Bacteriological profile and antibiogram of neonatal septicemia in a tertiary care hospital

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Abstract

Background: Neonatal sepsis is a clinical syndrome which is characterised by signs and symptoms of infection which may or may not be accompanied by bacteraemia within the first month of life. The bacteriological profile of neonatal sepsis is under constant change with the advances in the diagnosis and treatment. Blood culture is still the mainstay of investigation of potential sepsis in spite of the recent advances in molecular diagnosis of fungal and bacterial sepsis. Most common bacterial organisms responsible for neonatal septicaemia in developing countries like Bangladesh, India, Nigeria, Pakistan, are Klebsiella, Escherichia coli, Enterobacter etc. The present study was undertaken to describe the spectrum of the isolates in cases of neonatal septicaemia, and their antimicrobial susceptibility pattern. The study was carried out over a one year period.

Method: A total of 357 suspected patients of neonatal septicaemia were included in the study. Blood samples were collected with aseptic precautions preferably prior to the initiation of empiric antibiotic therapy. The bottles were incubated aerobically at 37^0 C for 7 days and subcultured on blood agar and Mac Conkey agar. If growth appeared the isolates were processed as per standard microbiological techniques and the isolates were identified. Antibiotic sensitivity testing was performed on Mueller-Hinton agar plates by modified Kirby-Bauer disk diffusion method as per Clinical Laboratory Standard Institute guidelines.

Results: Of the 357 samples studied, growth was observed in 154 samples. Gram positive organisms constituted 37.66% and gram negative bacilli constituted 62.34% of the isolates. The predominant pathogens isolated were Klebsiella species, Coagulase Negative Staphylococci, Staphylococcus aureus followed by Non fermenting gram negative bacilli. 71.43% of the Staphylococcus aureus isolates were found to be methicillin resistant.96.05% of the Enterobacteriaceae were found to be Extended Spectrum Beta Lactamase (ESBL) producers. 33.33% of the *Pseudomonas* isolates were found to be metallo-beta-lactamase (MBL) producers.

Conclusion: This study shows that *Klebsiella* species, Coagulase Negative Staphylococci, Staphylococcus aureus and Non-fermenting gram negative bacilli were the commonest organisms associated with Neonatal sepsis. An alarmingly high rate of MRSA and ESBL producers was observed. This study stresses that antimicrobial resistance is a universal problem and it stresses the need for surveillance.

Keywords: Sepsis, neonate, infection, bacterial, bacterial infections

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Introduction

Neonatal sepsis accounts for over half of the neonatal deaths in the community.[1] Approximately 25% of the neonatal deaths in the world are caused by Neonatal septicemia.[2] 1.6 million deaths occur globally every year due to neonatal infection as estimated by the World Health Organization.[3] As per the data of National Neonatal Perinatal Database 2005, 38% of neonatal deaths in India were attributed to sepsis. [4]

Neonatal sepsis is a clinical syndrome which is characterised by signs and symptoms of infection which may or may not be accompanied by bacteraemia within the first month of life.[1] The immature immune systems and poorly developed skin barrier of the neonates make them highly susceptible to infections.[5] Despite the advances in neonatal care having reduced complications and improved survival in neonates, sepsis is still contributing significantly to neonatal morbidity and mortality.[6]

Neonatal sepsis can be classified into early onset sepsis (EOS) and late onset sepsis (LOS) based on the timing of onset of sepsis-EOS presenting within 72 hours of birth and LOS presenting 72 hours after birth.[4] The importance of this classification is it helps to guide antibiotic therapy by implying differences in the mode of transmission and the predominant causative organisms.[6] EOS is caused mainly by bacteria transmitted from mothers to neonates during the intrapartum period,[6] these are bacteria prevalent either in the maternal genital tract or in the area of delivery.[1] LOS is caused by postnatal acquisition of the pathogens,[6] caused by the bacteria which thrive in the external environment of the hospital or home.[1]

Neonatal sepsis is difficult to diagnose clinically as it presents with non-specific signs and symptoms.[2] Neonatal sepsis can be defined clinically and\or microbiologically, either by positive blood and or

Cerebrospinal fluid cultures.[1] The growth of microorganisms from blood, cerebral spinal fluid or urine is still the gold standard for a definitive diagnosis.[1] Blood culture is still the mainstay of investigation of potential sepsis in spite of the recent advances in molecular diagnosis of fungal and bacterial sepsis.[7]

Overall it has been observed that gram negative organisms are more common and are mainly represented by Escherichia coli, Klebsiella and Pseudomonas.[8] Among the gram positive organisms, *Staphylococcus aureus*, Coagulase Negative *Staphylococci* (CONS) and Group B *Streptococcus* have been most commonly isolated.[8]

The microorganisms most commonly associated with EOS include Group B *Streptococcus,* CONS, *Escherichia coli*, and *Haemophilus influenzae* and LOS is caused by CONS, *Staphylococcus aureus, Klebsiella* spp., *Escherichia coli, Enterobacter* spp., *Pseudomonas* spp., *Candida* spp., GBS, *Serratia* spp., *Acinetobacter* spp. and the anaerobes.[2]

The organisms which are commonly implicated in neonatal sepsis in the developed countries vary from those seen in the developing countries.[8] In the developed countries, Group B Streptococcus and CONS are the most common causative agents for EOS and LOS, respectively, in the developing countries these bacteria are less common with a completely varying bacterial spectrum.[9] Most common bacterial organisms responsible for neonatal septicaemia in developing countries like Bangladesh, India, Nigeria, Pakistan, are *Klebsiella, Escherichia coli*, *Enterobacter* etc.[10] The aetiology of neonatal sepsis and their response to antimicrobial agents can vary significantly from time to time and even geographically which determines the effectiveness of empirical treatment.[11] Most of the aetiological agents of neonatal sepsis are bacteria, fungi, viruses and parasites, though having a smaller role, should also be considered.[5] The bacteriological profile of neonatal sepsis is under constant change with the advances in the diagnosis and treatment.[9]

The present study was undertaken to describe the spectrum of the isolates in cases of neonatal septicaemia, and their antimicrobial susceptibility pattern.

Materials and Methods

The aim of the study was to identify the bacterial isolates and study their antimicrobial susceptibility pattern in neonates admitted to the neonatal intensive care unit (NICU) of a tertiary care medical college and hospital. The present study was carried out from January 2015 to December 2015 in Microbiology department of a tertiary care medical college and hospital. A total of 357 suspected patients of neonatal septicaemia were included in the study. Two millilitres of blood was collected from the neonates aseptically preferably before administration of the antibiotics and inoculated into 20 ml of brain heart infusion broth. Blood culture bottles were transported immediately to the Microbiology Laboratory and the bottles were incubated aerobically at 37^0C for 7 days and subcultured on blood agar and Mac Conkey agar at 48 hours and 7 days or at an in-between period when visible turbidity appeared. The isolates were processed as per standard microbiological techniques and the isolates were identified.[12] Antibiotic sensitivity testing was performed on Mueller-Hinton agar plates by modified Kirby-Bauer disk diffusion method as per Clinical Laboratory Standard Institute guidelines. Screening for MRSA was done using a cefoxitin (30 μg) disc. Resistance to ceftazidime (30 μg) disk was used as a screening method for detection of ESBL confirmed by double disk synergy test.[13] The organisms were classified based on the time-point at which the blood was collected for culture: Up to 72 hrs as causing early onset sepsis and >72 hrs as causing late onset sepsis.

Results

A total of 357 blood samples from neonates suspected for septicaemia were tested. Of the 357 samples 203 showed no growth whereas 154 yielded growth. The culture isolation rate was 43.14%. Out of the 357 samples, 137 samples were evaluated for early onset sepsis and 220 samples for late onset sepsis. Of the 137 EOS samples, 90 showed no growth while growth was observed in 47 samples. Of the 220 LOS samples, 113 showed no growth while 107 samples yielded growth. The culture isolation rate was 34.31% for EOS and 48.64% for LOS.

Of the positive blood cultures, Gram positive organisms accounted for 37.66% (n=58) and Gram negative organisms accounted for 62.34% (n=96). There was an overall predominance of gram negative organisms. The major pathogens isolated were Klebsiella 64.58% (n=62), CONS 20.13% (n=31), *Staphylococcus aureus* 9.1% (n=14) and non-fermenting gram negative bacilli (NFGNB) 9.1% (n=14). Among the 58 Gram positive organisms isolated Coagulase Negative *Staphylococcus* (CONS) was 53.45% (n=31) followed by *Staphylococcus aureus* 24.14% (n=14) and then *Candida* 22.41% (n=13). Among the 96 Gram Negative organisms isolated *Klebsiella species* accounted for 64.58% (n= 62), NFGNB 14.58% (n=14), *Citrobacter* species 9.38% (n=9), *Pseudomonas species* 6.25 %(n=6%) and *Escherichia coli* 5.21% (n= 5).

CONS isolation rate was 29.79% in EOS and 15.89% in LOS. *Staphylococcus aureus* was isolated from 17.02% of the cases in EOS and 5.61% in LOS. *Candida* accounted for 4.26% of the isolates in EOS and 10.28% in LOS. *Klebsiella species* was isolated from 27.66% in EOS and 45.79% in LOS, *Escherichia coli* was isolated from 2.13% in EOS and 3.74% in LOS, *Citrobacter* species 6.38% in EOS and 5.61% in

LOS, NFGNB 6.38% in EOS and 10.28% in LOS, *Pseudomonas species* 6.38% in EOS and 2.80% in LOS.

71.43% (n=10) of the *Staphylococcus aureus* isolates were found to be methicillin resistant (MRSA). 96.05% (n=73) of the 76 Enterobacteriaceae were found to be Extended Spectrum Beta Lactamase(ESBL) producers of which 83.56% (n=61) were Klebsiella isolates, 10.96%(n=8) were *Citrobacter* species and 5.48%(n=4) were Escherichia coli. 33.33% (n=2) of the *Pseudomonas* isolates were found to be metallo-beta-lactamase (MBL) producers.

Most of the Gram positive isolates were resistant to amoxicillin (62.22%), erythromycin (57.77%) and cefoxitin (60%) and most of the Gram negative isolates were resistant to ceftriaxone (76.31%) and ampicillin (72.36%). Majority of the Gram positive cocci were sensitive to quinolones (82.22%) and clindamycin (64.44%) and majority of the Gram negative organisms were sensitive to imipenem (67.11%) followed by quinolones (52%).

Discussion

Culture positivity rate as observed in our study was 43.14% which is in conformity with several other earlier studies.[14,15,16] but the culture positivity rate is higher than few other studies.[11,17,18] The culture isolation rate for EOS was 34.31% which agrees with another study[9] and 48.64% for LOS which agrees with a study in Chennai.[3] However, we should note that even if the cultures are negative we cannot rule out the possibility of neonatal sepsis as the infection could be caused by anaerobic pathogens also which were not looked for in this study.

There was an overall predominance of gram negative organisms in our study. Of the positive blood cultures, Gram positive organisms accounted for 37.66% and Gram negative organisms accounted for 62.34%. This is in agreement with studies by several other authors.[17,18,19]

Few authors have found Gram negative organisms to predominate in EOS and Gram positive organisms in LOS.[14,20,9,11] In our study CONS (29.79%) & *Klebsiella species* (27.66%) i.e. Gram positive organisms and Gram negative organisms predominated in EOS and *Klebsiella species* (45.79%) i.e. Gram negative organism predominated in LOS.

In our study Escherichia coli accounted only 2.13% of EOS unlike other studies, where it was the predominant isolate.[9,21] *Candida* was isolated from 13 samples(22.41%) higher than in other studies where the rate was in the range of 5 to 7%.[16,22]

In the present study the major pathogens isolated were *Klebsiella species*, CONS, *Staphylococcus aureus* and NFGNB. CONS and *Klebsiella species* were most common offenders in EOS and *Klebsiella species* was the main offender in LOS. *Klebsiella species* has been detected as the main offender in numerous Indian studies.[9,3,16,23] National Neonatal Perinatal Database data 2002-2003 also shows that *Klebsiella species is* the most common pathogen isolated in neonatal sepsis in our country.[4]

In our study CONS(20.13%) was more frequently isolated than *Staphylococcus aureus* (9.1%) similar to findings in other studies.[16] The isolation rate of CONS correlates with various other Indian studies.[24,2] Overall our study showed *Staphylococcus aureus* (24.13%) and CONS (53.5%) as the most common Gram-positive organisms. The CONS isolation rate is quite high as compared to few studies.[18,19] We did not isolate group B *Streptococcus* from any of our cases and this agrees with the finding that it's not a common cause of neonatal sepsis in our country.[16]

NFGNB and *Klebsiella species* were the common Gram-negative isolates, which was comparable to the other studies in the region.[2,18,19]

In our study 71.43% of the *Staphylococcus aureus* were methicillin resistant similar to another study which reported that 66% of *Staphylococcus aureus* isolated from cases of neonatal sepsis were methicillin resistant,[25]. The various findings about MRSA isolation rates by other authors are 31.25%,[26] 41%,[2] 56%,[3]. 33.33% of the *Pseudomonas* isolates were found to be metallo-beta-lactamase (MBL) producers similar to findings in another study.[3]

96.05% of the Enterobacteriaceae were found to be ESBL producers, which is much higher than several other studies in the region. The various ESBL detection rates were 67.3%[3]; 48%[2]. In the present study ESBL was detected in 98.39% of Klebsiella spp., 88.89% of *Citrobacter* spp. and 80% of Escherichia coli isolates, this is closer to a study in which ESBL was in 86.6% of *Klebsiella pneumoniae* and 63.6% of *Escherichia coli*[27]; ESBL was detected in 70.9% of *Klebsiella pneumoniae* isolates and 57.14% of *Escherichia coli* isolates in a study[3]; and in another study ESBL production was seen in 52.9% of *Escherichia coli* and 50% of *Klebsiella pneumoniae* isolates.[26] The variations in the percentage of isolation of ESBL among several studies could be due to the regional variations.[3]

Staphylococcus epidermidis isolated in EOS could be because of contamination during the process of blood collection.[11] It is opined that this presence of the bacteria in the blood should not be taken as contamination especially in samples from patients in critical care units.[28] An incidence of 20% for CONS is a matter of concern. In the past decade it is being observed that there has been a shift of the predominance from the Gram-negative organisms to Gram-positive organisms like *Staphylococci* all over the world, the cause of which is unclear.[29,30] High rates of CONS infections have been reported from the Middle East, Southeast Asia and Latin America, which could be an indicator of invasive devices being used at a high rate.[19] As in our study many other recent studies

have reported the emergence of organisms such as CONS, NFGNB, and *Candida* spp. as a cause of neonatal sepsis.[18,19]

Gram negative bacteria of the Enterobacteriaceae family showed resistance to the penicillins and extended spectrum cephalosporins in huge numbers, hence the use of these antibiotics alone will not be effective. This can be combated by using the drugs to which most organisms were found to be sensitive. The emergence of the resistant bacteria in the NICU setup leads to failure in the treatment of neonatal sepsis.[2]

The widespread indiscriminate use of the broad spectrum β-lactam antibacterial agents has led to an exponential rise in the incidence of ESBL in Gram negative organisms.[3] Emergence of ESBLs is also due to the selective pressure which is imposed by the extensive use of antimicrobials in intensive care units which is a vital factor in the treatment of the sepsis.[10] These ESBL producers isolates show resistance to the beta-lactam antibiotics, including the third-generation cephalosporins, in addition they also often exhibit resistance to various other classes of drugs such as tetracycline, co-trimoxazole, aminoglycosides, and fluoroquinolones, hence, they pose an intimidating challenge with limited therapeutic options, especially in the developing countries.[10]

Conclusion

This study shows that Klebsiella species, Coagulase Negative *Staphylococci*, *Staphylococcus aureus and Non fermenting gram negative bacilli* were the commonest organisms associated with Neonatal sepsis. An alarmingly high rate of MRSA and ESBL producers was observed. This study stresses that antimicrobial resistance is a universal problem and it stresses the need for surveillance. Various measures to decrease the antibiotic resistance are implementing infection control measures, restricting the use of the broad spectrum antibiotics, rational use of antibiotics, prescribing only specific antibiotic therapy after evaluating the sensitivity pattern of isolates and cycling of antibiotics. To prevent the further spread of these resistant strains adopting of simple hygienic measures like hand washing and screening of the hospital staff and also the mothers for MRSA and ESBL can be helpful. This will help in slowing down the further spread of MRSA and ESBL and also improve the prognosis in cases of neonatal sepsis.

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Conflicts of Interest: None

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