



Speciation, Biofilm production and Antibiotic resistance pattern of Coagulase negative Staphylococci isolated from Neonatal septicemia

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Abstract

Nowadays Coagulase-Negative Staphylococci (CoNS) has become one of the major pathogen for neonatal septicemia as a result of increased use of intravascular devices and other invasive procedures. This prospective study was conducted to study about species distribution, biofilm production and antibiotic resistance pattern of Coagulase-Negative Staphylococci isolated from Neonatal Septicemia. During the ten months study period 68 CoNS were isolated from neonatal septicemia. Speciation of CoNS was done by simple, inexpensive biochemical tests and biofilm production was detected by Tube Method and Congo red agar method. On speciation of CoNS *Staphylococcus epidermidis* (53%) was the common isolate followed by *Staphylococcus haemolyticus* (41%), *Staphylococcus warneri* (3%) and *Staphylococcus schleiferi* (3%) CoNS isolates showed significant resistance to Ampicillin (82%), Clindamycin(76%), Cotrimoxazole (74%) Erythromycin (74%), and Methicillin (76%). CoNS isolates revealed 100% sensitivity to Vancomycin. CoNS isolates (53%) were biofilm producers by Congo Red Agar method and (47%) were biofilm producers by Tube adherence method. Due to their high antibiotic resistance and increased virulence of CoNS leads to increased morbidity and mortality in neonates. Preventive measures like hand hygiene, aseptic management of intravenous line should be followed to reduce the spread of virulent CoNS.

Keywords: CoNS, Speciation, Biofilm production.

Introduction

Neonatal septicemia remains one of the important causes of mortality and morbidity in neonates even after the introduction of new broad spectrum antimicrobial agents, advanced measures for diagnosis and treatment.⁽¹⁾ Neonatal septicemia is classified into early onset septicemia (EOS) and late onset septicemia (LOS) based on the age at onset. Neonatal sepsis is caused by a variety of

Gram-positive as well as Gram-negative bacteria, and sometimes yeasts.⁽²⁾ In the last two decades, the isolation of Gram-positive organisms has increased significantly. CoNS are the major cause of sepsis in neonatal intensive care units (NICU) worldwide⁽³⁾. With the advancement of medical sciences, especially with the increasing use of medical devices, the infections caused by CoNS are rapidly increased.⁽⁴⁾

S. epidermidis is the most frequently isolated species of CoNS in bacteremia cases, accounting for 50% to 80% of CoNS isolates. Other commonly recovered species include *S. haemolyticus*, *S. saprophyticus*, *S. lugdunensis*, *S. warneri*, *S. cohnii*, *S. schleiferi* and *S. hominis*^(5,6). CoNS are often resistant to multiple antibiotics. Methicillin resistance among CoNS is particularly important due to resistance to all beta-lactam agents and cross resistance to other anti-microbial classes.^(5,6,7) Main virulence determinant for CoNS is biofilm formation. Biofilm consists of multilayered cell clusters embedded in a matrix of extracellular polysaccharide which facilitates the adherence of these microorganisms to biomedical surfaces and protect them from host immune system and antimicrobial therapy^(8,9)

As neonatal septicemia is a life-threatening emergency and delay in diagnosis and treatment with the appropriate antibiotics may have grave consequences. The characterization of CoNS up to species level, their antimicrobial properties and biofilm production is very important for the treatment of neonatal sepsis.^(10,11)

Species	Tube coagulase test	Ornithine decarboxylase test	Urease test	Mannose fermentation test	Novobiocin sensitivity 5 µg
<i>S. epidermidis</i>	–	+	+	+	S
<i>S. saprophyticus</i>	–	–	+	–	R
<i>S. haemolyticus</i>	–	–	–	–	S
<i>S. lugdunensis</i>	–	+	+	+	S
<i>S. warneri</i>	–	–	+	–	S
<i>S. cohnii</i>	–	–	+	–	R
<i>S. schleiferi</i>	–	–	–	+	S

Susceptibility to antibiotics (concentration in µg) Ampicillin (10 µg), Linezolid (30µg), Cotrimoxazole (25 µg) Erythromycin(15µg) Clindamycin (10µg), Cefoxitin (30µg) (Hi Media) were performed on Mueller–Hinton agar plates by Kirby–Bauer disc diffusion method. Zone diameter was measured and interpreted as per the Clinical and Laboratory Standards Institute (CLSI) guidelines.

Phenotypic characterization of biofilm formation by Congo Red Agar Method and Tube Method.^(14, 15,16)

Materials and methods

This prospective study was conducted over the period of 9 months in a Tertiary care hospital. During the study period 68 coagulase negative Staphylococci that fit with the laboratory-confirmed bloodstream infection criteria defined by the Center for Disease Control and Prevention were isolated from Neonatal septicemia.

Identification and speciation of isolates

The isolates were initially identified by colony morphology, Gram staining, catalase, slide and tube coagulase test and acid formation from mannitol. Speciation of CoNS was done by urease test, mannose fermentation test, Novobiocin sensitivity test, ornithine decarboxylase test. These simple, inexpensive and easy to perform tests were selected from the scheme of Kloos and Schleifer to identify CoNS species.^(12, 13)

Tube method

A loopful of bacterial suspension from overnight culture was inoculated into the 10 ml of Trypticase Soy Broth with 1% glucose and incubated for 24 h at 37 °C. The culture tubes were decanted. Then the tubes were washed with phosphate buffer solution (pH 7.3). After drying, the tubes were stained with 0.1% crystal violet. After incubation for 10 min, excess stain was removed and tubes were washed away with water multiple times. Tubes were then kept in inverted position and finally observed for biofilm

formation. Biofilm formation was considered positive when a visible film lined the wall and bottom of the tube.

Congo red agar method

This test is based on the property of Congo red to stain polysaccharides black. The composition of the medium are BHI (37 gms/L), sucrose (50 gms/L), agar no.1 (10 gms/L) and congo red stain (0.8 gms/L). CoNS isolates were plated on congo red agar plate and were incubated at 37 °C for 24 h.. The biofilm forming strains produced black colonies while non-forming strains developed red colonies.

Results

During the study period 68CoNS were isolated from neonatal septicemia and they fitted with the laboratory-confirmed bloodstream infection criteria defined by the Center for Disease Control and Prevention (CDC).

In neonatal septicemia *Escherichia coli* (22%)was the most common organism followed by *Klebsiella pneumoniae* (19%), Coagulase negative *Staphylococcus aureus* (16%), *Pseudomonas aeruginosa* (16%) *Acinetobacterspp* (9%), *Enterobacterspp* (6%), *Citrobacterspp* (3%) *Staphylococcus aureus* (3%) and enterococci sp (3%).CoNS was the third most common organism isolated in this study.

On speciation of CoNS *Staphylococcus epidermidis* (53%) was the common isolate followed by *Staphylococcus haemolyticus* (41%), *Staphylococcus warneri* (3%) and *Staphylococcus chleiferi* (3%) CoNS isolates showed significant resistance to Ampicillin (82%), Clindamycin (76%), Cotrimoxazole (74%) and Erythromycin(74%).CoNS isolates revealed 100% sensitivity to Vancomycin. 52 (76%) CoNS isolates showed Methicillin resistance. Out of 68CoNS isolates 36 (53%) were biofilm producers by Congo Red Agar method and 32(47%) were biofilm producers by Tube adherence method.

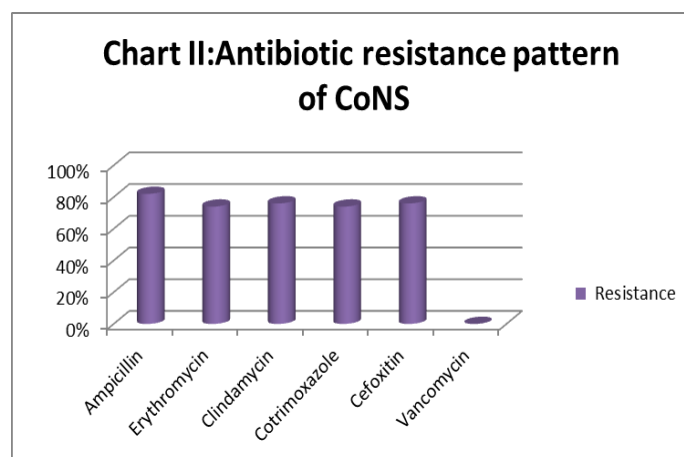
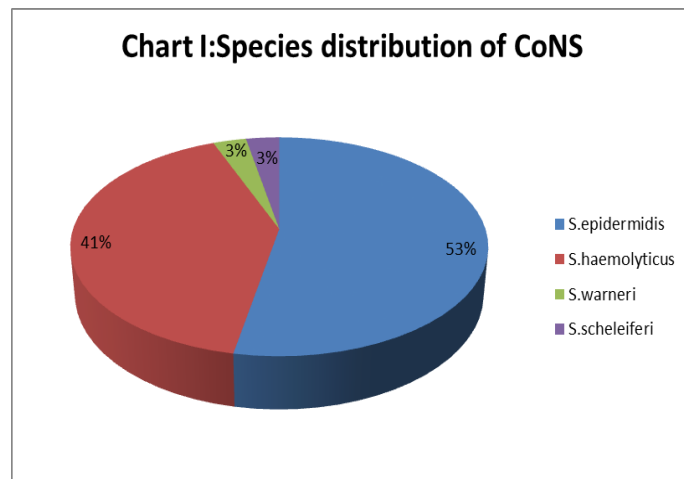


Fig I. Congo Red Agar Method



Black colored colonies positive for biofilm production

Discussion

CoNS are normal microbial flora of skin and mucus membrane of human beings. CoNS are considered one of the most common causes of the device related infections in the recent two decades, especially in extreme age group of patients such as neonates and old age.

In the present study CoNS were the third most common isolates in neonatal sepsis and 68 CoNS were isolated from neonatal septicemia.. This distribution pattern correlates well with the results published by Shahsanam Gheibi et al 2008 who reported that CoNS was the commonest cause of both early (48.8%) and late onset (69.8%) neonatal sepsis followed by Klebsiella, and E coli.⁽¹⁷⁾ Marchant et al and Vijaya Shivanna et al (66.6%), also identified CoNS as the commonest causative organism for neonatal sepsis.

The increasing prevalence of CoNS infections is attributable to their increasing antibiotic resistance and their ability to form biofilms on foreign bodies such as intravascular catheters.⁽¹⁸⁾

In the present study Staphylococcus epidermidis (53%) was the common isolate followed by Staphylococcus haemolyticus (41%). The studies conducted in clinically significant CoNS isolates from various samples by Vijayashri et al, Surekha et al, Saroj Golia et al and Manjushree et al reported S.epidermidis as the most frequent isolate (40%), (44.8%) (46.3%) and (58%) followed by (S.haemolyticus, (26%) (19.7%) (20.1%), and (15%) and S.saprophyticus, (15%), (27.1%) (28.4%), and (21%) respectively. In the present study S. saprophyticus was not obtained because we studied CoNS isolated only from neonatal sepsis, as S.saprophyticus is frequently isolated from urinary tract infections.

In our study, antibiotic susceptibility testing showed maximum resistance to ampicillin (82%), and all CoNS isolate were sensitive to vancomycin. Similarly Surekha et al and Vijayashri et al reported ampicillin resistance (88.5%) (79%), and no resistance to vancomycin. Manjushree et al also reported maximum resistance to Penicillin (98.08%; and all the

isolates were susceptible to Vancomycin. But Saroj Golia identified ampicillin resistance (88%) and vancomycin resistance (1.5%). In the present study Methicillin resistance was observed in 76%. This was concordant with Vijayashri et al who reported Methicillin resistance in 72% of CoNS and Surekha et al reported 64.6% of Methicillin resistance. But lower Methicillin resistance 19.2 % was published by Manjushree et al.

The ability of biofilm formation seems to play an essential role in the virulence of coagulase-negative staphylococci (CoNS).⁽²¹⁾ The most clearly characterized component of staphylococcal biofilms is the polysaccharide intercellular adhesin (PIA) encoded by the icaADBC operon. In the present study 53% were Biofilm producers by CRA method and 47% by TM method. Similar findings were observed by Vijayashri et al who reported (69%) as biofilm producers by CRA method and 33% by TM method. Riyaz Sheriff et al also reported biofilm production in 75% of CoNS isolates. Riyaz Sheriff et al and T Mathur et al identified that Tissue culture plate method was very sensitive and very specific method to detect the biofilm production. But in the present study biofilm production was detected by CRA method and TM method only. The high prevalence of MR-CoNS and biofilm producing strains demonstrated in this study indicates to follow the preventive measures to reduce the spread of virulent CoNS.

Conclusion

Increase in the use of medical devices, such as intravascular catheters and prosthetic devices, in neonates, will increase the prevalence of CoNS in neonatal septicemia.. The antibiotic resistance and biofilm production of CoNS produce negative impact on clinical outcome of neonatal sepsis. So it is necessary to study about the speciation, antibiotic resistance pattern and virulence determinants of CoNS isolated in neonatal septicemia.

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