Pravara Med Rev; December 2025, 17 (04), 34 - 41
DOI: 10.36848/PMR/2025/33000.10044

Original article

Characterization of Virulence Genes in Coagulase-Negative Staphylococci recovered from the anterior nares of healthy population.

Ramachandiran Ramamoorthi[1,2], Kesavaram Padmavathy[3*], Jebadass JasmineVinshia[4]

[1]Research Scholar, Department of Microbiology, Sree Balaji Dental College and Hospital, Bharath Institute Higher Education and Research, Velachery Main Road, Chennai- 600100, India.
[2]Tutor, Department of Microbiology, Indira Medical College and Hospital, Pandur – 602001, Tiruvallur, India.
[3]Professor, Department of Microbiology, Research Laboratory for Oral and Systemic Health, Sree Balaji Dental College and Hospital, BIHER, Velachery Main Road, Chennai- 600100, India.
[4]Reader, Department of Microbiology, Rajas Dental College & Hospital, Kavalkinaru, Tirunelveli, India.
**Corresponding author - Kesavaram Padmavathy*

Abstract

Coagulase-negative staphylococci (CoNS), once considered benign commensals, are now recognized as emerging opportunistic pathogens. This study aimed to characterize the presence of key virulence genes (sea, seb, pvl, and tsst-1) and methicillin resistance in CoNS isolated from the anterior nares of healthy dental students. Sixty CoNS isolates were recovered from 69 participants and identified using the VITEK-2® system. Methicillin resistance was determined phenotypically and confirmed by PCR detection of the mecA gene. SCCmec typing and PCR for virulence genes were performed using established protocols. Methicillin resistance was observed in 75% of isolates, and 45% carried the mecA gene. SCCmec type I variant and type V were most common. Only six isolates (10%) harbored virulence genes: sea (1), seb (2), and pvl (3), while tsst-1 was absent. Notably, pvl was exclusively found in methicillin-sensitive strains. One S. haemolyticus isolate co-harbored sea and mecA, indicating a potentially higher pathogenic profile. These findings suggest that nasal CoNS in healthy individuals can act as reservoirs of virulence and resistance genes, posing a potential risk for transmission in healthcare environments.

Keywords: *Coagulase-negative staphylococci (CoNS), Methicillin resistance, Virulence genes, mecA gene, SCCmec typing.*

Introduction

Staphylococcus species are among the most common pathogens found in both community and hospital settings, where they are responsible for a wide range of infections. Among them, Staphylococcus aureus is the major species implicated in both community-acquired and nosocomial staphylococcal infections worldwide (1,2). Historically, coagulase-negative staphylococci (CoNS) were considered non-pathogenic contaminants in clinical specimens. However, their role as opportunistic pathogens gained recognition in the 1970s (3). Advances in clinical microbiology have since confirmed that CoNS species, such as *S.epidermidis,* S. haemolyticus, and S. saprophyticus, can cause serious infections, especially in hospitalized and immunocompromised individuals. These infections include bloodstream infections, prosthetic device-associated infections, and neonatal sepsis (4,5).

Distinguishing between commensal and pathogenic CoNS remains diagnostically challenging, as many CoNS species possess virulence genes similar to those found in S. aureus (6). Among these virulence determinants, staphylococcal enterotoxins (SEs) and superantigens are notable for their ability to non-specifically specifically activate T-cells, resulting in massive cytokine release that can lead to symptoms such as vomiting, diarrhea, and toxic shock (7). Till date, 23 staphylococcal enterotoxins have been identified. Of these, five—*sea, seb, sec, sed,* and *see*—are classified as classical enterotoxins and have been studied most extensively. Other enterotoxins, including *seg, seh*, and *sei*, also possess pathogenic potential and should not be overlooked. (8,9).

While S. aureus is primarily associated with staphylococcal food poisoning (SFP), recent evidence has demonstrated that certain CoNS species also possess the ability to produce enterotoxins (10–12). The sea

gene, which encodes Staphylococcal Enterotoxin A (Sea), is frequently detected in S. aureus and is a heat-resistant superantigen often implicated in foodborne illnesses. Sporadic detection of this gene in CoNS raises concern about horizontal gene transfer and highlights the possibility that CoNS may act as reservoirs of virulence factors (13).

Similarly, the *sebs* gene encodes Enterotoxin B (*seb*), a potent superantigen involved in food poisoning and toxic shock syndrome. *seb* triggers extensive cytokine release through non-specific immune activation. Though predominantly associated with *S. aureus*, the *seb* gene has also been found in CoNS strains isolated from nasal and clinical samples, suggesting an under recognized virulence potential in these species.(14,15).

In addition to enterotoxins, other virulence genes such as pvl and tsst-1 are of clinical importance. Panton-Valentine leukocidin (PVL) is a bicomponent toxin known for its cytotoxic effects on white blood cells and is commonly linked to community-acquired methicillin-resistant S. aureus (CA-MRSA). Studies have examined the presence of the pvl gene in methicillin-resistant and methicillin-susceptible S. aureus strains (16,17), but the occurrence of *pvl* in CoNS strains, particularly those isolated from nasal swabs, remains unclear. Toxic shock syndrome toxin-1 (*tsst-1*), encoded by the *tsst-1* gene, is the causative agent of toxic shock syndrome, an acute systemic illness characterized by high fever, hypotension, rash, and skin exfoliation (18,19). Although *tsst-1* is predominantly produced by *S. aureus*, especially in cases associated with tampon use, studies have reported its production by CoNS strains as well (20,11).

Despite these findings, the pathogenic potential of CoNS remains underexplored, particularly with regard to their role as carriers of critical virulence genes. This study seeks to determine the presence of four major virulence genes—sea, seb, pvl, and tsst-1—in CoNS isolates obtained from nasal carriers. Understanding the distribution of these genes in CoNS may provide valuable insights into their potential pathogenicity and their role as reservoirs of virulence factors within the human nasal microbiota.

Materials and Methods

Bacterial Isolates and Preliminary Identification

A total of 69 nasal isolates of coagulase-negative staphylococci (CoNS) were collected from preclinical dental students (male: female ratio = 14:55). The study protocol was reviewed and approved by the Institute's Ethical Committee of Sree Balaji Dental College & Hospital, Bharath Institute of Higher Education & Research, Chennai, India (Ref No: SBDCH/IEC/06/2021/1). Informed consent was also obtained from all participants before sample collection. Nasal swabs were inoculated on 5% sheep blood agar and MacConkey agar, followed by selective growth on mannitol salt agar (HiMedia Laboratories Pvt. Ltd., India). All plates were incubated at 37 °C for 24–48 hours. Presumptive CoNS colonies were further characterized phenotypically using standard biochemical tests including catalase, oxidase, and coagulase production.

Species-level identification of the CoNS isolates was performed using the VITEK-2® automated identification system (bioMérieux, France) with the GP identification card, according to the manufacturer's instructions.

Methicillin Resistance Detection and SCC mecTyping

Methicillin resistance was phenotypically detected using cefoxitin (30 µg) as a surrogate marker, by the Clinical and Laboratory Standards Institute (CLSI) 2025 guidelines (21). Isolates exhibiting resistance to cefoxitin were designated as methicillin-resistant CoNS (MRCoNS). Molecular confirmation of methicillin resistance was carried out by PCR amplification of the *mecA* gene. Isolates positive for *mecA* were further subjected to *SCCmec* typing using specific primers, as previously described in established protocols (24,25).

DNA Extraction

Genomic DNA was extracted using the boiling lysis method. Briefly, 2–3 well-isolated colonies were suspended in 200 µL of nuclease-free water in a sterile 1.5 mL microcentrifuge tube and vortexed for 10 seconds. The suspension was heated at 95 °C for 10 minutes, followed by immediate cooling on ice at –20 °C for 10 minutes. After centrifugation at 8000 rpm for 5 minutes, the supernatant containing genomic DNA was transferred to a new sterile microcentrifuge tube and stored at –80 °C until use.

Molecular Detection of Virulence Genes

The presence of selected virulence genes, including staphylococcal enterotoxins (*sea*, *seb*), Panton-Valentine leukocidin (*pvl*), and toxic shock syndrome toxin-1 (*tsst-1*), was detected using conventional PCR. Primer sequences and amplification conditions were adopted from previously published protocols (22).

The expected PCR amplicon sizes were as follows:

- *sea*: 560 bp
- *seb*: 404 bp
- *pvl*: 433 bp and *tsst-1*: 180 bp

Amplified products were visualized by electrophoresis on a 1.5% agarose gel stained with ethidium bromide and viewed under UV transillumination.

Table 1: Primer Sequences for Detection of Virulence and Resistance Genes in CoNS Isolates

Gene	Primer5'-3'	Amplicon Size (bp)	Reference
Sea	F-CATTGCCCTAACGTTGAC	619	(23)
	R-CGAAGGTTCTGTAGAAGTATGG		
Seb	F-CTAAACCAGATGAGTTGCAC	489	(23)
	R-CCAAATAGTGACGAGTTAGG		
Pvl	F-CTGGTGCGATTCATGGT	433	(22)
	R-CGATATCGTGGTCATCA		
Tsst-1	F-ATCGTAAGCCCTTTGTTG	578	(22)
	R-GTGGATCCGTCATTCATTG		
mecA	F-TGAGTTCTGCAGTACCGGAT	777bp	(24)
	R-TGATTATGGCTCAGGTACTGCTATCCACC		

Table 2: Representing the Primer Sequences and Annealing Conditions for SCCmec Typing in CoNS Isolates.

Typing	Gene	Primer sequence	Amplicon	Reference
SCCmec typing	β	F-ATTGCCTTGATAATAGCCYTCT	937 bp	(25)
	α3	R-TAAAGGCATCAATGCACAAACACT		
	ccrC	F-CGTCTATTACAAGATGTTAAGGATAAT R-CCTTTATAGACTGGATTATTCAAAATAT	518 bp	
	1272	F-GCCACTCATAACATATGGAA R-CATCCGAGTGAAACCCAAA	415 bp	
	5RmecA	F-TATACCAAACCCGACAACTAC	359 bp	
	5R431	R-CGGCTACAGTGATAACATCC		

PCR Amplification for SCCmec Typing and Virulence Gene Detection

PCR reactions for SCCmec typing and detection of virulence-associated genes (*pvl*, *tst*, *sea*, *seb*, and *mecA*) were performed in 20 µl total reaction volumes.

For SCCmec multiplex PCR, the reaction mixture consisted of 10.8 µl of nuclease-free sterile water, 2 µl of 10× PCR buffer (containing 15 mM $MgCl_2$), 1 µl of dNTP mix (10 mM each), and 0.5 µl of each of eight primers (10 pmol) — including α3, β, *ccrC*, 1272-F1/R1, and 5RmecA/5R431. Additionally, 0.2 µl of Taq DNA polymerase (5 U/µl) and 2 µl of DNA template were added to each reaction tube.

For single-gene PCR amplification targeting *pvl*, *tst*, *sea*, *seb*, and *mecA*, the reaction mixtures were adjusted accordingly: 14.4 µl of nuclease-free water, 2 µl of 10× PCR buffer, 0.5 µl of dNTP mix, 0.5 µl of each gene-specific primer (10 pmol), 0.1 µl of Taq DNA polymerase (5 U/µl), and 2 µl of template DNA.

All PCR mixtures were prepared on ice and gently mixed to ensure homogeneity before amplification.

Thermal Cycling Conditions

PCR amplifications were carried out under the following conditions: an initial denaturation at 94°C for 3 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at gene-specific temperatures for 30 seconds, and extension at 72°C for 1 minute. The annealing temperatures used were: 58°C for *mecA* and *pvl*, 56°C for *tst* and *sea*, 60°C for *seb*, and 55°C for SCCmec typing. A final extension step was performed at 72°C for 7 minutes.

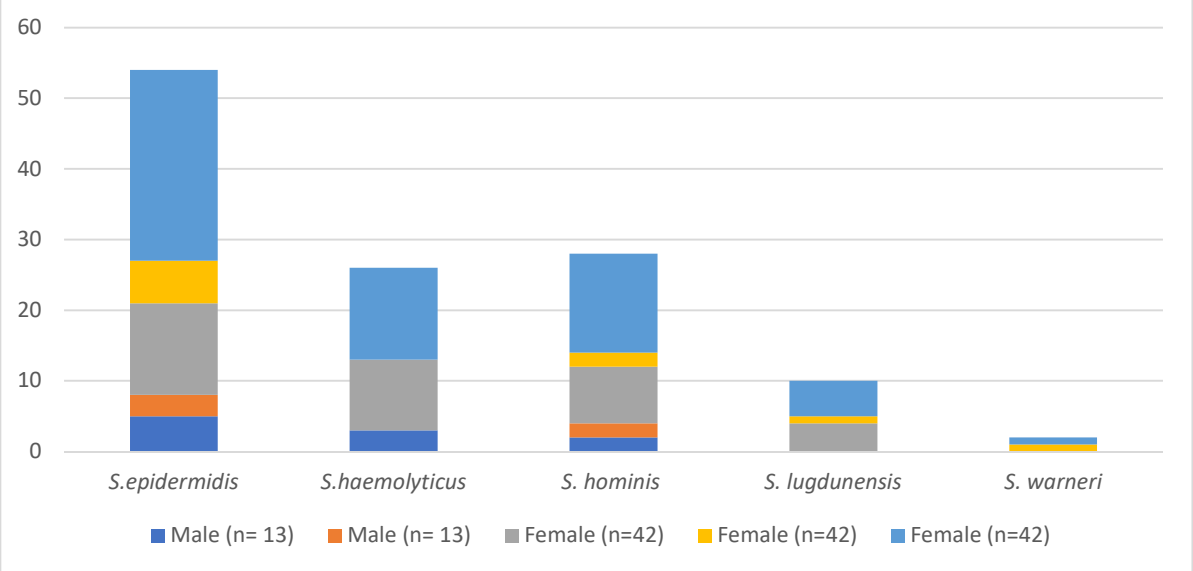
Results

Out of the 69 dental students enrolled in the study, 55 (13 males and 42 females) were found to carry *Staphylococcus* species in their anterior nares as part of their normal flora. From these 55 students, a total of 60 CoNS (coagulase-negative *Staphylococcus*) isolates were recovered. This included instances where two male and five female students were found to be colonized with dual isolates.

The gender-based distribution of CoNS colonization and methicillin resistance is illustrated in **Figure 1**. It was observed that female students harbored a higher number of isolates compared to males. Among the species identified, *Staphylococcus epidermidis* was the most frequently recovered isolate, followed by *S. haemolyticus* and *S. hominis* subsp. *hominis*.

Importantly, all *S. haemolyticus* isolates were found to be methicillin-resistant. Methicillin resistance was also observed in 80% of *S. lugdunensis*, 71.4% of *S. hominis* subsp. *hominis*, and 66.67% of *S. epidermidis* isolates, highlighting a significant burden of methicillin-resistant CoNS (MRCoNS) among the nasal carriage isolates.

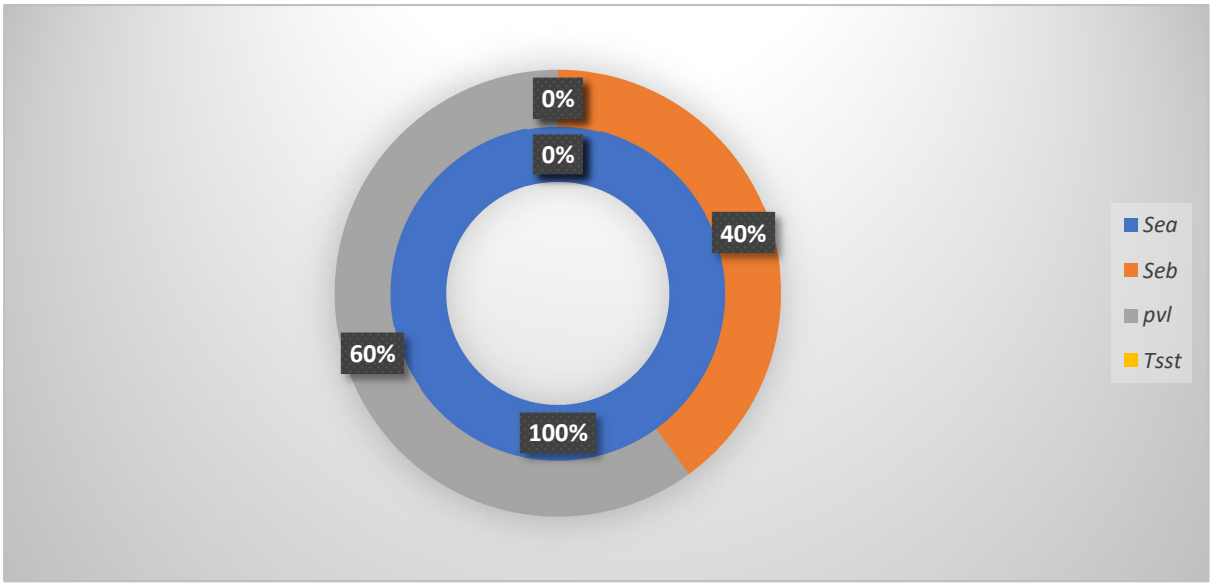
Figure 1: Distribution of Methicillin-Resistant and Methicillin-Sensitive CoNS Species Among Male and Female Dental Students.



Out of 60 CoNS isolates, only 6 were found to contain virulence genes. Among the screened genes, one isolate of *S. haemolyticus* carried the sea gene; two isolates of *S. epidermidis* carried the seb gene; and one isolate each of *S. epidermidis*, *S. warneri*, and *S. lugdunensis* carried the pvl gene. However, none of the CoNS isolates were positive for the tst gene. The distribution of these virulence genes among MRCoNS and

MSCoNS isolates is shown in **Figure 2**. Notably, all pvl-positive isolates were methicillin-sensitive, and the seb-positive *S. epidermidis* isolates were also methicillin-sensitive. In contrast, the sea-positive *S. haemolyticus* co-harbored the mecA gene and was further characterized as a variant of SCC mec type I*.

Figure 2: Distribution of Virulence Genes Among MRCoNS and MSCoNS Isolates.



Discussion

The nasal carriage rate of coagulase-negative staphylococci (CoNS) among dental students in our study was 79.7%, which aligns closely with previous studies by (26) (73.3%) and (27) (71.7%). However, several other studies reported comparatively lower carriage rates, including Khatter (58.8%), (28) (52.14%), (29) (39.3%), and (30, 31). Such variations might reflect differences in sampling populations, geographical settings, hygiene practices, and detection methodologies.

A significant finding in our study was the high prevalence (75%) of methicillin-resistant CoNS (MRCoNS) among the nasal isolates, which is considerably higher than those documented by (32) (45.9%), (33) (33.3%), (34) (22%), (35) (7.6%), (31) (5.5%), and (26) (2.1%). This elevated rate is clinically relevant as MRCoNS are known reservoirs of the mecA gene and have the potential to transfer methicillin resistance to *Staphylococcus aureus*, resulting in the emergence of MRSA, as noted by (36). Their study also suggested that MRCoNS nasal carriage could serve as a precursor for MRSA colonization in immunocompromised individuals, and possibly even in healthy individuals.

In our cohort, 45% (31/69) of the CoNS isolates harbored the mecA gene, including 40.6% MRCoNS and 4.4% methicillin-sensitive CoNS (MSCoNS). These findings exceed those reported by (37) (38%) and (27) (16.7%), but remain lower than those from clinical samples such as (22) (92.4%), (38) (82%), and (39) (78.6%).

Regarding SCCmec typing, our results revealed a predominance of SCCmec type I variant and type V among *S. haemolyticus* isolates. All *S. haemolyticus* isolates in our study were methicillin-resistant, and 92.3% were positive for the mecA gene, consistent with findings from (4) and (40). In contrast, *S. epidermidis* showed more SCCmec diversity, with 27.27% of isolates being non-typable. Interestingly, two *S. epidermidis* isolates were mecA-positive but phenotypically methicillin-sensitive, possibly due to gene suppression mechanisms, as described by (41). Our findings contrast with those of (42), who reported a higher prevalence of MRSA with SCCmec type IV among medical students, further supporting the emerging significance of MRCoNS in nasal colonization.

Virulence Gene Profile

The prevalence of virulence genes among nasal carrier CoNS isolates in our study was low. Among the 60 isolates, only six carried virulence genes: *sea*, *seb*, or *pvl*. Notably, *tst* was not detected in any isolate. One *S. haemolyticus* isolate harbored the *sea* gene, two *S. epidermidis* isolates carried *seb*, and *pvl* was found in one isolate each of *S. epidermidis*, *S. warneri*, and *S. lugdunensis*. Interestingly, all *pvl*-positive isolates were methicillin-sensitive.

This finding aligns with (43), who reported a low incidence (1.5%) of *tst* among nasal CoNS from medical students. Similarly, a study by (44) involving bus conductors reported the presence of *sea* and *tst* in 16.7% and 11.1% of isolates respectively, despite *pvl* was absent. These data support the notion that nasal colonizing CoNS strains have a lower frequency of virulence determinants compared to clinical isolates.

In contrast, several studies investigating clinical isolates have shown a significantly higher prevalence of virulence genes. For instance, (45) found that *seb* was more prevalent (27.5%) than *sea* (2.2%) in clinical CoNS. Another study demonstrated that *seb* was the most common virulence gene (62.3%) among clinical *S. epidermidis*, *S. capitis*, *S. hominis*, and *S. warneri*, whereas *tst* was the least frequent (2.1%). Neither *sea* nor *pvl* was detected in that study (22). These differences likely reflect the adaptation of clinical isolates to invasive and immune-evasive environments, in contrast to commensal carriage strains.

Conclusion

The presence of virulence genes (*sea*, *seb*, *pvl*) and the widespread occurrence of methicillin resistance via the mecA gene in nasal CoNS isolates from healthy dental students underscore their potential as opportunistic pathogens. These findings emphasize the need for continued surveillance of nasal CoNS, especially in healthcare-associated academic settings where students may serve as reservoirs and vectors of resistant strains. Infection control practices should be routinely enforced to minimize the risk of transmission and colonization.

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