The relevance of coagulase-negative *Staphylococcus* isolates in blood culture in the context of a tertiary neonatal unit from East India

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**ABSTRACT**

**Background:** Coagulase-negative *Staphylococcus* (CoNS) are known commensals and often contaminate neonatal blood cultures. Their unique ability to form biofilms, however, helps them evade immune mechanisms and antibiotics and cause neonatal sepsis (NS) in hospitalized neonates. True or probable Coagulase-negative *Staphylococcus* bloodstream infection (CoNS BSI) must be differentiated from contaminants so that antibiotics are used judiciously and hospital stay is minimized. **Aims and Objectives:** The primary objective of the study was to estimate the proportion of NS and contaminants among CoNS-positive blood cultures in the index neonatal unit and the host and health-care variables associated with CoNS BSI. The secondary objective was to estimate the susceptibility of CoNS isolates to antibiotics used. **Materials and Methods:** This was a retrospective study from digital records, from January 2018 to December 2022. **Results:** 25% of CoNS isolates were associated with NS (health-care infections) and 75% were contaminants. Over 90% of CoNS BSI was associated with central lines (CLs) and prolonged hospital stay. All isolates were resistant to oxacillin while resistance to gentamicin rose annually to over 68%. Susceptibility to linezolid and vancomycin was present, but a few strains were resistant to them. **Conclusion:** CoNS were an important cause of NS in the index hospital. Prolonged hospital stays and CLs were associated with increased incidence of CoNS sepsis and must be minimized where possible. Antibiotic resistance was high, and reserve drugs could also become ineffective. **Key words:** Coagulase-negative *Staphylococcus*; Neonatal sepsis; Contamination; Polymicrobial sepsis

**INTRODUCTION**

Neonatal sepsis (NS) is defined as bloodstream infection (BSI) within the first 28 days of birth. The estimated global burden of NS is 3 million per year, mostly borne by developing countries.¹ ² The true burden is likely unknown because of lack of data from many parts of the world. When symptoms of NS manifest prior to 72-h age, it indicates maternally acquired infection and is defined as early onset sepsis. Late-onset sepsis (LOS) manifesting beyond 72 h is acquired from the community, or in case of hospitalized neonates, they are health-care-associated infections (HAIs). With advances in neonatal care, more preterm and low birth weight (BW) neonates are surviving. However, the need for invasive central lines (CLs), parenteral nutrition, prolonged stay, and antibiotic use predisposes them to HAI. NS due to HAI negatively impacts mortality and causes long-term morbidity in surviving neonates leading to white
matter injury and poor neurodevelopmental outcome, bronchopulmonary dysplasia, retinopathy of prematurity, and necrotizing enterocolitis.

Coagulase-negative *Staphylococcus* (CoNS) are one of the most common causes of HAI in neonatal units worldwide. In South Asia, including India, Gram-negative pathogens such as *Klebsiella* species, *Acinetobacter* spp., and *Escherichia coli* are more common. However, recent studies show that the incidence of healthcare associated neonatal sepsis caused by fungal and Gram-positive organisms like CoNS and *Staphylococcus aureus* are rising in neonatal units in India.

CoNS are known commensals and, in hospital, rapidly colonize the neonatal skin and mucous membranes from the environment and hands of healthcare personnel (HCP) and parents. The complex relation between the host and commensals is influenced by the neonatal microbiome, which is affected by ethnicity, geography, climate, mode of delivery, hospital flora, and antibiotic use. These CoNS commensals can contaminate blood cultures during collection and be isolated as CoNS.

However, CoNS are also one of the most important causes of HAI in susceptible neonates because of their ability to evade immune mechanisms by several means.

i. Foremost is their unique ability to form biofilm matrix on indwelling CLs used for nutrition and antibiotics, making it difficult for antibiotics to penetrate and kill.

ii. Toxins and isoenzymes produced by CoNS help in biofilm formation.

iii. There is growing gene-transferred antibiotic resistance and antibiotic tolerance in CoNS isolates.

In a study from North America, 70% of NS in the neonatal unit were by Gram-positive organisms, the majority being CoNS. In another Western report, over 30% of CoNS isolates in the neonatal unit were definite CoNS sepsis, nearly 30% were contaminants, and the rest were undefined. The reported incidences of CoNS BSI in South Asian neonatal units range from 17.6% to 42%, though there is not much clarity with regard to definite or probable CoNS BSI, contamination, or polymicrobial CoNS BSI.

It is thus very important to differentiate true or probable CoNS infection from contamination and identify those who need to be treated with appropriate antibiotics, while avoiding unnecessary antibiotic use in those who do not. The National Healthcare Safety Network (NHSN) of Centre for Disease Control and Prevention (CDC), USA, gives guidelines to differentiate between:

i) Definite/true CoNS BSI

ii) Probable CoNS sepsis

iii) Contamination

iv) CoNS with polymicrobial BSI (PBSI)

**Aims and objectives**

The objectives of the current study were to throw some light on the relevance of CoNS isolated in blood culture in the neonatal unit of the index hospital from East India.

**Primary objective**

The primary objective of this study was to estimate the proportions of true or probable CoNS BSI, CoNS contaminants, and CoNS with PBSI, among CoNS-positive blood cultures in the index neonatal unit, over the study period and the association of CoNS with variables such as BW, gestational age (GA), presence of CLs, and duration of hospital stay.

**Secondary objective**

The secondary objective of this study was to estimate the antibiotic susceptibility of the CoNS isolates to the antibiotics used for treatment.

**MATERIALS AND METHODS**

**Study design**

The study was a retrospective observational study from digital hospital records.

**Study setting and location**

This study was conducted at the neonatal unit of the index hospital, a 250-bed tertiary referral children’s hospital with a 20-bed neonatal intensive care unit (NICU). The NICU is a tertiary referral center with around 580 admissions per year referred from peripheral hospitals and from the community.

**Study period**

The study period was 5 years from January 2018 to December 2022.

**Study population**

Neonates admitted to the NICU were enrolled.

**Inclusion criteria**

All neonates with CoNS-positive blood cultures were included in the study. CDC NHSN guidelines were used to classify CoNS isolates into true CoNS BSI, probable CoNS BSI, contaminant, and PBSI.
i. True/definite CoNS BSI case episode: Symptomatic neonate with two CoNS-positive blood cultures drawn within 24 h of each other, from two different aseptic sites.14,15

ii. Probable CoNS BSI: One blood culture positive for CoNS in a symptomatic neonate (hypothermia <36°C, fever >38°C, bradycardia, apnea, lethargy, and poor sucking) and hematological parameters of NS (leukopenia, thrombocytopenia, raised CRP, or procalcitonin) and agreed upon as sepsis by three specialists.16 These included case episodes where one of the two cultures sent was positive for CoNS and those where, due to technical difficulties, only one sample could be collected (extremely low BW <1000 g, very sick child, and technically difficult sampling).

iii. Polymicrobial coinfection BSI (PBSI): CoNS-positive blood culture and positive growth with another pathogenic organism isolated in the same culture specimen or within a 48-h time span in a symptomatic neonate.4,17

iv. Contaminant: Repeated positive cultures after 24 h of appropriate intravenous antibiotic therapy or only one CoNS-positive culture or two cultures positive with two different species of CoNS, without clinical signs or laboratory markers of sepsis.14 The reason why blood culture was sent in these cases is that some had subtle signs of sepsis, but some other non-infective causes such as hypoglycemia, hypocalcemia, and exaggerated physiological jaundice were later detected and antibiotics were discontinued. Ninety percent of admissions to the neonatal unit were referred from other hospitals/communities for various reasons. These were also screened for sepsis on admission.

Definite and probable CoNS sepsis case episodes were taken together as CoNS BSI in the index study. In keeping with the definition of LOS, positive blood cultures drawn after 72 h of index hospital stay were designated as HAI.14 CoNS blood cultures positive after 48 h of CL insertion were taken as CL-associated BSI as per CDC, NHSN definition.14 Microbiological data (not clinical) of other Healthcare associated blood stream infection (BSI) were collected to estimate the proportion of CoNS HAIs (definite and probable) among all HAIs.

Exclusion criteria
Neonates who did not have CoNS-positive blood cultures were excluded from the study.

Data management and quality assurance
Laboratory data, both microbiological and hematological, and clinical data were extracted from hospital digital records and entered into Excel sheets. Entered data were crosschecked from hospital records, and cleaned by the principal investigator. Personal identification data was anonymized to maintain confidentiality.

Statistical analysis
The proportion of CoNS BSIs among all CoNS isolates for each year was calculated by dividing the sum of definite and probable CoNS BSIs by the total number of CoNS-positive blood cultures that year and expressed as percentage (%). The proportion of CoNS PBSIs was calculated by dividing the number of CoNS PBSIs with total CoNS-positive isolates. The incidence of HAI per 1000 patient days was calculated by dividing the total number of HAIs (numerator) with total number of resident days that year (denominator). The average daily bed occupancy in 2018, 2019, and 2022 was 20 and in 2020 and 2021 was 17 (possibly because of COVID-19 lockdown with diminished accessibility to health care). Therefore, resident days for the years 2018, 2019, and 2022 were 7300 (20 X 365) and for the years 2020 and 2021 were 6205 (17 X 365). Statistical analysis was performed by SPSS 16 and Excel.

Consent and ethics
The study was approved by the Institutional Ethics Committee. Consent was waived as it was a retrospective study from hospital records. Personal data were anonymized and kept secure under lock and key and password protected.

RESULTS
A total of 2912 neonates were admitted to the neonatal unit from January 2018 to December 2022. The total number of CoNS-positive blood cultures was 189 (Table 1). Eight percent of these were monomicrobial true or probable CoNS BSI, 17% were polymicrobial CoNS BSI with another pathogen, and 75% were contaminants (Table 1). Therefore, CoNS monomicrobial and polymicrobial NS comprised 25% of CoNS-positive blood cultures. All cases of definite and probable CoNS BSIs, as well as PBSI, were HAI. The total number of HAIs in the neonatal unit from all isolates was 318 during this period. The overall incidence of HAI was 10.9% (318/2912). The average HAI rate per 1000 patient days was 9.36/1000 patient days. True and probable CoNS BSIs comprised 39% (15/38) of all Gram-positive HAI, and the rest were Enterococcus species and S. aureus.
On stratification of the clinical modifiers in the neonates with CoNS BSI, 73.3% had BWs >2 kg and 60% were >34-week GA (Table 2). Eighty percent of all CoNS BSIs had a duration of stay >2 weeks and 93% had CL.

Among CoNS PBSI (mostly CoNS with Candida species and Gram-negative organisms), more than 66% weighed <2 kg and were <34 weeks of gestation (Table 3). One hundred percent of CoNS PBSIs had CLs and stayed >2 weeks in the neonatal unit.

CFR in CoNS BSI was lower (6.6%) than CFR in CoNS with PBSI (24%) and CFR from all HAIs taken together (22%). However, the difference was not statistically significant (P=0.078) at 95% level of significance.

All (100%) of the CoNS isolates were resistant to the antibiotic oxacillin across the study period. Gentamicin resistance was observed in 30% of isolates in 2018–2019, 50% in 2020–2021, and 68.4% in 2022. Nearly all isolates were sensitive to vancomycin, teicoplanin, linezolid, and tigecycline. However, teicoplanin resistance was observed in one and linezolid resistance in two isolates in 2020, while one isolate was resistant to vancomycin, teicoplanin, and linezolid in 2021.

**DISCUSSION**

With advances in neonatal care, and increasing survival of very low BW neonates, HAI is a concern among hospitalized neonates. CoNS is an important and, in some

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**Table 1: Distribution of coagulase-negative *Staphylococcus*-positive blood cultures from 2018–2022**

<table>
<thead>
<tr>
<th>Year</th>
<th>2018</th>
<th>2019</th>
<th>2020</th>
<th>2021</th>
<th>2022</th>
<th>Total (mean) (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total CoNS+cultures (number)</td>
<td>45</td>
<td>38</td>
<td>22</td>
<td>40</td>
<td>44</td>
<td>189</td>
</tr>
<tr>
<td>Definite CoNS sepsis of all CoNS+cultures</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>Probable CoNS sepsis (number)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Definite+probable CoNS sepsis (number)</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>15</td>
</tr>
<tr>
<td>Percentage of definite+probable CoNS sepsis in all CoNS+ve</td>
<td>8.8% (4/45)</td>
<td>7.8% (3/38)</td>
<td>9% (2/22)</td>
<td>7.5% (3/40)</td>
<td>6.8% (3/44)</td>
<td>(8%) (4.08–11.8%)</td>
</tr>
<tr>
<td>CoNS contaminants (%)</td>
<td>34 (75.5)</td>
<td>29 (76.3)</td>
<td>16 (72.7)</td>
<td>30 (75)</td>
<td>32 (72.7)</td>
<td>141 (75) (68.39–80.8)</td>
</tr>
<tr>
<td>Polymicrobial bloodstream infection with others (percentage of CoNS +)</td>
<td>7 (15.5)</td>
<td>6 (15.7)</td>
<td>4 (18.2)</td>
<td>7 (17.5)</td>
<td>9 (20.5)</td>
<td>33 (17) (12–22.8)</td>
</tr>
</tbody>
</table>

CoNS: Coagulase-negative *Staphylococcus*, CI: Confidence interval

**Table 2: Clinical data of definite and probable coagulase-negative *Staphylococcus* sepsis**

<table>
<thead>
<tr>
<th>Year</th>
<th>2018</th>
<th>2019</th>
<th>2020</th>
<th>2021</th>
<th>2022</th>
<th>Total</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Deaths</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>6.6 (1/15)</td>
</tr>
<tr>
<td>Body weight &gt;2 kg</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>11</td>
<td>73.3 (11/15)</td>
</tr>
<tr>
<td>Body weight &lt;2 kg</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>26.6 (4/15)</td>
</tr>
<tr>
<td>Gestational age &gt;34 weeks</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>9</td>
<td>60</td>
</tr>
<tr>
<td>Gestational age &lt;34 weeks</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>6</td>
<td>40</td>
</tr>
<tr>
<td>Duration of hospital stay &gt;2 weeks</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>12</td>
<td>80</td>
</tr>
<tr>
<td>Duration of hospital stay &lt;2 weeks</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td>20</td>
</tr>
<tr>
<td>Central lines</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>14</td>
<td>93</td>
</tr>
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</table>

**Table 3: Clinical data of coagulase-negative *Staphylococcus*-positive blood culture with polymicrobial bloodstream infection in neonatal unit**

<table>
<thead>
<tr>
<th>Year</th>
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<th>2020</th>
<th>2021</th>
<th>2022</th>
<th>Total</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number CoNS +ve</td>
<td>45</td>
<td>38</td>
<td>22</td>
<td>40</td>
<td>44</td>
<td>189</td>
<td></td>
</tr>
<tr>
<td>CoNS with PBSI</td>
<td>7</td>
<td>6</td>
<td>4</td>
<td>7</td>
<td>9</td>
<td>33</td>
<td>17.4 (33/189)</td>
</tr>
<tr>
<td>Deaths</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>8</td>
<td>24.2 (8/33)</td>
</tr>
<tr>
<td>Body weight &gt;2 kg</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>11</td>
<td>33.3 (11/33)</td>
</tr>
<tr>
<td>Body weight &lt;2 kg</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>5</td>
<td>6</td>
<td>22</td>
<td>66.6 (22/33)</td>
</tr>
<tr>
<td>Gestational age &gt;34 weeks</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>10</td>
<td>30.3 (10/33)</td>
</tr>
<tr>
<td>Gestational age &lt;34 weeks</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>5</td>
<td>7</td>
<td>23</td>
<td>69.6 (23/33)</td>
</tr>
<tr>
<td>Duration of hospital stay &gt;2 weeks</td>
<td>7</td>
<td>6</td>
<td>4</td>
<td>7</td>
<td>9</td>
<td>33</td>
<td>100</td>
</tr>
<tr>
<td>Duration of hospital stay &lt;2 weeks</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CL</td>
<td>7</td>
<td>6</td>
<td>4</td>
<td>7</td>
<td>9</td>
<td>33</td>
<td>100</td>
</tr>
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</table>

CoNS: Coagulase-negative *Staphylococcus*, PBSI: Polymicrobial bloodstream coinfections, CL: Central lines
reports, the most important cause of HAI in hospitalized neonates worldwide. They are known commensals and can also be contaminants in blood cultures.

In the index study, 25% of all CoNS blood culture isolates were responsible for CoNS BSI (including monomicrobial and polymicrobial CoNS sepsis) and all of them were HAI, while 75% were contaminants. The reported rates of CoNS isolates from South Asia including India vary from 17% to over 40%. However, there is a paucity in clarity regarding the proportion of true infections and contamination. In one Western report, over 30% of CoNS isolates in the neonatal unit were definite CoNS sepsis, nearly 30% were contaminants, and the rest were undefined. In a previous study, the authors had reported that with improved neonatal infection control measures taken in the index NICU, HAI by the traditional Gram-negative pathogens declined significantly. However, CoNS HAI did not show a significant decline due likely to their immune mechanism evading abilities. CoNS HAI rates are influenced by GA, BW, and duration of hospital stay and presence of CL among others. In the index study, the majority of neonates with definite or probable CoNS BSI were >2 kg in weight (73%) and >34-week GA (60%) (Table 2). These findings were in contrast to those from Western countries where nearly 98% of CoNS BSIs in neonatal units were in those <2 kg and <34-week GA. Variables such as the proportion of neonates with BW >2 kg and of >34-week GA in the study cohort, climatic differences, ethnicity, and health-care practices could be some of the factors responsible for these findings. Among CoNS BSI with PBSI, however, a majority of cases (>66%) weighed <2 kg and were <34 weeks in GA (Table 3). More than 90% of all CoNS BSIs and 100% of CoNS with PBSI were associated with CLs, possibly helped by biofilm formation (Tables 2 and 3).

CFR from definite or probable CoNS BSI was low compared to CFR in PBSI and CFR in all HAIs combined; however, the difference was not statistically significant (P>0.05).

It must be emphasized that CoNS BSI may seem to present with less morbidity/mortality, but there is evidence to show that it is associated with neonatal morbidities such as white matter damage, poor neurodevelopmental outcome, chronic lung disease, ROP, and NEC.

With respect to antibiotic susceptibility, 100% of all CoNS isolates were resistant to oxacillin and over two-thirds were resistant to gentamicin, with resistance rates rising annually. Sensitivity to reserve drugs such as vancomycin, teicoplanin, and linezolid was present. However, alarmingly, a few strains in 2020 and 2021 showed resistance to linezolid and vancomycin reinforcing the need for judicious use of antibiotics. Non-antibiotic strategies such as biofilm-degrading enzymes, bacteriophages, and lysins, which are in the frontiers of research, may be part of future therapies along with antibiotics in CoNS BSI.

The present study merits attention because:

a. It fills a knowledge gap in the Indian context, about the relevance of CoNS-positive blood culture in the NICU.

b. The findings from the study would help in antibiotic stewardship and judicious use of antibiotics in the neonatal unit.

Follow-on future studies could explore:

b. Combined screening of HCP for CoNS carrier status and its impact on incidence of CoNS sepsis.
c. Multicentric studies in a wider Indian context with surveillance over time.

Limitations of the study
The limitations would include:

a. Being retrospective in nature, real-time clinical monitoring was not possible.
b. Correlation of CoNS infection to duration of CL and number of CLs could not be done.

CONCLUSION
In conclusion, CoNS were an important cause of HAI during the study in the index neonatal unit. Overall one-fourth of CoNS-positive blood cultures in the neonatal unit were CoNS; either monomicrobial or polymicrobial, the rest were contaminants. The presence of CLs and longer hospital stay had a higher association with CoNS BSI. Resistance to antibiotics was high among CoNS isolates. Reserve drugs such as vancomycin and linezolid were the mainstay in treatment; however, occasional strains were observed to be resistant to these. CoNS evade antibiotics by biofilm formation, so future treatment modalities could involve biofilm-degrading enzymes and bacteriophages along with antibiotics. Stricter infection control measures, judicious antibiotic use, minimizing use of CLs and where possible, reducing the duration of hospital stay are the needs of the hour.

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REFERENCES


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<tbody>
<tr>
<td><strong>MM</strong> - Literature survey, conceptualized, designed, and drafted the initial manuscript, defined intellectual content, approved final version of the manuscript, and submitted manuscript; <strong>SP</strong> - Data collection and substantial contribution to intellectual content and design; <strong>BD</strong> - Data collection and reviewed it critically for important intellectual content; <strong>AM</strong> - Statistical analysis and interpretation of data and preparation of tables; <strong>SD</strong> - Monitored adherence to protocol, revision, and editing the final manuscript; <strong>MadM</strong> - Literature survey, data collection, interpretation of data, and manuscript revision.</td>
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