Species distribution and antibiotic sensitivity pattern of coagulase negative staphylococci isolated from blood culture of NICU patients

Nisarg A Trivedi[1], Payal D Soni[2*], Kanu J Patel[3]

[1,2]Tutor, [3]Associate Professor, [1-3]Dept. of Microbiology, [1]GMERS Medical College Gandhinagar, [2]GMERS Medical College Valsad, [3]B J Medical College Ahmedabad, Gujarat, India

***Corresponding Author: Payal D Soni**
Email: innovativepublication@drnt007.fastmail.in

Abstract
Introduction: The study of blood cultures isolates was conducted on samples received from NICU of a tertiary care hospital at Ahmedabad during a period of two years.
Objectives: To isolate, identify & find out prevalence of various species of coagulase negative staphylococci (CoNS) from blood culture of neonatal intensive care unit (NICU) patients. To study antibiotic susceptibility pattern of those isolates.
Materials and Methods: Same staphylococcal species isolated multiple times from the each patient were considered true/probable pathogens and were considered for further analysis. Species characterization and antimicrobial sensitivity testing were done. The data was analyzed with WHONET 5.6 software.
Results: Out of 4889 blood cultures received from NICU during the study period, 1465 samples were found positive (positivity rate of 30.0%). The most common CoNS species were *S. epidermidis* (64.6%) followed by *S. haemolyticus* and S. hominis. Septicemia by CoNS was more frequent in male & low birth weight groups. Isolates were least susceptible to penicillin-G (9.38%) while they were most susceptible to vancomycin (100%), teicoplanin (100%) and linezolid (100%). Methicillin resistance was found to be 26.04% among CoNS isolates.
Conclusion: This study indicates importance of coagulase negative staphylococci isolated from NICU blood cultures and suggests that strategies to reduce such infections in neonates are needed urgently, to reduce their medical, social, and economic toll.

Keywords: NICU, Neonatal intensive care unit, Antimicrobial sensitivity, CoNS, Coagulase negative staphylococci, Blood culture.

Introduction
Staphylococci are distributed widely in nature and can be isolated from variety of environmental sources or as commensal inhabitants of the skin, mucosa and other body sites in humans and animals. Genomic studies have identified more than 40 species of the genus. Not all of them are pathogenic to humans. Traditionally, the pathogenicity of the staphylococci was associated with variety of characteristics like pigmentation, coagulase production, mannitol fermentation etc. Out of these, ability to produce coagulase - an extracellular enzyme has most consistent association with pathogenicity. Classically, staphylococci are divided into Coagulase positive staphylococci (i.e. *S. aureus*) and Coagulase Negative staphylococci (CoNS) (i.e. *S. epidermidis*, *S. saprophyticus* etc). In the past, coagulase negative staphylococci were considered nonpathogenic and many of them were found to be commensal. But in recent decades, increasing evidence has built up suggesting their role as pathogens in variety of diseases. Among coagulase negative staphylococcal infections, *S. epidermidis* is the most common isolate. Its ability to form biofilm on plastic surfaces has made it an important cause of prosthetic device infections. Other commonly found coagulase negative staphylococci are *S. saprophyticus* – causing urinary tract infection in women, S. hemolyticus – causing bacteremia and soft tissue infections. Last few decades has witnessed emergence of antibiotic resistance even in coagulase negative staphylococci. As a part of skin flora, they are frequently exposed to antibiotics used for treating systemic illness. The *mecA* gene responsible for methicillin resistance is frequently found in coagulase negative staphylococci. Such strains underscore the importance of correct identification and susceptibility testing of these isolates and monitoring of their spread within hospital environment.

Objectives
1. To isolate, identify & find out prevalence of various species of coagulase negative staphylococci (CoNS) from blood culture of neonatal intensive care unit (NICU) patients.
2. To study antibiotic susceptibility pattern of those isolates.

Materials and Methods
This study was conducted at a tertiary care level hospital, Ahmedabad. Infections in first 28 days of life, or up to 4 weeks after the expected due date for preterm infants were considered as neonatal sepsis.[1] Blood culture samples were collected as a part of routine diagnostic procedure from neonates admitted in NICU with sign and symptoms of neonatal sepsis. Number of blood cultures done per neonate varied. Proper aseptic precautions were taken before collection. Up to 4 ml of blood was collected and inoculated immediately into aerobic blood culture bottle aseptically. After sampling, the bottles were received in the laboratory. The received bottles were loaded in BacT/ALERT 3D™ automated blood culture monitoring system.[2] When the system signaled the bottle as positive, such bottles were taken out and subcultures and microscopy were done. Identification of staphylococcal species was done by standard microbiological protocols.[3] When CoNS could not be isolated multiple times from the each patient, those

isolates were considered possible contaminants and were excluded from further analysis. Same staphylococcal species isolated multiple times from the each patient were considered true/probable pathogens and were considered for further analysis. Isolates were tested for antimicrobial susceptibility testing according to CLSI 2014 guideline.[4,5] The Kirby-Bauer disc diffusion method was utilized for the susceptibility testing. Testing for various resistance mechanisms was also performed. Testing for β-lactamase production was done using acidimetric method.[6] Screening for methicillin (oxacillin) resistance was performed using cefoxitin disc diffusion test (30 µg)[5] and positives were confirmed by oxacillin epsilometer test.[7] Vancomycin screen agar was used to screen isolates for glycopeptide resistance.[5] Inducible clindamycin resistance was detected by D-test.[5] The isolates and its antimicrobial susceptibility data was saved and analyzed with WHONET 5.6 software.

Results

This study was conducted over a period of two years. During this period 4889 blood culture samples were received from NICU ward and processed for bacteriological culture at our laboratory. Out of these, organisms were isolated from 1465 samples. Thus positivity rate of blood culture was 30.0%.

In this study gram positive organisms predominated (58.2%) in positive blood cultures. The largest group of isolates consisted of coagulase negative staphylococci (49.1%). Other organisms in descending order of frequency were Klebsiella spp., Escherichia coli, Staphylococcus aureus, Acinetobacter spp., Pseudomonas spp., Enterococcus spp. etc. (See Table 1 & Fig. 1)

Table 1: Prevalence of various organisms isolated from blood culture of NICU patients

Organism	n	%
Gram Positive Organisms	852	58.2
Coagulase negative staphylococci	719	49.1
Staphylococcus aureus	73	5.0
Enterococcus spp.	49	3.3
Streptococus spp.	11	0.8
Gram Negative Organisms	481	32.8
Klebsiella spp.	266	18.1
Escherichia coli	99	6.8
Acinetobacter spp.	61	4.2
Pseudomonas spp.	49	3.3
Others gram negative rods	6	0.4
Fungi	132	9.0
Candida spp.	132	9.0
Total Positive	1465	100.0

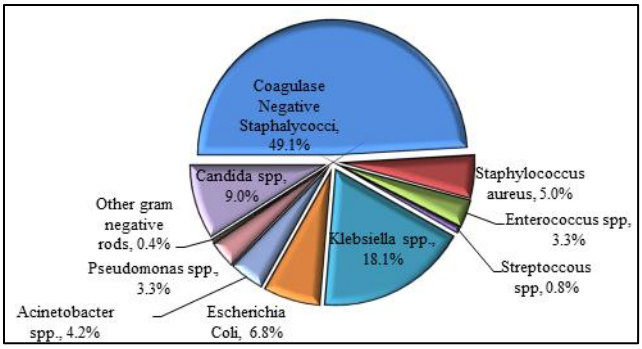

Fig. 1: Prevalence of various organisms isolated from blood culture of NICU patients

Of 623 patients from whom 719 strains of coagulase negative staphylococci were isolated, only 96(15.4%) patients had CoNS isolated from multiple blood cultures. The remaining strains were considered possible contaminants and excluded from further analysis. (See Table 2)

Table 2: Clinical significance of coagulase negative staphylococci isolated from blood cultures

Significance	No. of patients	No. of isolates
True / Probable pathogens	96(15.4%)	192(26.7%)
Possible contaminants	527(84.6%)	527(73.3%)
Total	623	719

Isolates from 79(82.3%) patients had the same species isolated from two separate blood cultures (Table-2). For remaining 17 (17.7%) patients, the isolates were not the same species. Largest numbers of isolates were identified to be *S. epidermidis* (64.6%) followed by *S. haemolyticus* and *S. hominis* (See Table 3 & Fig 2).

Table 3: Species distribution of coagulase negative staphylococci isolated from multiple blood cultures

	No. of pairs of blood culture	%
Same species	79	82.3
S. epidermidis	62	64.6
S. haemolyticus	11	11.5
S. hominis	6	6.2
Mixed species	17	17.7
S. epidermidis + *S. haemolyticus*	15	15.6
S. epidermidis + *S. hominis*	2	2.1
Total	96	100.0

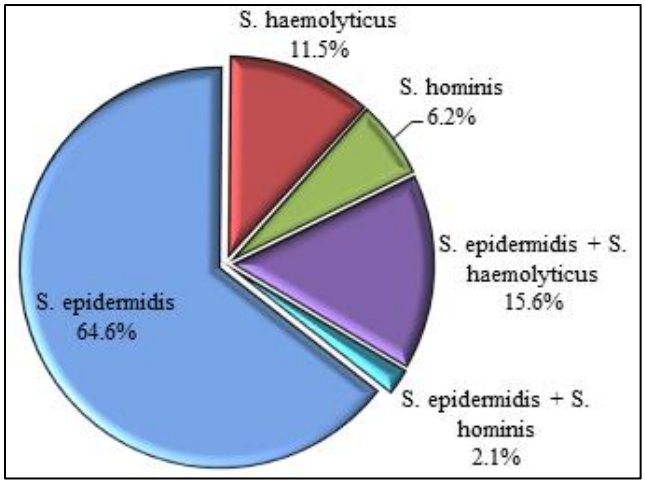

Fig. 2: Species distribution of coagulase negative staphylococci isolated from multiple blood cultures

Coagulase negative staphylococcal septicemia was more common among male (61.5%, n=59) than females (38.5%, n=37). (See Fig. 3)

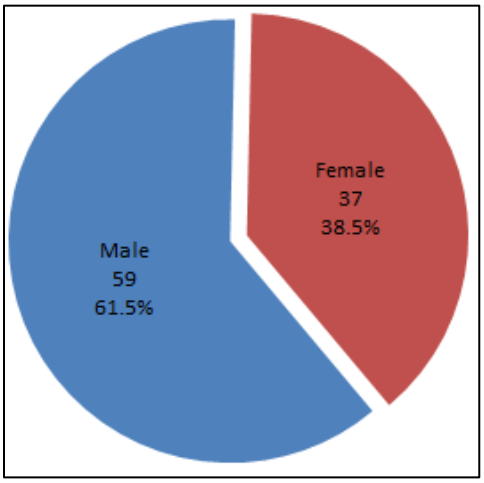

Fig. 3: Gender distribution of patients from which CoNS were isolated

All the patients were neonates (i.e. age < 28 days of life). Prevalence of coagulase negative staphylococcal neonatal sepsis was more in low birth weight babies (55.2%, n=53) when compared to normal babies (44.8%, n=43) (See Fig. 4)

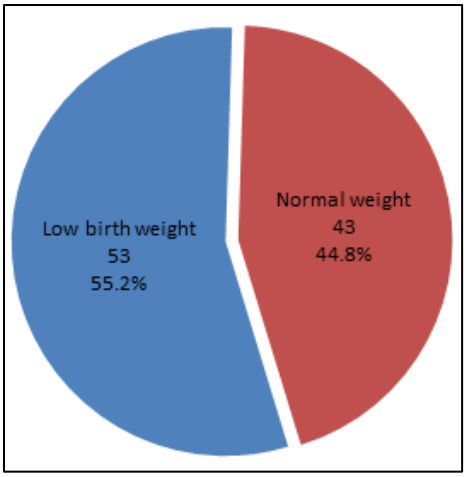

Fig. 4: Distribution of neonates according to birth weight

Antimicrobial susceptibility testing showed varying degree of resistance to commonly used drugs (Table 6). Almost all the isolates were resistant to Penicillin-G. Methicillin resistance was seen among 26.04% of isolates. All the isolates were sensitive to vancomycin, teicoplanin and linezolid. (See Table 4 & Fig. 5)

Table 4: Antimicrobial susceptibility pattern of CoNS isolates

		Susceptible		Resistant	
	Strength	**n**	**%**	**n**	**%**
Penicillin-G	10 IU	18	9.38	174	90.63
Oxacillin	1 µg	142	73.96	50	26.04
Vancomycin	30 µg	192	100.00	0	0.00
Teicoplanin	30 µg	192	100.00	0	0.00
Linezolid	30 µg	192	100.00	0	0.00
Azithromycin	15 µg	98	51.04	94	48.96
Clindamycin	2 µg	130	67.71	62	32.29
Ciprofloxacin	5 µg	158	82.29	34	17.71
Moxifloxacin	5 µg	186	96.88	6	3.13
Cotrimoxazole	25 µg	130	67.71	62	32.29
Chloramphenicol	30 µg	188	97.92	4	2.08
Tetracycline	30 µg	170	88.54	22	11.46
Gentamycin	10 µg	182	94.79	10	5.21

Isolates adopted variety of mechanisms for antibiotic resistance. Highest prevailing mechanism was β-lactamase (penicillinase) production which was found among 64.59% of isolates. However, all the isolates tested sensitive for glycopeptides (i.e. vancomycin & teicoplanin). Inducible MLS_B resistance was seen in 32.29% of isolates. (See Table 5)

Table 5: Antimicrobial resistance mechanism pattern of CoNS isolates

Identified Mechanism	**No. of Isolates**	**% of Isolates**
β-lactamase Production (Penicillinase)	174	64.59
Methicillin Resistance	50	26.04
Vancomycin Resistance	0	0.00
Inducible Clindamycin Resistance	62	32.29

Discussion

During the study period of 2 years, 4889 blood culture samples were received from NICU. Out of these, 1465 samples turned out to be positive. In our study, coagulase negative staphylococci had prevalence of 49.1% which is in concordance with study done by Stoll BJ,[8] USA (47.9%). But studies by Kumhar GD,[9] India (7.9%) and Kumar M,[10] India (19.99%) show very less CoNS isolation rate. The may be because some laboratories report the CoNS as contaminant when isolated in single culture and exclude their count from prevalence data.

In our study, 15.4% of coagulase negative staphylococcal isolates were considered as pathogens while the remaining 84.6% were considered contaminants. This was comparable with studies done at various places viz Weinstein MP,[11] USA (12.4%), Peacock SJ,[12] UK (10%), Ringberg H[13], Sweden (4.1%) and Bodonaik NC[14], West Indies (8.4%).

In our study, largest numbers of isolates belonged to *S. epidermidis* (64.6%), this is in concordance to studies done by Kleeman KT,[15] USA (59%), Marsik FJ,[16] USA (77.9%) and Croft SF,[17] USA (63.8%). Findings of our study differ significantly from study done by Jain A,[18] India (24%) where most common species is *S. haemolyticus* (34%). The possible reason may be because that study included only methicillin resistant CoNS isolates. Another possibility is prevalence of endemic antibiotic resistant strains in NICU.

All isolates showed varying degree of drug resistance to commonly used drugs. Lowest susceptibility was seen with penicillin-G (9.38%). Frequent use of macrolides and fluoroquinolones leads to emergence and spread of resistant strains. Methicillin resistance in our study was only 26.04% which is almost half of other study's findings.[16,18-20] The lower rate of MRCoNS (Methicillin Resistant Coagulase Negative Staphylococci) may be due to low prevalence of CoNS in hospital setting and can also be attributed to improvement in ICN practices. All the isolated strains were sensitive to glycopeptides (i.e. vancomycin and teicoplanin).

Conclusion

The prevalence rate of coagulase negative staphylococci in blood culture of NICU patients was 49.1%. Out of which only 26.7% isolates turned out to be clinically significant. Most common species of coagulase negative staphylococci found was *S. epidermidis* accounting for 64.6% among them, followed by *S. haemolyticus* 11.5% and *S. hominis* 6.2%. In present study, CoNS isolation from female was more than males. Also, low birth weight or premature neonates had higher incidence of neonatal sepsis than normal full term babies.

This study indicates importance of coagulase negative staphylococci isolated from NICU blood cultures and suggests that strategies to reduce such infections in neonates are needed urgently, to reduce their medical, social, and economic toll. Successful interventions should improve survival, shorten mechanical ventilation and hospital stay, decrease antibiotic usage, and reduce the high cost of caring for low birth weight infants.

Prevention and control of methicillin resistant coagulase negative staphylococci require co-ordinated efforts from various departments and can only be achieved by education of hospital staff regarding problem of drug resistance, prudent use of antimicrobials and early detection, reporting and immediate implementation of appropriate infection control measures.

Conflict of Interest: None.

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