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Original Research Article

Neonatal sepsis due to coagulase negative Staphylococci: a study from Kashmir valley, India

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ABSTRACT

Background: Neonatal sepsis is a leading cause of neonatal mortality and morbidity. Early diagnosis and treatment with appropriate antibiotics are important to improve the prognosis of neonatal sepsis. Coagulase-negative Staphylococci (CoNS) have emerged as prominent pathogens in the neonatal intensive care unit. These infections are rarely fatal, but they cause significant morbidity, especially among very low birth weight infants. This study was done to know the prevalence of Coagulase-negative Staphylococci in neonatal sepsis and to determine their antibiotic susceptibility pattern.
Methods: A prospective study was conducted on blood samples of suspected neonatal septicaemia between August 2017 and May 2018 received at Department of Microbiology, Government Medical College, Srinagar. Blood culture was done by automated blood culture system, (BacT/Alert) and identification and antibiotic susceptibility was done by VITEK2 method.
Results: Out of 356 neonates screened, there were 185 (53.4%) positive blood cultures. Among the culture positive cases, 107 (57.83%) were male and 78 (42.16%) were female. Early Onset Septicaemia cases (130 [70.27%]) were found to be three times higher than late onset Septicaemia (55 [29.72%]). Coagulase-negative Staphylococci (CoNs) (30.27%) were the most common organisms isolated followed by *Acinetobacter sp* (15.1%), *Klebsiella sp* (5.4%) *S. aureus* (4.8%) and *E. coli* (4.8%). All the isolates of CoNS were sensitive to linezolid and vancomycin and tigecycline. Methicillin resistance was seen in 84% isolates.
Conclusions: Present study highlights the emergence of Coagulase-negative Staphylococci *(CoNS)* as predominant cause of neonatal septicaemia. Most of the isolates were resistant to methicillin which is alarming and a cause for concern.

Keywords: Antibiotic susceptibility, Blood culture, Coagulase negative Staphylococci (CoNS), Neonatal sepsis

INTRODUCTION

Neonatal sepsis is a significant cause of morbidity and mortality and contributes to 30-50% of all deaths during the neonatal period in developing countries.[1] World health organization (WHO) estimates about 3.3 million neonatal deaths a year, majority of them occurring in developing countries.[2] In India it is estimated that neonatal septicaemia is responsible for approximately 4% intramural live births.[3] According to recent data from national neonatal perinatal database (NNPD) 2000, the incidence of neonatal sepsis has been reported to be 38 per 1000 intramural live births in tertiary care institutions.[4] Neonatal septicaemia can be divided into early onset in the first 7 days of life and late onset after the first 7 days of life.

Early onset sepsis usually presents within 72 hours. of life. Source of infection is generally the maternal genital tract. It often manifests as pneumonia causing acute

respiratory distress. Late onset sepsis usually presents after 72 hours of birth. The source of infection is either nosocomial or community acquired. Neonate usually presents with pneumonia, septicaemia or meningitis.[5] The spectrum of organisms that cause neonatal septicaemia varies in different countries, and sometimes changes from one center to another within the same country.[6] Group B streptococci (GBS) and E. coli predominate in the USA and Europe, whereas Staphylococci and Gram-negative bacilli are much more common in developing countries.[7]

Coagulase-negative staphylococci (CoNS) are the most frequent cause of late-onset sepsis among new-born infants in neonatal intensive care units (NICU) worldwide. Incidences of up to 66% of late-onset sepsis have been reported. [8] CoNS are a heterogeneous group of bacteria, consisting of approximately 40 species, of which, several species have been recognized as potential pathogens to humans. The most common species isolated from human specimens that result in disease are *S. epidermidis*, S. *haemolyticus*, *S. hominis*, and *S. saprophyticus*. Other species such as *S. warneri*, *S. lugdunensis*, *S. capitis*, *S. simulans*, *S. cohnii*, *S. saccharolyticus*, and *S. xylosus* have been considered as significant opportunistic pathogens but rarely isolated.[9]

Although CoNS sepsis is characterized by low mortality, the microorganism is known to be highly resistant to antibiotics-methicillin resistance exceeds 70% in most centres.[10] Neonates become colonized by microorganisms present in environment within first week of life.[11] During this period, the risk of CoNS infection increases substantially with the use of central venous catheters (CVC), mechanical ventilation, and par-enteral nutrition, and with exposure to other invasive skin or mucosa-breaching procedures.[12] Neonatal CoNS infections are rarely fatal, but they cause significant morbidity, especially among very low birth weight infants. In addition, CoNS frequently display multi resistance to antibiotics. Babies with CoNS infection are smaller and more premature than controls, but even after controlling for prematurity, they stay longer in neonatal intensive care units than non-infected babies, and at considerable cost.[13] In this paper, authors report the incidence and antibiotic susceptibility of CoNS infections studied in a prospective study of neonatal infections in Kashmir valley. Present study was designed to evaluate prevalence of CoNS in neonatal bacteraemia cases along with their antibiogram and help clinicians in making the proper choice of antibiotic for therapy as well as to take preventive measures to reduce the transmission of organism.

METHODS

A prospective study was carried out in Department of Microbiology, Government Medical College Srinagar during the period from August 2017 and May 2018. A total of 356 blood samples were collected from all clinically suspected neonatal sepsis patients. Institutional ethics committee clearance was taken before the start of the study. A detailed antenatal, natal and post-natal history was taken. Volume of 1-2ml blood was drawn aseptically before starting antimicrobial treatment and directly transferred into the BacT/ALERT PF plus disposable culture vials containing enriched broth and incubated in BacT/ALERT blood culture instrument at 35.5°C±1.5°C for 5 days. A positive result was indicated by an audible alarm and yellow illumination of the positive indicator lamp at the site of positive vial. On the computer instrument status display the station number was showed by flashing green in case of a positive vial. The bottles were incubated for five days before being reported as negative. A Gram stain and a subculture on Blood agar and MacConkey agar were performed from each presumptive positive vial. Identification was carried up to species level, and antimicrobial susceptibility testing was done with an automated microbiology system, Vitek 2 compact 60 system BioMerieux India ®) and interpreted according to CLSI criteria.[14] Cultures that yielded commensal species (CoNS) were reviewed using CDC criteria for CoNS related blood stream infection where minimum of two positive blood cultures are required to fulfil the criteria.[15]

Stastical analysis

Summary of measures was reported as mean and standard deviation (SD) for quantitative variables and percentages for categorical variables. Data analysis was done using Statistical Package for Social Sciences (SPSS) software version 14.

RESULTS

A total of 356 samples from clinically diagnosed septic emic neonates were received in the Department of Microbiology during the study period. Blood culture was positive in 185 samples (53.4%) and no growth was seen in 171 (47.6%) cases as shown in Figure 1. Among the culture positive cases, 107 (57.83%) were male and 78 (42.16%) were female. Early Onset Septicaemia cases (130[70.27%]) were found to be three times higher than Late Onset Septicaemia (55[29.72%]).

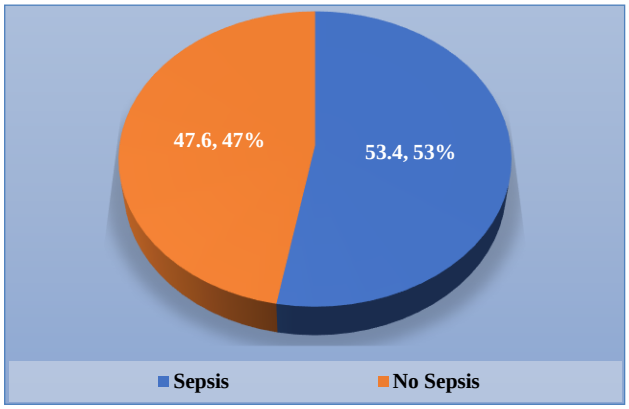

Figure 1: Incidence of sepsis.

Table 1: Distribution of various microorganisms isolated.

Name of organism	Number (%)
CONS	56 (30.27)
Acinetobacter sp	28 (15.13)
Klebsiella sp	10 (5.4)
S. aureus	09 (4.8)
E. coli	09 (4.8)
Enterococcus sp	07 (3.7)
Candida sp	62 (33.5)
Others	05 (2.7)

The culture positive organisms included Gram-positive cocci (71/185,38.37%) Gram-negative organisms (52/185, 28.10%) and *Candida sps* (62/185,33.51%). Coagulase-negative Staphylococci (CoNS) 56 (30.27%) and *Acinetobacter sp* 28 (15.13%) were the most common gram-positive and gram-negative organisms, respectively as shown in Table 1.

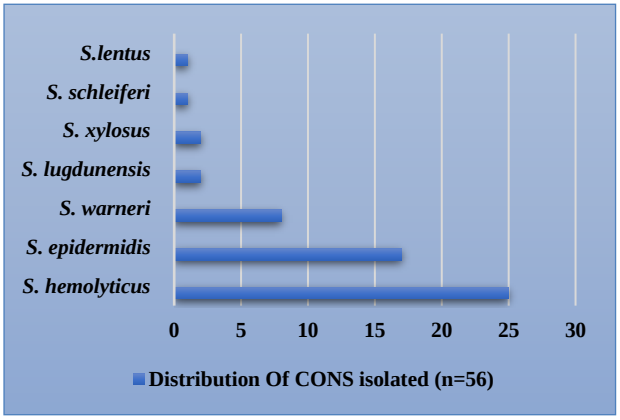

Figure 2: Distribution of coagulase negative Staphylococci (CoNS) isolated (n=56).

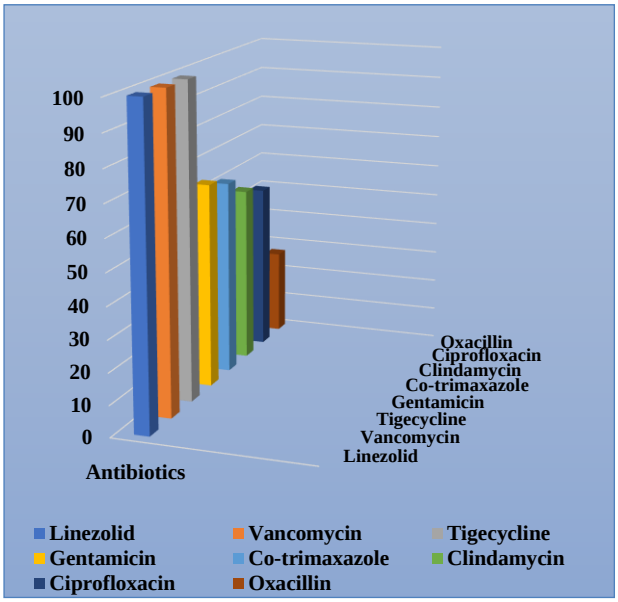

Figure 3: Antibiotic sensitivity profile of cons.

Out of the 56 isolates of Coagulase negative Staphylococci (CoNS) the most commonly isolated species was *S. hemolyticus* 25 (44.64%) followed by *S. epidermidis* 17 (30.35%), *S. warneri* 8 (14.28%), *S. lugdunensis* 2 (3.57%), *S. xylosus* 2 (3.57%) *S. schleiferi* 1 (1.78%) and *S. lentus* 1 (1.78%) as shown in Figure 2.

The antibiotic sensitivity profile of CoNS revealed that all the isolates were sensitive to linezolid and vancomycin and tigecycline. Methicillin resistance was seen in 84% isolates as shown in Figure 3.

DISCUSSION

Neonatal septicaemia refers to systemic bacterial infection with positive blood culture in the first 4weeks of birth. Sepsis is the commonest cause of neonatal mortality and is probably responsible for 30- 50% of the total neonatal deaths each year in developing countries. It is estimated that 20% of all neonates develop sepsis and approximately 1% die of sepsis related causes.[16]

The pattern of organisms causing neonatal sepsis has been constantly changing and indiscriminate use of antibiotics has resulted in the emergence of multidrug resistant and virulent organisms. Prior to 1980, most neonatal septicaemias were caused by gram negative bacilli and staphylococcus aureus. In the last two decades, however, *Coagulase negative staphylococci* (CoNS) has been emerging as the predominant causative organism.[13,17]

Coagulase-negative Staphylococci (CoNS) are now the most common organisms associated with late-onset septicaemia, accounting for more than 50 % of cases.[18,19] Although there is no consensus regarding the pathogenic potential of CoNS, as they are commensals of the skin and mucosa in humans and animals, studies have shown that they are major cause of neonatal morbidity and mortality.[20] Large number of risk factors have been identified for CoNS infections namely immunosuppression, extremes of age, any mucocutaneous breach, previous antibiotic exposure and most importantly presence of indwelling prosthetic devices, catheters.[21] This study was undertaken to determine the antimicrobial resistance pattern and species of coagulase-negative Staphylococci (CoNS) isolated from the blood and skin of neonates with clinical suspicion of septicaemia admitted to neonatal intensive care unit.

In present study the culture positivity rate was 53.4%. This is similar to findings of other studies showing positivity rates of 48%, 62.8% and 64% among neonates with sepsis.[22-24] However, some studies conducted by Joshi et al, and national neonatal perinatal database (NNPD) 2000, showed a low culture positivity rate.[3,25] The difference may be due to variable incidence of neonatal sepsis from place to place and due to many other factors like perinatal care, birth weight etc.

In the present study, males were more affected than their female counterparts, males contributing 107 (57.83%) of culture positive cases and the male: female ratio being 1.4:1. This may be because term male infants have an approximately two-fold higher incidence of sepsis than term females.[26] Also reason for male preponderance may be sex dependent factors as X-linked immunoregulatory genes may play some protective roles in females.[27]

In present study early onset Septicaemia cases (130[70.27%]) were found to be three times higher than late onset Septicaemia (55[29.72%]). Similar results have been reported by other workers also.[6,28]

This clustering of 70.27% cases in first 3 days of life reflects the immaturity of immunological responses in the first few days of life.[29] Also in developing countries, this could be due to prematurity, low birth weight or unhygienic conditions during labor.[30]

Present study showed a preponderance of gram-positive isolates 38% versus 28% gram-negative isolates. A similar preponderance of gram-positive organisms has also been reported by Thakur et al (60%), Ballot et al (54.9%) and Kaufman et al (68.2%).[31-33]

In this study Coagulase-negative Staphylococci (CoNS) 56 (30.27%) were the most common organism isolated followed by *Acinetobacter sps* 28 (15.13%), *Klebsiella sp* 10 (5.4%), *S. aureus* 09 (4.8%), *E. coli* 09 (4.8%) *Enterococcus sp* 07 (3.7%). Candida sp accounted for 33.5% of the species isolated. Among the CoNS, *Staphylococcus haemolyticus* was the predominant isolate constituting 44.64% of the cases. This corroborates with studies conducted by other authors as well.[34-36] Jain et al, isolated *S. haemolyticus* (58%) as the most common isolate, followed by *S. epidermidis* (17%).[37] Another study conducted by Kashid and Raghuraman in India showed that *S. haemolyticus* (30%) was the most common species isolated followed by *S. warneri* (14%).[38]

CoNS is often regarded as the contaminant, possibly from the skin, but de Leon P et al, stated that the presence of CoNS in blood cannot be taken as contamination, especially in patients in critical care units.[39] Also factors like lack of stratum corneum, lack of virulence factors, insufficient recruitment of neutrophils and biofilm formation in indwelling catheters are all important for CoNS pathogenesis in neonatal

The increased isolation of CoNS as a causative agent of neonatal septicaemia can be attributed to the overcrowding of NICU which helps in spreading of CoNS which colonies the nasopharynx and skin of health care workers to the babies. Further improper hand washing techniques lead to horizontal transmission of Gram-positive organisms. CoNS have become an important nosocomial pathogen partly because of the increasing use of medical devices such as long-term indwelling catheters, vascular grafts, and prosthetic heart valves and joints. [41]

The antibiotic sensitivity profile of CoNS revealed that all the isolates were sensitive to linezolid and vancomycin and tigecycline. Methicillin resistance was seen in 84% isolates, while as 65% were sensitive to gentamicin 62% to co-trimaxazole 56% to clindamycin and 53% to ciprofloxacin.

Similar results have been shown by Jyothi et al and Shivanna et al. [36,42] All these studies indicate increase in prevalence of methicillin resistant Coagulase negative staphylococci and increase in trend of multidrug resistance. Methicillin resistant Coagulase negative staphylococci is an important nosocomial pathogen mainly in neonatal intensive care units. It is also source of resistance gene to other gram-positive cocci including *Staphylococcus aureus* in hospital settings.

Methicillin resistance has been shown to be more common among *S. epidermidis* and *S. haemolyticus* isolates than among *S. aureus*.[43] Moreover, they may act as a reservoir of staphylococcal cassette chromosome (SCC) elements for *S. aureus*, including SCC harbouring the MR gene mec (SCCmec) and the SCC-like arginine catabolic mobile element which favors staphylococcal colonization of the skin.[44,45]

CONCLUSION

It is evident from this study that CoNS are one of the leading causes of neonatal sepsis, and alarmingly, most of them are resistant to multiple antibiotics. As CoNs are also part of the skin flora and a common contaminant, differentiating true infection from contamination is very challenging. The determination of the identity of CoNS isolates, whether being true pathogens or contaminants, is crucial to enhance the validity of a blood culture so that injudicious use of antibiotics is reduced. Regular antibiotic susceptibility surveillance and evaluation, and the enforcement and periodic review of the antibiotic policy of the hospital is a must to reduce rate of acquiring nosocomial infections and development of bacterial resistance.

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