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Original Research Article

Speciation Of Cons Isolated From Blood Culture And Their Correlation With Common Pathogenicity Attributes

[1]Dr. Deepa Hanamaraddi, [2]Dr. Manjunath Hosamani, [3]Dr. Sughosh Kulkarni, [4]Dr. Ashwita Alva, [5]Deepti .S. Patil, [6]Dr. RD Kulkarni

[1]Associate Professor, Department of Microbiology, JGM Medical College, Kotgondhunshi, Hubballi, Karnataka, India
[2]Associate Professor, Department of Microbiology, SDM College of Medical Sciences and Hospital, Sattur, Dharwad, Karnataka, India
[3]Assistant Professor, Department of Microbiology, SDM College of Medical Sciences and Hospital, Sattur, Dharwad, Karnataka, India
[4]Junior Resident, Department of Ophthalmology, SDM College of Medical Sciences and Hospital, Sattur, Dharwad, Karnataka, India
[5]Assistant Professor, Department of Microbiology, JGM Medical College, Kotgondhunshi, Hubballi, Karnataka, India
[6]Professor, Department of Microbiology, SDM College of Medical Sciences and Hospital Sattur, Dharwad, Karnataka, India

Corresponding Author:
Dr. Deepti .S. Patil

Abstract

Purpose: Bacteremia is considered as a major cause of hospital acquired infections. The longer hospital stays and instrumentation on patients has led to increase in incidence of bacteremia. The common source being the normal commensal of the skin, anterior nares and the ear canals of the patients. The common commensal being Coagulase negative staphylococci have not been considered as pathogen for true bacteremia, but because use of intravascular devices and prolonged hospital stay has led these bacteria as a major causative agent. The aim of this study is to know the CoNS species distribution, its biofilm activity and to understand the various pathogenicity attributes causing bacteremia.
Material and Methods: A minimum of 50 CoNS species were collected from positive blood culture samples and identified along with its antibiogram assay. Production of slime was demonstrated by Tissue culture plate method. Other pathogenicity factors were analysed to clinically correlate the true bacteraemia.
Results: Out of 47 isolates, 68.08% were Methicillin sensitive CoNS and 32% were Methicillin resistant CoNS. 53.1% demonstrated slime production and 46.8% were negative for slime production.
Conclusion: CoNS are the major cause for nosocomial infections causing true bacteremia. The identification of CoNS species along with its anti-biogram pattern and its ability to produce biofilm, helps in the better management of cases and to prevent the infections.

Keywords: Slime, bacteremia, blood culture, CoNS

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Introduction

Coagulase negative staphylococci (CoNS) have been considered as non-pathogenic in causing serious infections. The CoNS being the normal commensal in ear canal, anterior nares and skin in humans never been the true cause of bacteremia. However, as a result of long hospital stay, use of instruments on patient and immunocompromised status of patient, the CoNS is the leading cause of the hospital acquired blood stream infection [1].

The CoNS being the common skin commensal, this bacterium is most commonly isolated from blood culture. Because of lack of diagnostic reference criteria for blood stream infection (BSI), isolation of CoNS from blood culture will not help in clinical correlation of true bacteremia case [2].

The definition of primary BSI is used according to the CDC guideline [2] and it requires presence of infection with antibiotic therapy, instrumentation on patient and at least two times positive blood culture. The presence of persistent temperature of ~38°C or body temperature below 36 °C, hypotension (BP<90mmHg), disseminated intravascular coagulopathy and leucocytosis or neutropenia with a left shift differential count are the minimum criteria for a true case of bacteremia.

The long-term use of catheterization, immunocompromised status of patient with IV lines and the ability of biofilm production by the bacteria will increase the risk of infection by commensal flora [3, 4].

S. epidermidis accounts for 50% to over 80%, the causative agent of bacteremia, as it is distributed all over the body surface and it is the most common species among coagulase negative staphylococci *S. haemolyticus*, *S. lugdunensis*, *S. schleiferi*, *S. warneri*, *S. hominis*, *S. simulans, and S. saccharolyticus* are the other commonly isolated species [5].

The main aim of this study is to know the different species of CoNS, its ability to produce biofilm and to understand the various pathogenicity factors causing blood stream infections.

Materials and Methods

A minimum of 50 Coagulase negative Staphylococcus species were collected from positive blood culture samples. Blood samples were inoculated and incubated using automated blood culture system (BacT/Alert). The positive blood culture bottles were then sub-cultured on 5% sheep blood agar and incubated at 35 ºC for 24 hours. The isolated colonies were subjected for different biochemical tests to identify the coagulase negative staphylococci [6].

By using the Kloos and Schleifer and Koneman identification scheme different species of CoNS were isolated [7, 8]. The different biochemical tests used were ornithine decarboxylase test, nitrate reduction test, Voges-Proskauer test, urease test and fermentation of sucrose, lactose, maltose, mannose, mannitol, xylose and trehalose sugars. Susceptibility to novobiocin and polymyxin B were performed for the identification [8, 9].

The antibiotic sensitivity testing was done by using Vitek2 automated system. The antibiotic discs discussed in this study were Penicillin (P), Cloxacillin (Cx), Erythromycin (E), Clindamycin (Cd), Tetracycline (Te), Gentamycin (G), Ciprofloxacin (Cip), Co-trimoxazole (Cot), Vancomycin (Va), Linezolid (Lz), Amikacin (Ak), Imipenem (Ipm), Cefipime (Cpm), Amoxycillin-Clavulanic acid (AMC), Piperacillin-Tazobactum (Pit), Cefotaxime (Ctx) and Cefixime (CXM). Production of slime activity was demonstrated by Tissue culture plate method [10].

Speciated CoNS were inoculated in Trypticase soy broth (TSB) with 0.5% sucrose and incubated for 18 to 24 hours. After incubation it was inoculated in 1:100 diluted fresh TSB. 0.2-ml aliquots of the diluted culture were filled in individual wells of sterile, polystyrene, 96-well, flat-bottomed tissue culture plates. The tissue culture plates were then incubated for 24 hours at 37 °C. The contents of each well were gently aspirated by tipping the plate and

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placing the aspirator tip in the lowest corner of the well. The wells were washed four times with 0.2 ml of phosphate-buffered saline (pH 7.2). Adherent organisms were fixed in place with 2% sodium acetate for 15 minutes and stained with 0.1% crystal violet for 30 seconds. Excess stain was removed by washing with de-iodinised water for four times. Plates were inverted and gently tapped to remove excess water, then decolourised with ethanol acetone (80:20, v/v). After drying, the ODs of stained adherent bacterial films were read with a Micro ELISA Reader [10]. Other pathogenicity factors such as presence of fever, hypotension, leucocytosis, neutropenia, raised C-reactive protein and disseminated intravascular coagulation was noted down. In addition, major risk factors for potential infection by skin flora is required, which includes long term intravascular catheterization (mainly used in critical care units) and immunocompromised patients with central lines, to clinically correlate the true bacteraemia. Ethical clearance was obtained from Institutional Ethics Committee.

Results

In this study, a total of 50 Coagulase negative Staphylococci were collected from blood culture positive samples during the study period from April 2016 to June 2016. Out of 50 CoNS isolates, 47 isolates were subjected for speciation, antibiogram and demonstration of slime activity by tissue culture plate method.
The species distribution of 47 CoNS isolates was shown in the Table no 1. *Staphylococcus caseolyticus* was the predominant species (28%) followed by *Staphylococcus epidermidis* (21%).

Table 1: CoNS species

CoNS species	Number	Percentage
Staphylococcus caseolyticus	13	28%
Staphylococcus epidermidis	10	21%
Staphylococcus haemolyticus	5	11%
Staphylococcus warneri	5	11%
Staphylococcus caprae	3	6%
Staphylococcus capitis sub sp *capitis*	3	6%
Staphylococcus saprophyticus	2	4%
Staphylococcus auricularis	1	2%
Staphylococcus hominis	1	2%
Staphylococcus cohnii sub sp *cohnii*	1	2%
Staphylococcus sciuri sub sp *sciuri*	1	2%
Staphylococcus capitis sub sp *urealyticus*	1	2%
Staphylococcus hominis sub sp *novobiosepticus*	1	2%

Out of 47 isolates, 32 (68.08%) were Methicillin sensitive CoNS and 15 (32%) were Methicillin resistant CoNS as shown in the Figure no 1.

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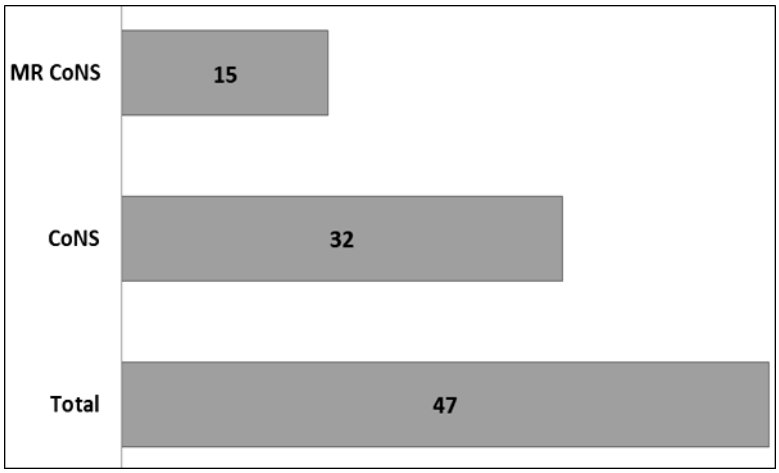

Fig 1: Shows CoNS and Methicillin Resistant CoNS distribution (n=47)

Of the 47 CoNS isolates 53.1% (n=25) demonstrated the production of slime activity and 46.8% (n=22) were negative for slime production. The detailed species wise slime activity was shown in Table no 2.

Table 2: Species distribution based on slime activity

CoNS species (n=47)	**Biofilm Positive (n=25)**	**Biofilm Negative (n=22)**
Staphylococcus caseolyticus (n=13)	13	0
Staphylococcus epidermidis (n=10)	5	5
Staphylococcus haemolyticus (n=5)	1	4
Staphylococcus warneri (n=5)	1	4
Staphylococcus caprae (n=3)	1	2
Staphylococcus capitis sub sp *capitis* (n=3)	1	2
Staphylococcus saprophyticus (n=2)	0	2
Staphylococcus auricularis (n=1)	1	1
Staphylococcus hominis (n=1)	0	1
Staphylococcus cohnii sub sp *cohnii* (n=1)	0	1
Staphylococcus sciuri sub sp *sciuri* (n=1)	0	0
Staphylococcus capitis sub sp *urealyticus* (n=1)	1	0
Staphylococcus hominis sub sp *novobiosepticus* (n=1)	1	0

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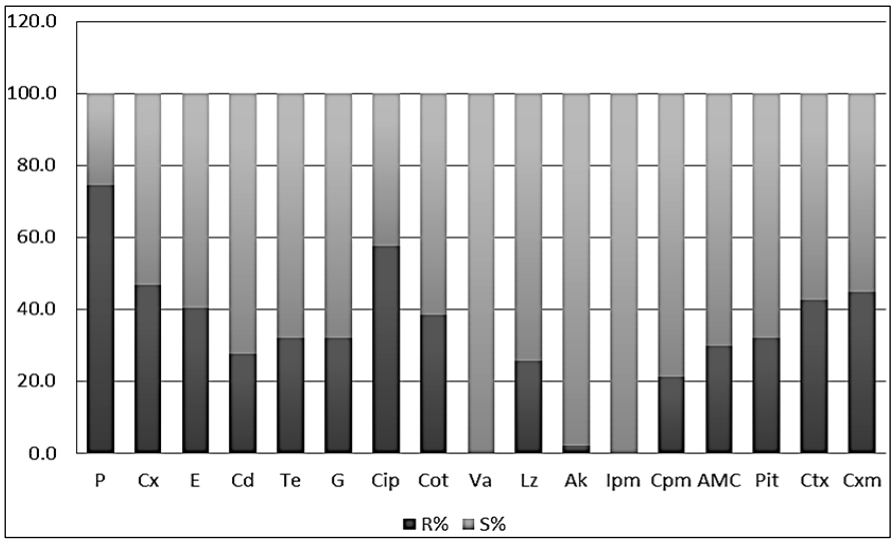

Fig 2: Antibiogram profile of CoNS isolates (n=47)

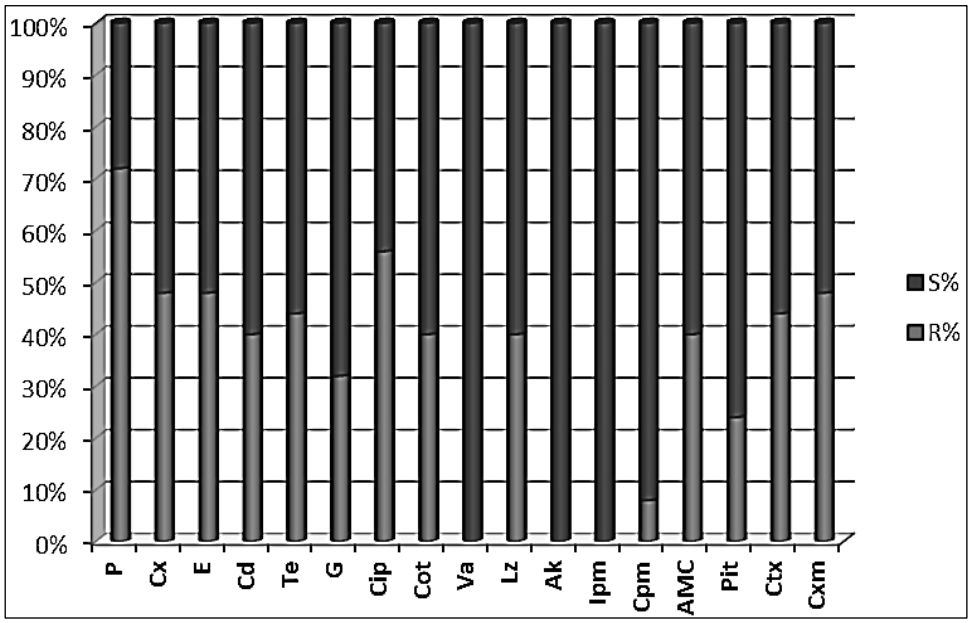

Fig 3: Antibiogram profile of Biofilm positive CoNS isolates (n=25)

Table 3: Biofilm Positive CoNS and MR-CoNS

Total Biofilm positive	**25**
CoNS	16
MR-CoNS	9

The antibiotic sensitivity profile of all the CoNS isolates was shown in Figure no 2. The vancomycin and linezolid have shown 100 percent sensitivity pattern, followed by amikacin with 97.9% sensitivity pattern.

The biofilm producing CoNS antibiogram picture was shown in Figure no 3. The drugs amikacin, vancomycim and imipenem showed 100 percent sensitivity profile. Biofilm positivity of CoNS and MR-CoNS was shown in Table no 3.

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Discussion

Blood culture is the commonly done investigation for suspected cases of fever in hospitalised patients. The isolation of true pathogen from a blood culture needs utmost attention for the proper treatment with appropriate antibiotic.

If the isolated pathogen turns to be a doubtful one such as CoNS, which is a known commensal in humans, then it needs to be evaluated carefully and needs extra investigation to rule out it as a causative agent of bacteremia. Also, it helps the clinician in proper patient management. It becomes important for a clinical microbiologist to correlate the CoNS as a true pathogen. The repeated isolation of the same pathogen and understanding the other pathological parameters are considered commonly in determining the clinical correlation of the isolate to the bacteremia. However, there is a variation in diversity and strain, speciation of CoNS is not commonly done in the laboratory [11].

The most commonly isolated pathogen in blood cultures is coagulase negative staphylococci (CoNS) and it plays an important role in hospital acquired blood stream infections (BSI) [1, 2]. The CoNS are the most common contaminants of blood culture, as it is the part of the rich commensal flora [3]. It becomes very important to differentiate between the contamination and blood stream infection [4, 5], to give proper treatment and prevent the occurrence of antimicrobial resistance [2].

The most repeated cause of nosocomial blood stream infections is CoNS, 27% to 32% seen among adults and 50% of infections in paediatrics, respectively [11].

In this study we collected 50 isolates of CoNS, 47 were subjected for speciation, antibiogram, biofilm activity demonstrated by tissue culture plate method and we tried to correlate with other pathogenicity attributes such as total count, C-reactive protein level, critical care stay and instrumentation. In this study, *Staphylococcus caseolyticus* (n=13) was a predominant species accounting for 28% followed by *Staphylococcus epidermidis* (n=10) with 21% and *Staphylococcus haemolyticus* (n=5) and *Staphylococcal warneri* (n=5) accounting for 11% each and detailed list of all speciation CoNS was shown in Table no 1.

In majority of studies *Staphylococcal epidermidis* is the predominant species isolated form blood culture followed by *Staphylococcal haemolyticus* [11, 12, 13].

Among the total CoNS tested, 31.9% showed resistance to Methicillin (MR-CoNS) and 68.1% were Methicillin sensitive CoNS as shown in Figure no 1. However, many other studies have showed higher resistance to methicillin [8, 12, 14].

In this study, the males accounted for 65.9% of CoNS infection and in females it was 34.1%. Some studies showed the similar findings [14]. The most common age group affected was between 50 to 70 years, followed by in neonates, which was 27.6%.

Among the 47 CoNS isolates, total count was asked for 17 patients and C-reactive protein was asked for four patients. Total count parameters did not show any rise in count. C-reactive protein showed raised values in all the four patients.

Thirteen CoNS isolates were from neonatal care unit and all were suspected cases of early onset of sepsis and isolation of CoNS in these neonates can be taken as pathogen for true bacteremia [15].

It is observed that, in neonates the frequency of isolation of CoNS is high due to the change in hospitalised neonatal population. There are other host factors which play role in this population, such as immune system immaturity, instrumentation, long hospital stay and along with technology dependency which leads to hospital acquired blood stream infections [16].

To consider CoNS as true bacteremia agent according to CDC one of the criteria is instrumentation being done on patients, in our study total of 36 (76.5%) cases underwent instrumentation and these cases can be attributed to nosocomial blood stream infections. As compared to other study where CoNS was reported as contaminant more than a true pathogen [13].

The CoNS accounts for 30% hospital acquired central line associated blood stream infections, the most common type of nosocomial infections related to CoNS, according to CDC and

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Prevention National Healthcare Safety Network [17].

The antibiotic sensitivity testing was done on all the 47 isolates and it showed different sensitivity pattern. The different studies showed similar reports [18, 19, 20, 21, 22].

Maximum resistance was seen with penicillin with 74.5%. None of the isolates showed resistance to vancomycin and imipenem. A detailed antibiogram bar diagram with other drugs was shown in figure no 2.

The importance of CoNS and its species becomes important in understanding its clinical relevance, and to assess their role in true bacteremia cases. For the same reason it is best to speciate the CoNS to possible level and analyse its antibiogram, before doing any typing procedure for epidemiological studies [15,23].

The CoNS demonstrates few virulence factors such as, ability to produce biofilm, presence of delta toxin and MecA gene. The biofilm or exopolysaccharide production has been an important epidemiological marker of infection [4, 24, 25].

The production of biofilm or slime is demonstrated in CoNS, either it is a contaminant or true pathogen for bacteremia. However, the frequency CoNS associated with true bacteremia agents is known to produce biofilm higher than the contaminant CoNS.

Al the 47 CoNS isolates were subjected for Biofilm activity testing by tissue culture plate method [10]. 25 CoNS isolates were positive (53.2%) for Biofilm activity and 22 were negative (46.8%). All the 13 isolates of Staphylococcal caseolyticus were Biofilm producers followed by 5 isolates of Staphylococcal epidermis (n=10). Table no 2 explains the detailed list of speciated CoNS producing biofilms.

Out of 25 Biofilm positive CoNS, 16 were Methicillin sensitive CoNS (n=32) and 9 were from MR-CoNS (n=15). Though 2*2 contingency table shows 60% of MR-CoNS are Biofilm producers compared to 50% of CoNS, but statistically it is not significant as p value is more than 0.05.

Antibiotic sensitivity pattern of Biofilm producers was shown in figure no 3. The maximum resistance was shown for penicillin (72%). Ciprofloxacin showed 56% resistance pattern. No resistance was seen for vancomycin, amikacin and linezolid. Similar findings were seen in other studies where CoNS isolates has shown multidrug resistance pattern.

Conclusion

Clinical isolates of CoNS isolated from blood culture are not usually identified till the species level, by as they are normal inhabitants of skin and anterior nares. Demonstration of Biofilm activity helps us to treat effectively as these strains have more affinity for catheter material and by nature, they are multidrug resistant. Studying the antibiogram profile of Biofilm producers will guide us treating patients effectively and preventing untoward drug resistance.

To summarize, CoNS isolated from blood culture should be speciated, Biofilm activity tested and antibiogram should be performed. Along with these, minimum history related to case should be evaluated before reporting CoNS as contaminant, details such as history of hospital stay, instrumentation information, total count levels, C-reactive protein count, presence of fever etc. CoNS as a true cause of true bacteremia should be assessed thoroughly before considering it as contaminant. CoNS have become one of the major causes of nosocomial infections. The antibiotic resistance pattern of CoNS in our study shows resistance to routinely used antibiotics.

The increased prevalence of CoNS and its drug resistance pattern is mainly due to multiple factors. This emphasis on rapid identification of CoNS and correlating with other pathogenicity factors, to say it as a true cause of bacteremia. This helps in improved management of patients and prevention of emergence of drug resistant pathogens.

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