Speciation, Biofilm Formation, and Antimicrobial Resistance in Clinically Significant Coagulase-Negative Staphylococci at a Tertiary Care Hospital

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ABSTRACT

Background

Coagulase-negative staphylococci (CoNS), which were considered harmless commensals or contaminants, have recently emerged as potential pathogens as medical technology has advanced. Their role as pathogens has increased mainly due to an increase in the number of immunocompromised patients and increased use of implants in hospitals. As they are normal inhabitants of human skin, this makes it difficult to differentiate harmless contaminants from potentially lethal pathogens. It is necessary to speciate CoNS and understand their pathogenic potential due to various virulence factors. Failure to identify them may lead to inappropriate therapy resulting in antimicrobial resistance, treatment failure and increased mortality.

Methods

A total of 200 isolates of CoNS were collected from various clinical samples, such as urine, pus, drain tips/catheter tips/intravenous canulas, blood, skin and conjunctival swabs and various body fluids from patients suspected of bacterial infection at Victoria Hospital, Vanivilas Hospital, Minto Hospital and Bowring & Lady Curzon hospital attached to Bangalore Medical College and Research Institute during the period November 2015 - May 2017. The isolates were identified by standard biochemical reactions and virulence factors were determined by various methods. Antibiotic susceptibility testing was done according to the Kirby-Bauer disk diffusion method and the results were interpreted according to CLSI guidelines.

Results

The majority of the isolates were from pus samples with S. epidermidis as the most common species followed by S. haemolyticus. S. epidermidis was the predominant biofilm-producing species. S. epidermidis, S. haemolyticus and S. saprophyticus produced most of the virulence factors among CoNS species. Methicillin resistance was 25% among the CoNS isolates. Antibiotic sensitivity pattern revealed 100% sensitivity to linezolid and maximum resistance was observed for Penicillin.

Interpretation and Conclusion

The present study aimed to identify the most prevalent clinical isolates of CoNS by minimum number of tests and to study pathogenic potential virulence factors were identified. Antibiotic susceptibility revealed methicillin resistance. Taking into consideration their etiological importance this present study confirmed that they should not be ignored or classified as mere contaminants.

Keywords: CoNS, Virulence factors, Methicillin resistance, Contaminants, S. epidermidis.

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INTRODUCTION

Staphylococci are Gram-positive, catalase-positive, non–spore-forming cocci arranged in clusters and are classified into coagulase-positive and coagulase-negative staphylococci (CoNS) based on their ability to clot plasma.[1]

CoNS are part of the normal flora of human skin and mucous membranes, making it difficult to distinguish contamination from true infection when isolated from clinical specimens.[2] Historically regarded as harmless commensals, they are now recognized as important opportunistic pathogens, particularly in healthcare settings.[2]

The increasing clinical significance of CoNS is attributed to the widespread use of indwelling medical devices such as catheters, prosthetic joints, cardiac implants, and cerebrospinal fluid shunts, along with predisposing factors such as immunosuppression, malignancy, intensive care unit stay, and invasive procedures.[2]

Among CoNS, *Staphylococcus epidermidis* is the most frequently isolated species, followed by *Staphylococcus haemolyticus* and *Staphylococcus saprophyticus*.[2] A major factor contributing to their pathogenicity is biofilm formation, which facilitates adherence to biomaterials and confers resistance to host immune responses and antimicrobial agents.[4–5]

The increasing prevalence of methicillin resistance among CoNS further complicates treatment and

highlights their growing clinical importance in nosocomial infections.[3]

Therefore, this study was undertaken to speciate CoNS isolates, assess their virulence factors, and determine their antimicrobial susceptibility patterns

Objective

To speciate coagulase-negative staphylococci isolated from clinical samples and to evaluate their virulence factors and antimicrobial susceptibility patterns.

MATERIALS AND METHODS

Study design and setting

A hospital-based cross-sectional study was conducted on 200 CoNS isolates obtained from various clinical samples including urine, pus, blood, catheter tips, conjunctival swabs, skin swabs, and body fluids received at the Department of Microbiology, Bangalore Medical College and Research Institute and its attached hospitals between November 2015 and May 2017.

Inclusion and exclusion criteria

All clinically significant CoNS isolates obtained in pure culture from infected sites were included in the study. Coagulase-positive staphylococci, Gram-negative organisms, Gram-positive bacilli, and fungi were excluded.

Isolation and identification of CoNS

Clinical samples were cultured on standard media including blood agar, MacConkey agar, and chocolate agar as appropriate. Identification of CoNS was based on colony morphology, Gram staining, catalase test, bacitracin sensitivity, slide and tube coagulase tests, and standard biochemical reactions.

Speciation of CoNS

Speciation of CoNS isolates was performed using standard biochemical tests including phosphatase test, ornithine decarboxylase test, Voges–Proskauer test, carbohydrate utilization tests, and novobiocin sensitivity testing.

Detection of virulence factors

Biofilm production was detected using tissue culture plate method, tube adherence test, and Congo red agar method. Hemolysin, DNase, thermonuclease, lipase, protease, and lecithinase production were detected using standard microbiological methods.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed by modified Kirby–Bauer disk diffusion method on Mueller–Hinton agar according to CLSI 2015 guidelines. The antibiotics tested included penicillin, erythromycin, clindamycin, ciprofloxacin, linezolid, cotrimoxazole, chloramphenicol, cefoxitin, amikacin, azithromycin, doxycycline, gentamicin, and nitrofurantoin.

Detection of methicillin resistance

Methicillin resistance among CoNS isolates was detected using cefoxitin (30 µg) disk diffusion test according to CLSI 2015 criteria.

Detection of inducible clindamycin resistance

Inducible clindamycin resistance was determined using the D-test as per CLSI 2015 guidelines.

Statistical analysis

Data were analyzed using descriptive statistics and expressed as percentages.

Ethical considerations

The study was conducted using anonymized data obtained as part of postgraduate thesis work approved by the Institutional Ethics Committee

RESULTS

A total of 200 clinically significant CoNS isolates were included in the study. All isolates were negative for both slide and tube coagulase tests. Of the 200 patients, 115 (57.5%) were males and 85 (42.5%) were females. Bloodstream infections due to CoNS were more common among neonates, whereas other infections were predominantly observed in patients aged 30–60 years.

Clinical sample distribution

The majority of CoNS isolates were obtained from pus and wound swab samples (106/200, 53%), followed by blood cultures (47/200, 23.5%) and urine samples (28/200, 14%). Other isolates were obtained from sputum, vaginal swabs, catheter tips, ear swabs, endotracheal aspirates, and conjunctival swabs.

Table 1. Distribution of CoNS isolates according to clinical specimens

Specimen	Number of isolates (n=200)	Percentage (%)
Pus/Wound swab	106	53
Blood	47	23.5
Urine	28	14
Vaginal swab	5	2.5
Sputum	4	2
Ear swab	4	2
Catheter tips	3	1.5
Endotracheal aspirate	2	1
Conjunctival swab	1	0.5

Species distribution

Among the 200 CoNS isolates, *S. epidermidis* was the predominant species (58%), followed by *S.*

haemolyticus (23.5%), *S. saprophyticus* (7.5%), and *S. lugdunensis* (5%). Other species isolated included *S. xylosus*, *S. cohnii*, *S. hominis*, *S. warneri*, and *S. simulans*.

S. epidermidis was the most common isolate from pus and wound infections as well as blood cultures, whereas *S. saprophyticus* predominated among urine isolates.

Table 2. Species distribution of CoNS isolates

Species	Number of isolates (n=200)	Percentage (%)
Staphylococcus epidermidis	116	58
Staphylococcus haemolyticus	47	23.5
Staphylococcus saprophyticus	15	7.5
Staphylococcus lugdunensis	10	5
Staphylococcus xylosus	3	1.5
Staphylococcus cohnii	3	1.5
Staphylococcus hominis	2	1
Staphylococcus simulans	2	1
Staphylococcus warneri	2	1

Virulence factors

Among *S. epidermidis* isolates, lipase production (90.5%) and slime production (67.2%) were the most commonly detected virulence factors. *S. haemolyticus* also demonstrated significant lipase production (85.1%) and slime production (55.3%). TNase production was observed only in *S. lugdunensis* isolates.

Biofilm production

By the tissue culture plate (TCP) method, 29 isolates were strong biofilm producers, 83 were moderate producers, and 88 were weak or non-biofilm producers. Biofilm production was most commonly observed among *S. epidermidis* isolates, followed by *S. haemolyticus*.

The tube adherence method showed comparable results, whereas the Congo red agar method detected biofilm production in only eight isolates.

Methicillin resistance

Methicillin resistance was observed in 25% of CoNS isolates. Resistance was highest among *S. epidermidis* isolates (29.3%), followed by *S. haemolyticus* (25.3%) and *S. saprophyticus* (20%).

Inducible clindamycin resistance

D-test positivity indicating inducible clindamycin resistance was observed in 16 (8%) isolates. Forty isolates showed isolated erythromycin resistance, while 144 isolates were sensitive to both erythromycin and clindamycin.

Antimicrobial susceptibility pattern

All isolates were sensitive to linezolid. Maximum resistance was observed against penicillin (94.5%), followed by gentamicin (75.5%) and ciprofloxacin (63.5%). Methicillin-resistant CoNS isolates demonstrated higher resistance to multiple antimicrobial agents compared to methicillin-sensitive isolates.

Oxacillin MIC

Oxacillin MIC testing by E-test method showed that 75% of isolates were sensitive (MIC ≤0.25 µg/ml), while 25% were resistant (MIC ≥0.5 µg/ml). Resistant isolates demonstrated MIC values ranging from 0.5 µg/ml to 32 µg/ml.

DISCUSSION

Coagulase-negative staphylococci (CoNS) are increasingly being recognized as important nosocomial pathogens because of their opportunistic nature and the widespread use of invasive procedures in critically ill and immunocompromised patients. Differentiating true pathogens from contaminating skin flora remains a major challenge in clinical microbiology. Therefore, identification of CoNS up to the species level is essential for understanding their distribution, pathogenic potential, and contribution to hospital-acquired infections.

In the present study, 200 clinically significant CoNS isolates were obtained from various clinical specimens. Among the patients, 115 (57.5%) were males and the majority were above 40 years of age. Similar findings were reported by Manikandan et al.6

The majority of isolates in the present study were obtained from wound infections (53%), followed by blood cultures (23.5%) and urine samples (14%). Similar findings were reported by Sewell et al.7, who identified wound drainage as the most common source of CoNS isolates, followed by blood and urine samples. Comparable observations were also made by Goyal et al.8, who reported wounds as the predominant source of CoNS isolates.

In contrast, Gill et al.9 reported blood as the most common source of CoNS isolates. Similarly, Mohan et al.10 and De Paulis et al.11 reported urine as the predominant source. These variations among studies may be attributed to differences in patient populations, specimen selection, hospital settings, and criteria used for defining clinically significant CoNS isolates.

In the present study, *Staphylococcus epidermidis* was the predominant species (58%), followed by *S. haemolyticus* (23.5%), *S. saprophyticus* (7.5%), *S. lugdunensis* (5%), *S. xylosus* (1.5%), *S. cohnii* (1.5%), and *S. hominis*, *S. warneri*, and *S. simulans* (1% each). Similar species distribution patterns have been reported by De Paulis et al.11, Goyal et al.8, Iorio et al.12, and Manikandan et al.6

Among the wound and pus isolates, *S. epidermidis* was the predominant species, followed by *S. haemolyticus*. Similar findings were reported by Singh et al.13, Sewell et al.7, and Kleeman et al.14

Among blood culture isolates, *S. epidermidis* was the predominant species, followed by *S. haemolyticus*. These findings correlate well with studies conducted by Eng et al.15 and Cunha et al.16 Both Gill et al.9 and Cunha et al.16 reported *S. epidermidis*, *S. haemolyticus*, and *S. hominis* as the first, second, and third most frequently isolated species, respectively.

In urine samples, *S. saprophyticus* was the predominant isolate, followed by *S. epidermidis*. Similar observations were reported by De Paulis et al.11 and Goyal et al.8 In contrast, Gill et al.9 reported *S. epidermidis* as the predominant urinary isolate. Singh et al.13 also reported isolation of *S. epidermidis* and *S. hominis* from urinary specimens, while *S. saprophyticus* was not isolated.

The pathogenicity of CoNS in the present study was evaluated by detection of various virulence factors including haemolysin, DNase, TNase, lipase, protease, lecithinase production, and biofilm formation. Haemolysin production was observed among isolates of *S. epidermidis*, *S. haemolyticus*, and *S. saprophyticus*. Similar findings were reported by Cunha et al.16 and Hébert et al.17

Lipase and protease production were observed predominantly among *S. epidermidis*, *S. haemolyticus*, *S. saprophyticus*, and *S. lugdunensis* isolates. Similar findings were reported by Priya Ravindran et al.18, whereas Cunha et al.16 reported lower rates of lipase production among *S. epidermidis* isolates.

DNase production was detected in *S. epidermidis*, *S. haemolyticus*, and *S. saprophyticus*, whereas TNase production was observed exclusively in *S. lugdunensis*. Similar findings were reported by Cunha et al.16

Lecithinase production in the present study was observed among *S. epidermidis*, *S. haemolyticus*, and *S. saprophyticus*. Similar findings have been reported by Cunha et al.16 and Krzymińska et al.19

Biofilm formation, one of the most important virulence factors associated with CoNS pathogenicity, was detected in 112 (56%) isolates by the tissue culture plate (TCP) method. Among the species studied, *S. epidermidis* showed the highest rate of biofilm production (67.2%), followed by *S. haemolyticus* (55.3%), *S. lugdunensis* (40%), and *S. saprophyticus* (28.5%). Similar findings have been reported by Deka et al.20, Agarwal et al.21, and Ishak et al.22

Among the methods used for biofilm detection, the TCP method was found to be the most sensitive, accurate, and reproducible. The tube adherence method correlated reasonably well with TCP for strong biofilm producers, whereas the Congo red agar method showed poor correlation. Similar observations were reported by Christensen et al.23 and Mathur et al.24

Antimicrobial susceptibility testing revealed 25% methicillin resistance among CoNS isolates. Similar rates have been reported by Uma C et al.25 and Mohan et al.10, whereas Usha MG et al.26 documented higher resistance rates.

Oxacillin MIC testing by E-test demonstrated that 75% of isolates were sensitive to oxacillin (MIC $\leq$0.25 µg/ml), while 25% were resistant (MIC $\geq$0.5 µg/ml). These findings were comparable to those reported by Sharma et al.27

All isolates in the present study were sensitive to linezolid. Similar findings have been reported by Priya Ravindran et al.18, Begum et al.28, Hamad et al.29, and Sharma et al.27

Maximum resistance was observed against penicillin (94.5%), followed by gentamicin (75.5%) and ciprofloxacin (63.5%). Methicillin-resistant CoNS isolates demonstrated higher multidrug resistance compared to methicillin-sensitive isolates. More than 80% of isolates remained sensitive to doxycycline, erythromycin, clindamycin, and nitrofurantoin. Similar variability in antimicrobial susceptibility patterns among CoNS isolates has been reported by Priya Ravindran et al.18, Begum et al.28, Mohan et al.10, and Singh et al.13

Inducible clindamycin resistance was observed in 8% of CoNS isolates by D-test. Similar findings were reported by Angel et al.30, who documented inducible clindamycin resistance in 5% of isolates.

Overall, the present study highlights the increasing clinical significance of CoNS as opportunistic pathogens associated with biofilm production and multidrug resistance. Accurate species identification, detection of virulence factors, and antimicrobial susceptibility testing are essential for appropriate clinical management and effective infection control practices.

CONCLUSION

Coagulase-negative staphylococci (CoNS) have emerged as important causes of nosocomial infections, particularly in severely debilitated and immunocompromised patients and in those with indwelling medical devices and implants. Their increasing clinical significance highlights the need for accurate identification and differentiation from contaminating flora.

The present study aimed to identify the prevalent clinical isolates of CoNS using a minimum number of tests necessary for species-level identification.

Among the 200 clinically significant CoNS isolates studied, *Staphylococcus epidermidis* was the predominant species, followed by *S. haemolyticus*. The pathogenic potential of CoNS was evaluated by detecting various virulence factors, among which biofilm formation was the most important. Biofilm production was predominantly associated with prosthetic device-related infections such as orthopedic wound infections and intravenous catheter-associated infections. Methicillin resistance was also observed, particularly among *S. epidermidis* isolates. Multidrug resistance was more common among methicillin-resistant and biofilm-producing isolates, indicating the role of biofilm in enhanced antimicrobial resistance.

Afaq N et al., Coagulase negative Staphylococci (CoNS) are one of the most common bacteria found on human skin and on mucous membranes as a normal flora. The presence of CoNS in clinical specimens is frequently associated with an infectious aetiology or contamination. CoNS are significant and commonly encountered pathogens in hospitals and they are occurring as the most preponderant isolates of all nosocomial infections. [31].

Among the methods used for biofilm detection, the tissue culture plate (TCP) method was found to be the most sensitive and reliable. Early identification of virulence factors along with antimicrobial susceptibility testing can help in timely institution of appropriate antimicrobial therapy and may aid in preventing treatment failure and implant removal.

The present study emphasizes that CoNS should not be dismissed as mere contaminants, as they possess significant pathogenic potential and contribute substantially to hospital-acquired infections. Accurate species identification, detection of virulence factors, and appropriate antimicrobial susceptibility testing are essential for effective patient management and infection control practices.

DECLARATIONS:

Conflicts of interest: There is no any conflict of interest associated with this study

Consent to participate: There is consent to participate.

Consent for publication: There is consent for the publication of this paper.

Authors' contributions: Author equally contributed the work.

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