Silent Nasal Carriers: Nasal Colonization and Genetic Profiling of Methicillin-Resistant Coagulase Negative Staphylococci among College Students

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Abstract

Coagulase-negative staphylococci (CoNS) have emerged as important opportunistic pathogens and reservoirs of antimicrobial resistance, particularly methicillin resistance mediated by the mecA gene. Their ability to colonize the anterior nares makes healthcare students potential carriers, posing a risk of transmission in clinical environments. This study aimed to assess nasal colonization of methicillin-resistant CoNS (MR-CoNS) among preclinical dental students and to characterize the molecular mechanisms of methicillin resistance including detection of the mecA gene and SCCmec typing. Nasal swabs were collected from 71 students and processed using standard microbiological techniques. Identification of CoNS isolates was carried out by biochemical tests and VITEK-2, while methicillin resistance was determined using cefoxitin and oxacillin susceptibility testing, confirmed by PCR amplification of the mecA gene. SCCmec typing was performed through multiplex PCR.

Among the 71 isolates, 51 (71.83%) exhibited methicillin resistance, with Staphylococcus epidermidis (43.13%) and Staphylococcus haemolyticus (25.4%) as predominant species. The mecA gene was detected in 31 isolates including 28 MR-CoNS and 3 methicillin-susceptible CoNS, suggesting silent mecA carriage. SCCmec analysis showed a predominance of type 1 variant and type 5, especially in S. haemolyticus, while several isolates remained non-typable, indicating possible novel SCCmec elements. These findings demonstrate significant nasal carriage of genetically diverse MR-CoNS among dental students, underscoring their role in the dissemination of antimicrobial resistance within healthcare settings.

Keywords: Coagulase-negative staphylococci, Methicillin resistance, Staphylococcal Cassette Chromosome mec, Polymerase chain reaction.

Introduction

Staphylococci are frequently encountered human pathogens, divided into two main groups: coagulase-positive, mainly *Staphylococcus aureus* and coagulase-negative Staphylococci (CoNS). In healthcare settings, their spread can significantly increase the likelihood of infections, ranging from minor skin irritations to serious, potentially fatal illnesses[11]. *Staphylococcus* species account for nearly 30% of hospital-acquired infections and are associated with healthcare costs estimated at two billion annually[24]. Over the past few decades, the rapid increase in methicillin-resistant Staphylococcus strains has significantly hindered the effective management of hospital-acquired infections. International surveillance reports reveal that methicillin-resistant coagulase-negative Staphylococci (MR-CoNS) may account for up to 80% of isolates found in clinical settings[21].

The methicillin resistance among Staphylococcal species is largely associated to the *mecA* gene, embedded within staphylococcal cassette chromosome mec (SCCmec)[2,4,22]. MR-CoNS are primarily spread through direct contact, typically between healthcare workers (HCWs) and patients. As a result, monitoring methicillin resistance in Staphylococcus strains carried by HCWs is crucial for preventing and controlling hospital-associated infections[26]. Since the anterior nares serve as a primary reservoir for both methicillin-resistant *S. aureus* (MRSA) and MR-CoNS, the presence of these organisms in the nasal passages of healthcare workers (HCWs) can be a valuable indicator for assessing the antibiotic resistance patterns of Staphylococcus strains within hospital settings[16,18]. SCCmec are transposable segments that enable the exchange of genes imparting resistance among coagulase positive as well as coagulase negative Staphylococcal spp[6,8,9,12].

Another study on the MR-CoNS nasal carriage incidence among healthcare workers revealed that nursing students had a higher carriage rate (50%) compared to other groups of healthcare workers[1]. A comparable study in India found a notable prevalence of MR-CoNS carriage among doctors (41.6%) and nurses (32.4%)[14]. Most existing research focuses primarily on the nasal colonization of MR-CoNS, often overlooking the molecular epidemiology of MR-CoNS

strains isolated from nasal samples. Furthermore, there is a lack of studies on dissemination of SCCmec types among nasal isolates, especially in India. In this context, the current study investigates the colonization of MR-CoNS in the anterior nares of preclinical dental students along with the incidence of varied SCCmec types among MR-CoNS isolates among the study participants.

Material and Methods

Nasal swabs collected under aseptic conditions from the anterior nares of (n=71) preclinical dental students (male n=14, female n=57) were recruited for the study. The study protocol was reviewed and approved by the Institute's Ethical Committee (Ref No: SBDCH/IEC/06/2021/1). Informed consent was also obtained from all participants before sample collection.

The samples were processed using standard microbiological methods. Staphylococci recovered from the samples were phenotypically identified by Gram staining and the production of catalase, oxidase and coagulase was detected by standard biochemical tests. The samples have been seeded on 5% sheep blood agar and MacConkey agar plates and selective isolation by plating onto mannitol salt agar (HiMedia laboratories Pvt. Ltd., India) and incubated at 37°C. Isolated pink colonies of CoNS were suspended in sterile saline; cell density was adjusted to 0.5 MacFarland standard for species identification to assess the susceptibility testing pattern using VITEK-2 (BioMe´rieux) automated system. *Staphylococcus aureus* ATCC25923 was included as control.

Detection of *mecA* by PCR: DNA isolation was done by boiling lysis method. The extracted DNA was stored at -80ºC until further use. PCR was carried out for detection of *mecA* in the CoNS isolates (n =71) using previous described primers[5]. Briefly, PCR was set up (20µL reaction volume) with 10× PCR buffer ,10 mM of dNTP mix, 1 unit Taq DNA, DNA template and PCR grade water. The PCR amplification was conducted in Veriti 96-Well Thermal Cycler (Applied Biosystems, USA) with the cyclic conditions of initial denaturation at 94°C for 3 min followed by 35 cycles at 94°C for 30s, 58°C for 30s for *mecA* gene and 72°C for 1min and final extension at 72°C for 7 min.

After PCR run, the amplicons were resolved along with DNA markers in 1% agarose with ethidium bromide (10 mg/mL) by gel electrophoresis for ~15min at 135 V using Mupid-exU system (Takara, Japan) and gel was then analyzed using BioGlow UV Transilluminators (Crystal Technology, USA). The amplicon size was 777bp for *mecA* gene (Table 1).

SCCmec typing by multiplex PCR: Amplification was performed in a mixture of 30 µL volume containing PCR master mix and specific primers [βR, α3F, ccrCF, ccrCR, 1272F, 1272R, 5RmecAF, 5R431R (10 pmol/µL of each primer)] and template DNA (Table 2). Initial denaturation at 94°C for 3 minutes, 35 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, extension at 72°C for 1 minute, final extension at 72°C for 7 minutes were performed.

Results and Discussion

Of the 71 nasal carrier isolates of coagulase negative staphylococci that were screened, majority of the isolates were *S. epidermidis* followed by *S. hominis subspp hominis*, *S. haemolyticus*, *S. lugdunensis*, *S. lentus* and *S. warneri*. Of these CoNS, nearly 51/71 (71.83%) were found to be resistant to cefoxitin and oxacillin and hence were scored as MRCoNS. Of the 51 MRCoNS, majority of the isolates were found to be *S. haemolyticus* (25.4%), *S. epidermidis* (43.13%) followed by *S. hominis subspp hominis* (19.6%) (Table 3). Nearly 12/13 (92.3%) of *S. haemolyticus* isolates were found to be MRCoNS and all of the them were found to harbor *mecA* gene (Fig. 1).

Table 1
A PCR targeting *mecA* was performed using the following primers.

Gene	Primer sequence [5]	Annealing Temp	Amplicon
mecA	F:5′-TGAGTTCTGCAGTACCGGAT-3′ R:5′-ATGATTATGGCTCAGGTACTGCTATCCACC-3′	55˚C	777 bp

Table 2
PCR for SCCmec typing was performed using the following primers.

Gene	Primer sequence	Annealing Temp	Amplicon
B A3	F: 5'-ATTGCCTTGATAATAGCCYTCT-3′ R: 5'-TAAAGGCATCAATGCACAAACACT-3′	55˚C	937 bp
ccrC ccrC	F: 5'CGTCTATTACAAGATGTTAAGGATAAT-3′ R: 5'CCTTTATAGACTGGATTATTCAAAATAT-3′		518 bp
1272 1272	F-GCCACTCATAACATATGGAA R-CATCCGAGTGAAACCCAAA		415 bp
5RmecA 5R431	F-TATACCAAACCCGACAACTAC R-CGGCTACAGTGATAACATCC		359 bp

Table 3
Methicillin Resistance and *mecA* Gene in Nasal CoNS

Total isolates (n=71)	**MRCoNS (n=51)**			**MSCoNS (n=20)**		
	Cx^R	**Oxa^R**	***mecA* positive**	**Cx^S**	**Oxa^S**	***mecA* positive**
S. epidermidis (n=36)	22 (43.13)	22 (43.13)	11(21.5)	14 (70)	14 (70)	2(10)
S. haemolyticus (n=13)	13(25.4)	13(25.4)	12 (23.5)	0 (0)	0(0)	0(0)
S.hominis sub species hominis (n=14)	10 (19.6)	10(19.6)	4 (7.8)	4 (20)	4 (20)	1(5)
S. lugdunensis (n=5)	4 (7.8)	4(7.8)	1(1.9)	1 (5)	1(5)	0(0)
S. warneri (n=2)	1(1.9)	1(1.9)	0 (0)	1(5)	1 (5)	0(0)
S. lentus (n=1)	1(1.9)	1(1.9)	0(0)	0 (0)	0 (0)	0(0)

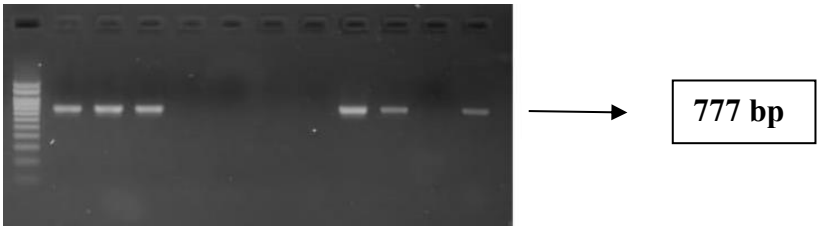

Figure 1: Representative Gel image of *mecA*
Figure legend: Lane M- DNA ladder, Lanes 1,2,3,8,9- *mecA* positive, Lanes 4,5,6,7- *mecA* negative, NC-negative control, PC-positive control.

Table 4
SCCmec Type Distribution Among *mecA*-Positive CoNS Species

*CoNS*isolates (n=68)	**MRCoNS (n=28)**							**MSCoNS (n=3)**		
	***mecA* positive**	**Type 1**	**Type 1 variant**	**Type 4**	**Type 4 variant**	**Type 5 variant**	**NT**	***MecA* positive**	**Type 1**	**Type 1 Variant**
S. epidermidis (n=36)	11	2	2	1	2	1	3	2	2	0
S. haemolyticus (n=13)	12	3	5	0	0	0	4	0	0	0
S.hominis sub species hominis (n=14)	4	1	0	2	0	0	1	1	0	1
S. lugdunensis (n=5)	1	0	0	0	0	0	1	0	0	0

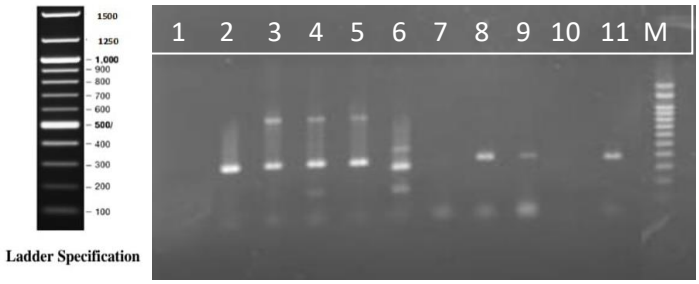

Figure 2: Representative Gel image of SCCmec Typing
Figure legend: Lanes 2,8,9,11- SCCmec Type 1; Lanes 3,5- SCCmec Type 4; Lane 4- SCCmec Type 4 variant, Lane 6 - SCCmec Type 5 variant, Lanes 1, 7,10 SCCmec non-typable.

Among the *S.epidermidis* isolates, only 50% of the MRCoNS isolates were found to harbor the *mecA* gene. Nevertheless, only 40 % of MR *S. hominis sub spp hominis* isolates were found to harbor *mec A* gene, followed by MR *S. lugdunensis* 25% (1/4) isolates, found to harbor the *mecA* gene. None of the *S. warneri* (n=1), *S. lentus* (n=1) isolates harbored *mec A* gene but exhibited a Methicillin resistant phenotype. Among the 20 MSCoNS isolates included in the study, only 3 isolates (S.*epidermidis* (n=2), S.*hominis sub spp hominis* (n=1) carried *mecA*. These isolates are of particular interest as they were found be of harbored by the *mecA*$^+$ genotype, but exhibited a MSCoNS phenotype. This could be attributed to the fact that the gene was not expressed.

Thirty-one CoNS isolates were found to habor*mecA* gene [MRCoNS (n=28), MSCoNS (n=3)]. 22 isolates [MRCoNS (n=19), MSCoNS (n=3)], were found to belong to the

following SCCmec types (Table 4, Fig. 2). However, 9 MRCoNS isolates were non-typable.

Despite possessing less virulence characteristics than *S. aureus*, CoNS still contribute significantly to opportunistic nosocomial infections, mainly because strains that are a reservoir of antibiotic resistance, are frequently seen[3]. Numerous coagulase-negative staphylococci SCCmec cassettes (1-7) have been found and documented. However, it is difficult to make an accurate and clear identification because new combinations of the ccr and mec genes are found. Furthermore, it is stated that not all of the seven varieties previously identified, are found throughout the world[23].

The distribution of CoNS species in our study is consistent with earlier research from India, where *S. haemolyticus* and *S. epidermidis* were the most prevalent species coinciding with the previous study done by Marincola et al[17] and Jain et al[10]. In our present study, SCC mec type 1 variant and type 5 cassette were predominant followed by type 1, type 4 and type 4 variant. The occurrence of the SCCmec type 1 variant was predominantly found in *S. haemolyticus.* Interestingly, all the *S. hemolyticus* isolates (100%) were found to be MRCoNs. It noteworthy that 92.3% of them harbored *mecA.* Of the *mecA* harboring *S. hemolyticus* isolates majority belonged to SCCmec type 1 variant (41.67%) followed by type 1 (25%) and 33.33% were non-typable.

However, the study done by Katkowska et al[13] showed SCCmec type V was only found in *S. haemolyticus*[13].CoNS has a large number of SCCmec cassettes that cannot be categorized as existing types because they most likely comprise as undescribed allotypes or a combination of existing ones[10].

In the current study, *S. epidermidis* isolates (15%), harbors the *SCC*mec type of the *mecA* were found to be diverse, type 1 (18.18%), type1 variant (18.18%), type 4(9.09%), type 4 variants (18.18%) and type 5 variants (9.09%), while, 27.27% were non-typable. 2 isolates of MS *S. epidermidis* were found to harbor *mecA*, nevertheless the gene was not expressed. Both these isolates belonged to SCCmec type1. Nevertheless, many studies revealed about dispersion of SCCmec type for *S. Epidermidis* alone[20]. Also, a greater prevalence of SCCmec type V was observed in commensal samples, which could be associated with the high occurrence of SCCmec type V in *S. epidermidis* reported in certain studies[19].

Among the *S. hominis* isolates harbored *mecA*, SCCmec type 4 was the most prevalent (50%), followed by type 1 (25%) and 25% were non-typable. Our results were in line with the report by Ibrahem et al[7]. Of note, 1 isolate of MS *S. hominis* was found to harbor *mecA*, nevertheless the gene was not expressed. The mecA positive MS *S. hominis* isolate belonged to SCCmec type1variant. In our study, *S. lugdenensis* isolates (20%) harbored *mecA* and this isolate *was* SCCmec- nontypable, whereas the study conducted by Yen et al[25] showed *S. lugdenensis* that harbored *mecA* and of SCCmec type V.

Conclusion

Coagulase-negative staphylococci (CoNS) have increasingly been recognized as clinically significant pathogens, rivalling *Staphylococcus aureus* in both hospital and community settings. Their role as potent SCCmec transmitters highlights the importance of strict phenotypic and genotypic analyses, particularly focusing its characterization. Such investigations are essential for advancing our understanding of antimicrobial resistance, improving infection control strategies and supporting the development of novel therapeutic approaches.

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