Section: Bacteriology

Original Research Article

CHARACTERIZATION OF CLINICALLY RELEVANT ISOLATES OF COAGULASE NEGATIVE STAPHYLOCOCCI (CONS) IN A TERTIARY CARE CENTRE

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ABSTRACT

Background: Coagulase Negative Staphylococci (CoNS) are a diverse group of Gram-positive bacteria, commonly isolated as normal flora of skin and mucous membranes. The emergence of multidrug resistant strains, particularly methicillin resistance further aggravates the situation. Therefore, the present study was undertaken to predict the potential pathogenicity of the CoNS isolates by characterizing, determining their antibiotic susceptibility pattern, detecting the presence of mecA gene among the Methicillin Resistance Coagulase Negative Staphylococci species (MRCoNS) from various clinical samples.

Materials and Methods: The study was a hospital-based prospective study conducted in the Department of Microbiology, NEIGRIHMS, Shillong from May, 2021 to June, 2022. CoNS isolates were identified using conventional microbiological procedures and speciation was done following Kloos and Schleifer's scheme. Antibiogram was determined by Kirby Bauer's disk diffusion method and broth microdilution method. Detection of methicillin resistance was performed using Cefoxitin disk diffusion method. *MecA* gene detection was done among the MRCoNS isolates using real time PCR. Data analysis was done using descriptive statistics.

Results: Fifty-three CoNS isolates were identified from different clinical specimens, which included Staphylococcus epidermidis (39.6%) followed by S. simulans (15%), S. haemolyticus (13.2%), S. hominis (9.4%). Most isolates were resistant to penicillin (83%), and least to vancomycin (1.9%). No resistance to linezolid was seen. Methicillin resistance was detected in 34 of the isolates. Out of the 34 isolates identified as MRCoNS by phenotypic methods, *mecA* gene was detected in 17 isolates by Real- time PCR.

Conclusion: CoNS are emerging multidrug resistant pathogens, and hence, studies on their local species distribution and antibiotic sensitivity pattern are very important. The present study will be a guide for the clinicians in establishing their role as significant pathogens and initiate proper antimicrobial therapy.

Keywords: CoNS, MRCoNS, PCR, MecA.

INTRODUCTION

Staphylococci are important pathogenic bacteria and responsible for causing hospital acquired infections or nosocomial infections.[1] They are gram-positive, non-motile, catalase-positive cocci grouped primarily in grape-like clusters but also in singles, pairs, tetrads, short chains and are ubiquitous in nature.[1,2] Based on the ability to produce an enzyme called coagulase, they are usually divided into two groups. The coagulase positive staphylococci (CoPS) group which includes Staphylococcus aureus, an important human pathogen, and the coagulase negative staphylococci (CoNS), which is a large heterogeneous group with a diverse natural habitat.[3] CoNS are an important microbiota of skin and mucous membranes and are frequently isolated in a clinical microbiology laboratory but are usually discarded as contaminants.[4] One of the major problems faced by the microbiology laboratory is differentiating contaminants from clinically significant pathogenic strains.[5] CoNS are becoming the reservoirs of multiple antimicrobial resistant determinants, owing to their presence in greater number on the skin, their selection due to rampant usage of broad-spectrum antibiotics, their ability to form biofilms and multidrug resistant.[6,7] Methicillin resistance which is encoded by mecA gene, has been reported in about 80% of the CoNS isolates. The rates of methicillin and vancomycin resistance are generally higher in CoNS than in coagulase positive staphylococci (CoPS).[8] Therefore, the present study was undertaken to characterize the various clinically relevant isolates of CoNS and detect the presence of mecA gene among the Methicillin Resistance Coagulase Negative Staphylococci Species(MRCoNS) from various clinical samples.

MATERIALS AND METHODS

Sample collection

This was a hospital-based prospective study which was conducted from May, 2021 to June, 2022.All the CoNS isolates from various clinical samples like peritoneal fluid, pleural fluid, high vaginal swab, blood samples, ear swabs, CSF, urine samples, synovial fluid, deep wounds, tracheal aspirate, sputum obtained from patients attending the out-patient and in-patient departments during the study period were included.

Phenotypic Identification

Speciation of CoNS was done following the scheme of Kloos and Schleifer which was based on slide and tube coagulase tests, ornithine decarboxylase, Voges Proskauer (VP) test, urease test, novobiocin (5 µg) disk test, and fermentations of sugars i.e mannitol, mannose, lactose, trehalose, and xylose.[9,10]

Antibiogram of the isolates: Antibiotic susceptibility was determined by using the Kirby Bauer's disk diffusion method and interpretations was done as per the 31st edition of Clinical and Laboratory Standards Institute (CLSI), M100 guidelines 2021. Mueller Hinton agar (Hi-Media, Mumbai, India) was used for antibiotic susceptibility test and tested against the commercially available disks i.e penicillin (10 µg), erythromycin (15 µg), clindamycin (2 µg), levofloxacin (5 µg), cotrimoxazole (1.25/23.7 µg), ciprofloxacin (5 µg), gentamicin (10 µg), chloramphenicol (30 µg), linezolid (30 µg). Antimicrobial susceptibility testing of vancomycin was performed using microbroth dilution test. Detection of methicillin resistance among CoNS was performed using cefoxitin disk (30 µg) diffusion method.

Molecular method for detection of mecA gene among the CoNS isolates

All the Methicillin-resistant CoNS isolates detected by phenotypic method were put up for PCR to detect the presence of mecA gene. Microbial DNA real-time polymerase chain reaction (q-PCR) was performed for detection of mecA gene using QIAGEN's real-time PCR cycler, Rotor-Gene Q.

Extraction of bacterial DNA

DNA extraction was carried out using Chromous BiotechTM Bacterial Genomic DNA Spin Kit following the kit instructions. DNA concentration was determined by UV spectrophotometry using Eppendorf BioSpectrometer® basic.

Real-time Polymerase Chain Reaction:

Real-time PCR for detection of mecA gene was carried out using Microbial DNA q-PCR from Eurofins Genomics India Pvt. Ltd., Bengaluru, Karnataka, India and Chromous Biotech Pvt. Ltd., Bengaluru, Karnataka, India.The primers and probe sequence (from 5' to 3')

P mecA [FAM]
TTGGCCAATACAGGAACAGCA[BHQ1]
F mecA GAATGCAGAAAGACCAAAGC
B mecA TTCTTTGGAACGATGCCTAT

Cycle threshold (Ct) value for each reaction tube was calculated using the real-time cycler's software. ATCC S. aureus 33591 was used as positive control. ATCC S. aureus 25923 and ATCC S. haemolyticus 29970 were used as negative control. A reaction tube without DNA template was also set up as no template control (NTC)

Statistical Analysis

Descriptive statistics like percentage and proportion were used to present the data. Bivariate analysis was done and the results were interpreted in terms of odds ratio with confidence interval of 95%. Analysis was done using SPSS v.25 software. $p < 0.05$ was considered significant.

RESULTS

During the study period 53 CoNS were obtained from various clinical samples, which included Staphylococcus epidermidis (39.6%) followed by S. simulans (15%), S.haemolyticus (13.2%), S.

hominis (9.4%), S. saprophyticus (5.7%), S. xylosus (3.8%), S. lugdunensis (3.8%), S. capitis (3.8%), S. schleiferi (3.8%) and S. warneri (1.9%). Among the 53 isolates of CoNS, 17 (32%) were isolated from pus, nine (17%) from the blood, six (11.3%) from urine. [Table 1]

Table 1: Distribution of CoNS among the different clinical samples

CLINICAL SAMPLES	NUMBER OF SAMPLES OBTAINED(%)
PUS	N=17(32%)
BLOOD	n= 9, (17%)
URINE	n= 6(11.3%)
CENTRAL LINE TIP	n= 4(7.5%)
SPUTUM	n= 4(7.7%)
ENDOTRACHEAL ASPIRATE	n= 3(5.7%)
PERICARDIAL FLUID	n=2(3.8%)
CEREBROSPINAL FLUID	n= 3(3.8%)
HIGH VAGINAL SWAB	n= 2(3.8%)
BRONCHOALVEOLAR LAVAGE	n = 1(1.9%)
SHUNT TIP	n =1 (1.9%)
PERCUTANEOUS NEPHROSTOMY SAMPLE	n =1 (1.9%)

A total of 32 (60.4%) CoNS were identified from males and 21 (39.6%) were from females. The majority of the isolates were from the age group of 21 – 31 years (26.4%), followed by the age group of 31 – 40 years (18.9%) as shown in figure 1.

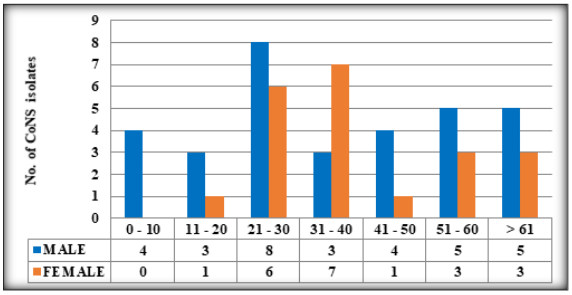

Figure 2: Age and gender wise distribution of the CoNS isolates

Table 2: Distribution of CoNS species among the different clinical samples

Cons Species	Samples												
	Pus (%)	Blood(%)	Urine(%)	Central Line Tip(%)	Sputum(%)	Endotracheal Aspirate(%)	Pericardial fluid (%)	Cerebrospinal fluid(%)	High Vaginal fluid(%)	Brochoalveolar lavage(%)	Shunt tip(%)	PCN(%)	Total(%)
S.epidermidis	9 (43)	3(14.3)	0	2 (9.5)	0	1(4.8)	1(4.8)	2	1(4.8)	0	1(4.8)	1(4.8)	21
S.simulans	1(12.5)	2	1(12.5)	1(12.5)	0	1(12.5)	1(12.5)	0	1(12.5)	0	0	0	8
S.hemolyticus	3(43)	1(14.3)	1(14.3)	0	0	1(14.3)	1(14.3)	0	0	0	0	0	7
S.saprophyticus	1(33.3)	0	2(66.7)	0	0	0	0	0	0	0	0	0	3
S.lugdunensis	2(100)	0	0	0	0	0	0	0	0	0	0	0	2
S.capitis	1(50)	0	0	0	1(50)	0	0	0	0	0	0	0	2
S.xylosus	0	0	1(50)	0	0	0	0	0	0	1(50)	0	0	2
S.schleiferi	0	0	0	0	2(100)	0	0	0	0	0	0	0	2
S.warneri	0	0	0	0	1(100)	0	0	0	0	0	0	0	1
S.hominis	0	3(60)	1(20)	1(20)	0	0	0	0	0	0	0	0	5
Total	17	9	6	4	4	3	3	2	2	1	1	1	53

CoNS isolates showed maximum susceptibility to linezolid (n=53,100%), gentamicin (n=46,86.8%), chloramphenicol (n=43,81.1%), co-trimoxazole (n=40,75.5%), levofloxacin (n=37,69.8%), tetracycline (n=36,67.9%), and ciprofloxacin (n=28,52.8%). Maximum resistance was shown by penicillin (n=48,90.6%), erythromycin (n=36,67.9%), and clindamycin (n=26,49.1%).

All the CoNS isolates were subjected to Microdilution Vancomycin susceptibity testing to determine their Minimum Inhibitory Concentration (MIC). Out of the 53 isolates tested, 52 (98.11%) were found to be sensitive and one (1.9%) was resistant to vancomycin.

Methicillin-resistance among the CoNS isolates was detected phenotypically by cefoxitin (30µg). Out of the total 53 CoNS isolates, 34 (64.2%) were methicillin-resistant CoNS (MRCoNS) and 19 (35.8%) were methicillin-susceptible.

Among the 34 MRCoNS isolates, sixteen (47%) were S. epidermidis, six (17%)were S. hemolyticus, four (12%) were S. simulans, three (9%) were S.

hominis, two (6%) were S. saprophyticus, and one (1%)each were S. lugdunensis, S. capitis, and S. xylosus.

Comparison of the antimicrobial resistance pattern of MRCoNS and MSCoNS

The MRCoNS isolates (n=34) showed higher degree of resistance to most of the antibiotics tested and it was statistically significant as shown in table 3

Table 3: Antimicrobial resistance pattern of the MRCoNS and MSCoNS

Antimicrobials	MRCoNS (*n* = 34)	MSCoNS (*n* = 19)	*p* value (95% CI)	Level of Significant
Penicillin	34 (100%)	14 (73.7%)	---	*
Erythromycin	26 (76.5%)	10 (52.6%)	<0.001	Significant
Clindamycin	21 (61.8%)	5 (26.3%)	<0.001	Significant
Levofloxacin	15 (44.1%)	1 (5.2%)	<0.001	Significant
Ciprofloxacin	18 (53%)	7 (36.8%)	<0.001	Significant
Tetracycline	15 (44.1%)	2 (10.5%)	<0.001	Significant
Gentamicin	4 (11.8%)	2 (10.5%)	<0.001	Significant
Chloramphenicol	10 (29.4%)	0	---	*
Linezolid	0	0	---	*
Cotrimoxazole	10 (29.4%)	3 (15.8%)	<0.001	Significant
Vancomycin	1 (2.9%)	0	---	*

***p* value of <0.05 was considered significant.**
*** Comparison could not be made as the percentage of resistance to antimicrobials for both MRCoNS and MSCoNS were either 0 or 100% and hence, *p* value could not be determined.**

Detection of mecA gene

The methicillin-resistant CoNS (MRCoNS) isolates were tested for the presence of mecA gene by Real-time PCR. Out of the 34 isolates identified as MRCoNS by phenotypic methods, mecA gene was detected in 17 isolates.

Among the 17 mecA detected isolates, eight (47%) were S. epidermidis, two (12%) each were S.hemolyticus, S.hominis, and S.saprophyticus, and one (6%) each were S. simulans, S.schleiferi and S.warneri.

DISCUSSIONS

In the present study, 53 CoNS were isolated from different clinical samples and the results were analysed by comparing with similar studies which were conducted all over the country and the globe Maximum CoNS were isolated from the male patients (53%) than female patients (39.6%) which are in par with the studies conducted by Mane et al,[11] Sardar et al,[12] but contrasting findings were observed in the study conducted by Goudarzi et al.[13] Maximum CoNS were isolated from the patients belonging to age group between 21 to 30 years (26.4%), which is in par with the studies conducted by Mane et al (22.85%),[11] and Chikkaraddi et al (26.8%),[14] but contrasting results were reported in the study conducted by Roopa et al,[15] and Sardar et al,[12]

Majority of the CoNS isolates were from pus sample, followed by blood cultures, then urine samples which was similar to results of the studies conducted by Roopa et al,[15] Chikkaraddi et al,[14] Asangi et al.[16] About 32% of the CoNS species were isolated from the pus samples which was in comparison with the results of the studies conducted by Chikkaraddi et al,[14] Asangi et al.[16]

S. epidermidis was the predominant species isolated which is in accordance with most of the previously conducted studies done by Usha et al,[17] Chikkaraddi et al,[14] Singh et al.[18]

CoNS show maximum resistance to penicillin 90.6%, followed by erythromycin 62.3%, clindamycin 49.1%, ciprofloxacin 47.2%, and vancomycin 1.9%. No resistance to linezolid was seen. Similar findings had been reported by Gunti et al,[19] Singh et al.[18]

The prevalence of methicillin-resistant CoNS in the present study was 64.2% which was similar to the finding of Manadhar et al (66.8%).[20] The highest methicillin resistance was found in S. hemolyticus (85.7%) and similar findings were also reported in the studies conducted by Singh et al,[18] Manadhar et al.[20]

All the MRCoNS isolates were resistant to penicillin (100%), followed by erythromycin (67.7%), clindamycin (61.8%), ciprofloxacin (53%) and similar findings were reported by Singh et al,[21] and Singh et al.[18]

MecA gene was detected in 17 (50%)isolates which was in par with the studies conducted by Kilic et al,[22] who detected mecA gene in 19 (59.4%) isolates out of 32 MRCoNS isolates. Detection of mecA gene by PCR is the "gold standard" to determine the methicillin-resistance in Staphylococcus species and is a beneficial adjunct to the standard susceptibility testing. However, it should be kept in mind that the susceptible strains by phenotypic methods may possess mecA gene and might not be expressed.[22,23]

The strength of this study was that detection of mecA-mediated resistance and the presence of mecA gene was done using both the phenotypic as well as molecular methods. The limitation of this study was that advance methods, such as sequencing could not be done due to the lack of infrastructure during the study period.

CONCLUSION

The clinical significance of CoNS is increasing day by day which warrants the need for a rapid and accurate identification. Given the limited number of therapeutic options for CoNS with multidrug resistance, the rise in the incidence of both methicillin resistance and multidrug resistance should be taken very seriously. Therefore, a strict hospital antibiotic policy is a must to eradicate the infections caused by CoNS and also to reduce their resistance. There is a need for preventive strategy such as surveillance and empirical treatment for better patient management which will reduced hospital associated infections, halt development of drug resistance and spread of resistant strains.

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Ethical Statement: The procedures used in this study were approved by the Institution Ethics Committee, NEIGRIHMS, vide no. NEIGR/IEC/M14/T15/2021, dated 26th April, 2021.

Conflict of Interest: The authors declare no Conflict of Interest.

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Submission Declaration

The work described has not been published previously and it is not under consideration for publication elsewhere, the publication has been approved by all the authors and that if accepted it will not be published elsewhere in the same form in English or in any other language including electronically without the written consent of the copyright holder

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