoniae	25 (80.6)	8 (42.1)	0.0130
Escherichia coli	0	1 (5.3)	0.3800
Enterobacter spp.	2 (6.5)	1 (5.3)	1.0000
Acinetobacter spp.	0	3 (15.8)	0.0494
Pseudomonas stutzeri	1 (3.2)	0	1.0000
CONS	1 (3.2)	5 (26.3)	0.0244
Streptococcus pneumoniae	2 (6.5)	1 (5.3)	1.0000
Total	31	19	

In this study, the authors compared the etiological agents of neonatal sepsis in babies born in their hospital and those born outside. *Klebsiella pneumoniae* was more significantly implicated in sepsis occurring among in-born babies, while *Acinetobacter* species and CONS were significantly associated with out-born babies. *Klebsiella pneumoniae* is commonly found in the environment of the neonatal intensive care units and nursery [20]. It can also be present as colonizers on the hands of the health care workers. There are also frequent reports of neonatal septicemia outbreaks due to *Klebsiella pneumoniae* in nursery and NICUs [20]. In the present study, the authors observed an outbreak of ESBL producing *Klebsiella pneumoniae* during May-June 2006. The occurrence of *Acinetobacter* species among out-born babies may be due to prior hospitalization during delivery of these babies, which could have exposed them to such nosocomial pathogens.

In the present study, majority of the *Klebsiella pneumoniae* isolates were resistant to all the antibiotics tested except

amikacin and meropenem and 32% of them were found to be ESBL producers. In a similar study, 50–100% of the *Klebsiella pneumoniae* isolates were observed to be resistant to commonly used antibiotics especially gentamicin and the second and third generation cephalosporins [10]. In another study from North India, 30–80% of the Gram negative isolates were resistant to third-generation cephalosporins [2]. This suggests that the third-generation cephalosporins cannot be used alone for empirical treatment of neonatal sepsis and amikacin which shows good activity against the gram negative bacteria should always be included in the empirical regimen. This also emphasizes the need to routinely test for cephalosporin resistance and ESBL production among the gram-negative bacterial isolates.

Conclusions

The blood culture positivity rate was 41.6%. *Klebsiella pneumoniae* was the commonest agent causing both early-onset and late-onset sepsis. The authors also documented an outbreak of ESBL producing *Klebsiella pneumoniae*. Adequate care of the low birth weight babies is of utmost importance to prevent infection by *Klebsiella pneumoniae*. Amikacin should be used along with third-generation cephalosporins for empirical treatment of gram-negative neonatal sepsis. This empirical regimen should be modified later based on the antibiogram of the isolates.

Contributions VB, HBN and ZBP were involved in the conception of the study. VB and ZBP conducted the study and collected the data. ABT and JNM did literature search, analyzed the data and drafted the manuscript which was critically reviewed and approved by VB. VB will act as the guarantor of this study.

Conflict of interest None.

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