Original Article

Detection of Coagulase negative Staphylococcal blood stream infections among Infants and Neonates and their Antibiotic susceptibility pattern in a Tertiary care Hospital

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ABSTRACT

Introduction: There has been an increased emergence of Coagulase negative *Staphylococcus* causing blood stream infections in Neonates and Infants leading to morbidity and mortality. Catheter related blood stream infections are most common in critically ill very low birth weight neonates and infants. Colonization and biofilm formation are the significant risk factors. Another concern is the rising incidence of Methicillin-resistant Coagulase negative *Staphylococcus* (MRCONS) in hospitalized childrens.

Aim & Objectives: This study was conducted to know the Prevalence of Coagulase negative *Staphylococcus* associated septicemia, to speciate them and to know the Antibiotic susceptibility pattern of these isolates.

Materials and Methods: The blood samples were collected from Neonates and Infants. Brain heart infusion (BHI) was used for blood culture. 1-2ml of blood was collected in 10ml of BHI broth. By repeated isolation, clinically significant Coagulase negative *Staphylococcus* was identified. From the positive isolates Gram staining, biochemical tests and Antibiotic susceptibility tests were done.

Results: Out of 362 blood samples, 92 (25%) shows clinically significant CONS. Biotyping shows the commonest species isolated from blood samples were *Staphylococcus epidermidis, Staphylococcus haemolyticus* and *Staphylococcus warneri.* All the isolates were resistance to Penicillin. Many isolates were resistance to Methicillin (61%) and other commonly used drugs. All *MRCONS* isolates were 100% sensitive to Vancomycin.

Conclusion: The study demonstrates higher prevalence of CONS in neonatal and infant bacteremia. *Staphylococcus epidermidis* is the commonest pathogen among Coagulase negative Staphylococcus in our study.

Keywords: Coagulse negative Staphylococcus, Neonates and Infants septicemia, *Staphylococcus epidermidis,* Antibiotic susceptibility tests, Methicillin resistant Coagulase negative Staphylococcus.

INTRODUCTION:

Coagulase negative *Staphylococcus* is a normal skin commensal and it acts as a contaminants in many clinical samples[1]. But from the past years, it is recognized to cause bacteremia mostly in Neonates and Infants.

Coagulase-negative *Staphylococcus* (CONS) is the most common bacteria in neonatal healthcare associated infections[2]. Colonization of CONS is a risk factor among neonatal infections[3]. The neonatal hospital population has several factors that leads to development of blood stream infections; these include the immature immune system of the neonates, the use of invasive procedures and aggressive antibiotic therapy[4,5]. Predominantly, *Staphylococcus epidermidis* is the most common causative agent of neonatal sepsis[5].

It is most commonly isolated from blood culture causing nosocomial bloodstream infections[6].

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Common species of Coagulase negative *Staphylococcus* isolated from blood stream infections are *Staphylococcus epidermidis* and *Staphylococcus haemolyticus.* Other species of Coagulase negative *Staphylococcus* are *Staphylococcus simulans, Staphylococcus hominis, Staphylococcus warnerii* and *Staphylococcus xylosus.* Newly isolated Coagulase negative *Staphylococcus* species causing human infections in clinical samples are *Staphylococcus petrasii, Staphylococcus massiliensis, Staphylococcus jettensis* and *Staphylococcus pettenkoferi*[7,8].

The pathogenesis of CONS infections depends on their ability to form biofilms on polymer surfaces[9]. Health care-associated blood stream infections (HABSI) among infants hospitalized in the neonatal intensive care unit (NICU) are a leading cause of morbidity and mortality. Central venous catheter is mandatory in the critically ill child including very low birth weight (VLBW) infants with short-gut syndrome, children with cancer and those with chronic diseases such as cystic fibrosis and hemophilia[10]. Among the CONS, *Staphylococcus epidermidis* is the most recovered species in biofilm-associated infections.

Common clinical manifestations of both Neonates and Infants includes temperature instability, hypoxemia, apnea, bradycardia, hypotension, irritability, lethargy and feeding intolerance[11,12]. Collection of greater than or equal to two blood cultures during a neonatal sepsis evaluation has proven superior to only one blood culture[13,14]. Hematological indices (e.g., numbers of white blood cells, neutrophils, platelets, etc) and biochemical markers for inflammation, such as C-reactive protein and procalcitonin are routinely used in clinical practice and can aid in the diagnosis of neonatal sepsis[15,16].

Treatment of CONS infections is more difficult, due to certain unique characteristics of these organisms, most notably their ability to express resistance to multiple antibiotics. Resistance to Methicillin is almost universal among isolates recovered from hospitalized individuals. Vancomycin is usually the antibiotic of choice in the treatment of multi drug resistant CONS infections. However, it has been reported that the Glycopeptide susceptibilities of clinically significant CONS are decreasing[17,18]. Newer drugs like Linezolid, Daptomycin, Quinupristin / Dalfopristin can be given to the resistant individuals.

The present study was undertaken with the aim of studying the prevalence of Coagulase negative Staphylococcus in blood stream infections and their antibiotic susceptibility pattern in Neonates and Infants.

AIM AND OBJECTIVES:

1. To isolate and detect the prevalence of Coagulase-negative *Staphylococcus* in blood stream infections among neonates and infants
2. To speciate Clinically significant Coagulase negative *Staphylococcus*
3. To know their Antibiotic susceptibility pattern.

MATERIALS AND METHODS:

The blood samples were collected from Neonates and Infants with clinical suspicion of septicemia during the period of February 2018 to January 2019.

BLOOD COLLECTION AND CULTURE:

The clinical significance of Coagulase negative *Staphylococcus* was established by repeat isolation. The isolation of the same Coagulase negative Staphylococcus species from two blood

culture samples are considered as significant. Brain Heart Infusion broth (BHI broth) was used for blood culture. Two samples were collected from each patient. In catheterized patients, sample was collected from catheter site and peripheral site. In non-catheterized patients, two samples were collected from different sites. When Catheter related blood stream infections (CRBSI) was suspected, the catheter hub was cleaned with alcohol and allowed to dry to minimize blood culture contamination; 1-2 ml of blood was drawn from lumen of Central venous catheter (CVC)[19]. For peripheral blood, 1 ml of blood collected under sterile aseptic precautions by venipuncture and inoculated in 10ml of BHI broth (1:10 dilution). Blood collected was immediately transported to the laboratory and incubated at 37°C. The broth was observed daily for macroscopic evidence of growth (Turbidity or Hemolysis).

Once turbidity was observed after incubation at 37°C, subculture was done on MacConkey agar and Blood agar plates for further identification and the plates were incubated at 37°C for 24 hours. After incubation, the plates were examined for colony characteristics such as size, shape, consistency and hemolytic properties and subjected to Gram staining and Biochemical tests.

GRAM STAINING AND BIOCHEMICAL TESTS:

All the isolates which were Gram positive cocci in clusters were subjected to further study. To differentiate CONS from *Staphylococcus aureus,* slide and tube coagulase test were done. Once the isolates were confirmed as Coagulase negative *Staphylococcus,* they were subjected to biochemical tests to identify the species. The tests includes: Urease test, Acetoin production, Ornithine decarboxylase, Nitrate test and Mannitol fermentation test.

ANTIBIOTIC SUSCEPTIBILITY TESTING:

The isolates were subjected to routine Antibiotic susceptibility testing by modified Kirby Bauer's disc diffusion method on Muller Hinton agar plates as per CLSI guidelines using following antibiotics: Penicillin G, Cephalexin, Cefoxitin, Amoxy-clav, Ciprofloxacin, Clindamycin, Erythromycin, Gentamicin, Cotrimaxazole and Vancomycin by E-strip method.

METHICILLIN SUSCEPTIBILITY:

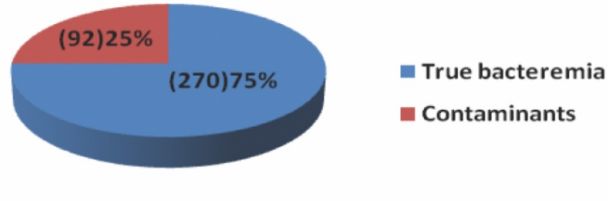
In our study, the Methicillin susceptibility was detected by using Cefoxitin 30µg disk by disc diffusion method on the Muller Hinton agar plate (MHA). The zone of inhibition and zone of resistant was clearly visible and it was read according to CLSI guidelines. Methicillin susceptible CONS showed $\geqslant$ 22mm diameter of zone. If the isolates were resistant to Methicillin, the zone diameter is <21mm.

MIC FOR VANCOMYCIN BY E-STRIP:

All the MRCONS were subjected to Vancomycin MIC by E-strip. The E strip is coated with Vancomycin and has a range of 0.016µg/ml to 256µg/ml. The standard E-test procedure was performed with CONS isolates by using Muller-Hinton agar plate. After incubation, a inhibition ellipse is clearly visible and was read under the CLSI guidelines.

RESULTS:

Figure 1: Prevalence of CONS in blood culture



Out of 362 blood samples from Neonates and Infants, 92 (25%) samples were significantly positive (true bacteremia) for Coagulase negative Staphylococcus. And 270 (75%) samples showed contaminants.

Table 1: Species isolated from CONS

S. No	Species of CONS	No. of isolates	Percentage
1	*Staphylococcus epidermidis*	46	50%
2	*Staphylococcus haemolyticus*	38	41%
3	*Staphylococcus warneri*	8	9%

From 92 isolates, the most commonly isolated species were *Staphylococcus epidermidis 46 (50%), Staphylococcus haemolyticus 38 (41%)* and *Staphylococcus warneri 8 (9%).*

Table 2: Antibiotic susceptibility Pattern

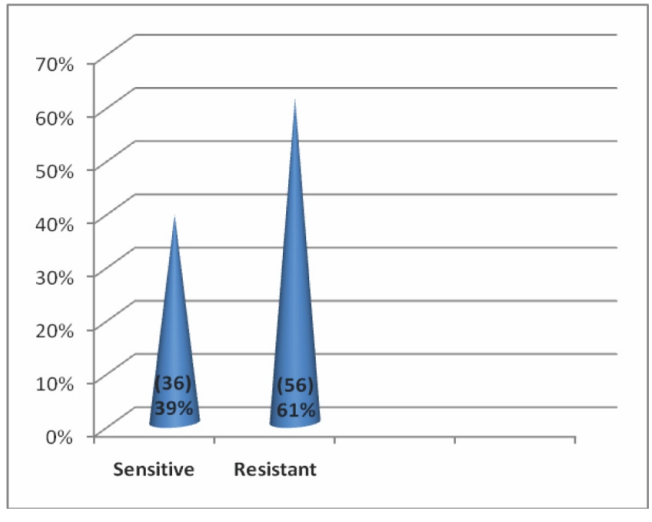
S. No	Antibiotics		*Staphylococcus epidermidis (46)*	*Staphylococcus haemolyticus (38)*	*Staphylococcus warneri (8)*
1	Clindamycin	S	(35) 76%	(32) 84%	(7) 88%
		R	(11) 24%	(6) 16%	(1) 12%
2	Cotrimaxazole	S	(31) 67%	(30) 79%	(7) 88%
		R	(15) 33%	(8) 21%	(1) 12%
3	Erythromycin	S	(31) 67%	(29) 76%	(6) 75%
		R	(15) 33%	(9) 24%	(2) 25%
4	Gentamicin	S	(29) 63%	(27) 71%	(5) 63%
		R	(17) 37%	(11) 29%	(3) 37%
5	Ciprofloxacin	S	(29) 63%	(25) 66%	(5) 63%
		R	(17) 37%	(13) 34%	(3) 37%
6	Amoxyclav	S	(20) 43%	(21) 55%	(4) 50%
		R	(26) 57%	(17) 45%	(4) 50%
7	Cefoxitin	S	(18) 39%	(15) 39%	(3) 38%
		R	(28) 61%	(23) 61%	(5) 62%
8	Cephalexin	S	(13) 28%	(10) 26%	(3) 38%
		R	(33) 72%	(28) 74%	(5) 62%
9	Penicillin	S	(0) 0%	(0) 0%	(0) 0%
		R	(46) 100%	(38) 100%	(8) 100%

In the Antibiotic Susceptibility pattern, according to CLSI guidelines the isolates were highly sensitive to Clindamycin 86 (93%), Cotrimaxazole 78 (85%), Erythromycin 71 (77%), Gentamicin 71 (77%), Ciprofloxacin 68 (74%), Amoxyclav 60 (65%), Cefoxitin 36 (39%), Cephalexin 30 (33%). And all the isolates were highly resistant to Penicillin 92 (100%)

METHICILLIN SUSCEPTIBILITY OF CONS:

Out of 92 isolates of CONS, 36 (39%) were sensitive to Methicillin and 56 (61%) were resistant to Methicillin.

Figure 2: Methicillin susceptibility



MIC FOR VANCOMYCIN:

56 MRCONS isolates were subjected to MIC for Vancomycin by using E-strip. All the isolates of MRCONS showed 100% sensitive to Vancomycin in our study.

Table 3: MIC for Vancomycin by E-strip

Isolates	<1.5µg/ml	>1.5µg/ml
MRCONS	56	-

DISCUSSION:

In our study, we have isolated Coagulase negative Staphylococcus from Neonates and Infants, the organism is more prevalent in causing blood stream

infections. In our study, 25% of samples were significantly positive for Coagulase negative Staphylococcus among Neonates and Infants. L.F. Nimri et al declared that the majority of CONS species were isolated from children less than 18 months[20]. Adrie Bekker et al showed that the blood stream infections (BSI) prevalence rate reported from NICU is 11.5%[21].

Study by V.S. Vatkar showed 25.5% of BSI in Pediatric patients[22], which is similar to our study. Indian studies of Bhattacharjee.A reported 32% of BSI in Neonates and Infants were by CONS[23]. Indian studies of Bhat reported 47% of CONS causing BSI among Neonates[24].

Our study shows the commonest CONS species isolated from blood culture were *Staphylococcus epidermidis (50%), Staphylococcus haemolyticus (41%)* and *Staphylococcus warneri (9%).* Yves Mauro Ternes et al showed in their study, *S.epidermidis* (38.3%) and *S.haemolyticus* (38.0%) were the most frequent species colonizing the neonates in blood stream infections[25]. Similar to our study, Monika Brzychczy-Wloch et al in 2013 showed that the most frequently isolated species were *Staphylococcus epidermidis* (50% - 76%) and then *Staphylococcus haemolyticus* (14% - 32%) from the very low birth weight neonates[26]. Jeannie P. Cimiotti et al showed that most of the CONS-associated neonatal infections were caused by *S.epidermidis* (68%), followed by *S.warneri* (8%)[27].

In our study, the isolates were highly sensitive to Clindamycin (93%), Cotrimaxazole (85%), Erythromycin (77%), Gentamicin (77%), Ciprofloxacin (74%), Amoxyclav (65%), Cephalexin (33%). And all the isolates were highly resistant to Penicillin (100%). Indian studies of Narayan Prasad in 2017 showed that, isolates from Pediatric patients were highly resistant to Ampicillin (80%), Erythromycin (51.1%), and Clindamycin (51.1%) and less susceptible to Cephalexin (37.7%), Cefoxitin (37.7%), and Amoxyclav (37.7%), respectively[28]. Yves Mauro Ternes et al showed that rate of Methicillin resistance up to 80% have been observed among CONS, isolated from bloodstream infections in NICU patients[25]. Yue Qu et al showed that most CONS were resistant to Penicillin G (100%), Gentamicin (83%), and Oxacillin (92%) and remained susceptible to Vancomycin (100%)[29].

Our study showed the Methicillin resistant isolates were 61%. Jeannie P. Cimiotti et al showed that most NICU isolates were resistant to Methicillin (84%)[27]. Alissa Craft et al showed that majority of the infants with CONS BSI were treated with Vancomycin and many hospital acquired CONS were Methicillin resistant[30].

CONCLUSION:

Coagulase negative *Staphylococcus*, which is a normal commensal, has become a major nosocomial pathogen, having an impact on human life and health. They are particularly associated with the use of indwelling or implanted foreign devices, which are indispensable in modern medicine and these are more commonly seen in Paediatric age groups. And also the organism is frequently isolated from blood cultures in all the age groups.

In 20th century, CONS emerged as the foremost pathogen of neonatal sepsis in developed countries. VLBW neonates contribute disproportionately to CONS-related morbidity and mortality, in contrast to their full-term counterparts, who usually suffer milder symptoms. Advances in medical technology have dramatically increased the survival rate of premature neonates. This corresponds to a growing burden of both short and long-term problems

associated with neonatal sepsis. Effective prophylactic measures, prompt and accurate diagnosis and subsequent administration of targeted therapy are vital to curb the excessive burden of disease[31].

The study concludes the Prevalence of Coagulase negative *Staphylococcus* in bloodstream infection is moderately increased in Pediatric age groups, commonly affecting Neonates and Infants. The commonest species isolated from these patients were *Staphylococcus epidermidis, Staphylococcus haemolyticus* and *Staphylococcus warneri.* Methicillin-resistant Coagulase negative *Staphylococcus* isolated from blood cultures of patients with true bacteremia shows that, there is a high level of resistance to commonly used agents.

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