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Original article

Molecular Detection of Mupirocin Resistance Genes in Coagulase-Negative Staphylococci from Nasal Carriers Among Pre clinical Dental Students

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Abstract

Coagulase-negative Staphylococci (CoNS) are increasingly recognized as significant nosocomial pathogens, frequently colonizing the nasal cavity of healthcare workers (HCWs) and serving as reservoirs for the spread of antimicrobial resistance. Mupirocin, a topical antibiotic widely used for nasal decolonization, is facing challenges due to the emergence of resistant strains, particularly those carrying the mupA gene. This study aimed to determine the prevalence of mupirocin-resistant CoNS colonizing the anterior nares of preclinical dental students and to assess associated methicillin resistance. Nasal swabs were collected from 69 dental students (14 males, 55 females), and 60 CoNS isolates were recovered from 55 individuals. Identification of the species and antibiotic susceptibility pattern of CoNS was performed using conventional microbiological methods and automated system i.e.VITEK-2. PCR was used to detect the presence of mupA, mupB, and mecA genes. The CoNS species isolated were Staphylococcus epidermidis (45%), S. hominis (23.3%), S. haemolyticus (21.7%), S. warneri (8.3%) and S. lugdunensis (1.7%). Of the total isolates, 26.7% were found to harbor mupA, while none carried mupB. Among the mupA-positive isolates, 68.75% co-harbored mecA gene, indicating methicillin resistance. High rates of mupA carriage were observed in S. hominis (42.9%) and S. epidermidis (29.6%). Antibiotic resistance profiling revealed universal resistance to benzyl penicillin in several species and significant resistance to cefoxitin and oxacillin among methicillin-resistant isolates. These findings highlight the nasal carriage of Mu^{R}MRCoNS among dental students and under scores the need for routine surveillance and targeted decolonization strategies to prevent the transmission of resistant pathogens in clinical settings.

Key words: *CoNS, mupA, mupB, mecA, mupirocin resistance.*

Introduction

Nasal colonization of *Staphylococcus* species among healthcare workers is of concern as they frequently come in contact with patients in clinical settings. Nasal colonization serves as a likely pool for the spread of infections

within the hospital environment. To combat this, mupirocin, a topical antibiotic, has been widely recognized as an effective agent for eradicating nasal colonizers, thereby reducing the risk of infections originating from these bacterial reservoirs (1).

Nasal colonizers not only contribute significantly to the spread of infections but also play a critical role in the dissemination of antibiotic-resistant strains. This makes them a key factor in both nosocomial infections and broader public health concerns related to antimicrobial resistance (2).

Most of the *Staphylococcus* colonization is endogenous, meaning that it originates from within the host rather than being acquired externally. This type of colonization often predisposes individuals to subsequent infections, particularly in immunocompromised patients or those undergoing invasive medical procedures. Such infections pose a significant challenge in clinical settings, as they can lead to prolonged hospitalization and escalated health care costs (3).

With the rising prevalence of antibiotic-resistant bacteria and nosocomial infections, eliminating nasal colonization has become a crucial component of infection control measures. The ability of resistant strains to spread rapidly within healthcare facilities necessitates targeted interventions to curb their transmission. Therefore, strategies aimed at decolonizing healthcare workers and high-risk patients are essential in reducing infection rates and improving patient outcomes (4).

Mupirocin, formulated as a nasal antimicrobial agent, is widely used to eliminate *Staphylococcus* colonization in the nasal cavity. Despite its effectiveness, continuous monitoring of resistance patterns is necessary to prevent the emergence and spread of mupirocin-resistant strains. While the application of mupirocin is strongly recommended, its use must be carefully managed to balance its benefits with the risk of developing resistance. Healthcare workers, being in close contact with patients, act as key vectors in the transmission of resistant infections. Consequently, routine screening and treatment of colonized healthcare workers can significantly reduce the risk of spreading multidrug-resistant organisms to vulnerable patients (5).

Mupirocin has been advocated for decolonization of the anterior nares of carriers. This targeted approach is particularly important in hospital settings, where healthcare workers serve as a major source of pathogen transmission. By decolonizing healthcare workers, the risk of nosocomial infections can be minimized, contributing to improved infection control and patient safety (6).

Mupirocin resistance arises through two primary mechanisms: genetic mutations and the horizontal transfer of resistance genes. Acquired *mupA* resistance allows the bacteria to withstand mupirocin treatment, thereby reducing the effectiveness of this antibiotic. These resistance mechanisms can spread within bacterial populations, further complicating treatment strategies (7,8).

MupL (low-level) and MupH (high-level) resistance are known to exist. MupL is attributed to point mutations in *ileS* that encodes the native isoleucyl-tRNA synthetase, which slightly reduces the affinity of the enzyme for mupirocin. In contrast, MupH is mediated by plasmid encoded *mupA* (*ileS2*), that encodes a modified isoleucyl-tRNA synthetase which significantly reduces susceptibility to mupirocin. Recently, another gene, *mupB*, has been identified as a contributor to high-level mupirocin resistance. The increased prevalence of MupH may be associated with selective pressures in hospital environments, particularly during outbreaks of *Staphylococcus aureus* especially methicillin resistant -MRSA. However, studies on mupirocin resistance in carrier isolates of

coagulase-negative *Staphylococci* (CoNS) remains limited. Additionally, molecular studies exploring the MupR in *Staphylococcus* species, particularly in Indian isolates, are scarce and warrant further investigation (9).

The present study aimed to determine the prevalence of mupirocin-resistant isolates of coagulase-negative Staphylococci (CoNS) colonizing the anterior nares of preclinical dental students.

Materials and Methods:

Species identification of CoNS:

Nasal swabs collected under aseptic conditions from the anterior nares of preclinical dental students (n=69, male: female=14:55). Staphylococci recovered from the samples were phenotypically identified by Gram reaction, biochemical tests including, production of catalase, oxidase and coagulase were detected by standard biochemical tests. The samples were inoculated on 5% sheep blood agar and MacConkey agar and selective isolation was done by plating onto Mannitol salt agar and incubated at 37°C. Isolated colonies were adjusted to 0.5 MacFarland standard for species identification and to assess the susceptibility testing pattern using VITEK-2 (BioMe´rieux) automated system. *Staphylococcus aureus* ATCC25923 was included as Control.

DNA Isolation:

DNA was extracted by boiling lysis method. Briefly, 2 – 3 isolated colonies were inoculated into 200µl of Nuclease free water in a sterile 1.5 ml eppendorf tube and were vortexed. Followed by boiling at 95ºC for 10 mins. Tubes were immediately cooled at -20ºC for 10mins. The tubes were centrifuged at 8000rpm for 5mins. The supernatant was transferred to a sterile 1.5 ml microfuge tube and stored at -80ºC until further use.

mupA and *mupB* gene targeted PCR was performed for the bacterial isolates using previously described primers (10). A total of 25µL reaction mixture comprising of appropriate primers (10 p moL of each primer, *mupA*-Forward and reverse, *mupB* -Forward and Reverse), 10× PCR buffer, dNTP mix (10 mM each), Taq DNA (1U), DNA template (0.1-1 µg) and PCR grade water. PCR was performed using the Veriti 96-Well Thermal Cycler (Applied Biosystems, USA) with the following cycling conditions, 94°C for 3 minutes (initial denaturation), 35 amplification cycles (94°C for 30 seconds: 55°C for 30 seconds, and 72°C for 45 seconds - denaturation, annealing and extension respectively) and a finally 72°C for 7 minutes (final extension). A positive control was included in each run to validate the reaction. The expected amplicon sizes were 456 base pairs for the *mupA* gene and 674 base pairs for the *mupB* gene.

mecA gene targeted PCR was performed for the bacterial isolates using previously described primers (11). A total of 25µL reaction mixture comprising of appropriate primers (10 p moL of each primer, *mecA*-Forward and reverse), PCR buffer (10×), dNTP mix (10 mM), Taq DNA (1U), DNA template (0.1-1 µg) and PCR grade water. The PCR using the Veriti 96-Well Thermal Cycler (Applied Biosystems, USA) and the following cycling conditions were adopted, 95°C for 3 minutes (initial denaturation), 35 amplification cycles (94°C for 60 seconds: 55°C for 60 seconds, and 72°C for 120 seconds - denaturation, annealing and extension respectively) and a finally 72°C for 5 minutes (final extension). A validated positive control was included to ensure the reliability of the amplification process.

Following amplification, agarose (1%) gel electrophoresis was carried out at 135 V for approximately 15 minutes using the Mupid-exU system (Takara, Japan) to visualize the amplicons. Imaging of the DNA bands was carried out with a BioGlow UV Transilluminator (Crystal Technology, USA).

Results:

Among sixty nine dental students screened, 55 (13 males and 42 females) were found to possess *Staphylococcus* species as commensals in their anterior nares. A total of 60 coagulase-negative *Staphylococci* (CoNS) isolates were isolated and identified, with 7 students (2 males, 5 females) exhibiting polymicrobial colonization, co-existence of 2 different CoNS species.

The most prevalent species was *S. epidermidis* (27 isolates), followed by *S. hominis* (14), *S. haemolyticus* (13), *S. lugdunensis* (5), and *S. warneri* (1). Among these 60 CoNS isolates, 16 (26.7%) carried the *mupA* gene. The distribution of *mupA*-positive isolates was as follows: *S. epidermidis* (8/27, 29.6%), *S. hominis* (6/14, 42.9%), *S. haemolyticus* (1/13, 7.7%), and *S. lugdunensis* (1/5, 20%) (Table 1). Nevertheless, none of the CoNS isolates were found to harbor *mupB*.

Table 1: Distribution of *mupA* in nasal carrier isolates of coagulase negative staphylococci :

CoNS Isolates (n=60)	*mupA* positive (n=16) %	*mup A* negative (n=44) %
S. epidermidis (n=27)	8 (29.6)	19 (70.4)
S. haemolyticus (n=13)	1(7.7)	12(92.3)
S. hominis ssp hominis (n=14)	6 (42.9)	8(57.1)
S. lugdunensis (n=5)	1(20)	4(80)
S.warneri(n=1)	0(0)	1(100)

Of the 16 CoNS isolates that harbored *mupA,* majority (11(68.75%)) were found to co-harbor *mecA* gene, hence were MRCoNS while, 5(31.25%) were MSCoNS. Of note, nearly 62.5% of MR *S.epidermidis* isolates were found to harbor *mup A* gene. Among the MR *S. hominis spp hominis* isolates, only 66.7% harbored the *mup A*. Remarkably, both MR *S. haemolyticus* (100%) and MR *S. lugdunensis* (100%) isolates were found to be *mupA* positive (Table 2). Interestingly, *mupA* was detected in 37.5%, 33.3% of the *S. epidermidis* and *S. hominis spp hominis* isolates with a MSCoNS phenotype respectively (Table 2).

Table 2: Distribution of *mupA* among MRCoNS and MSCoNS:

mupA positive CoNS Isolates (n=16)	MRCoNS % (n=11)	MSCoNS % (n=5)
S.epidermidis (n=8)	5 (62.5)	3 (37.5)
S.haemolyticus (n=1)	1(100)	0 (0)
S. hominis ssp hominis(n=6)	4 (66.7)	2(33.3)
S. lugdunensis (n=1)	1(100)	0(0)

Antibiotic sensitivity testing by VITEK-2 (bioMe´rieux)showed that, all the *S. haemolyticus* isolates exhibited resistance to benzyl penicillin (100%), cefoxitin (100%) and oxacillin (100%). Nevertheless, the resistance percentage of *S. epidermidis* was relatively less to benzyl penicillin (87.5%), cefoxitin (62.5%) and oxacillin (62.5%). *S. lugdunensis* showed 100% resistance to benzyl penicillin, cefoxitin oxacillin, erythromycin and,

clindamycin. *S. hominis sub spp hominis* showed higher resistance to tigecycline (100%) followed bybenzyl penicillin (83.3), cefoxitin (66.7) and oxacillin (66.7).

Discussion:

CoNShad gained attention as an inevitable nosocomial pathogen with increasing resistance to antibiotics (12). Also, data related to CoNS especially recovered from healthy volunteers in healthcare facilities are under reported (13). Since 1980's the existence of MR-CoNSwere reported and their degree of resistance is increasing over the years (14). Previous Indian studies had reported a lower nasal carriage rate (4.3%, 8%) of mupirocin resistant CoNS among healthcare workers (15,16). However, very few studies had screened for nasal carriage of *mupA* positive CoNS among healthcare workers. A recent study conducted among healthcare workers in an European teritary care hospital had reported that 14.3% of the CoNS were positive for *mupA* (17).To the best of our knowledge, this is the first Indian report on carriage of *mupA* positive CoNS among healthcare workers. Of note, 26.6%of our nasal carrier isolates were found to harbor *mupA* gene. This report is of clinical significance, as HCWs have been documented to be reservoirs for the transmission of resistant genes including *mupA*. Another study by Abdullahi etal., 2024 had reported a very high (64.9%) nasal carriage rate of *mupA* positive CoNS among pet owners (dog) (18).

In the present study the MR status of CoNS was 11%, whereas study performed by Eed and Coworkers in 2019 showed 32.1% which coincides with the present study, Similar kind of results were also generated by Bhatt etal in 2016 Showed 32.7% of MRCoNS status by PCRfor *mec A* gene targeting (19,20). This is in concordant with our study also showed 43.3% of *mec A* gene positivity by PCR. In contrast, a Swedish study reported no methicillin-resistant coagulase-negative staphylococci (MRCoNS) among hospital isolates (13).

Conclusion:

Unrestrained and extended use of mupirocin has been linked with the widespread emergence of mupirocin resistance in both MRCoNS as well as MSCoNS (21). Increasing resistance to mupirocin among methicillin-resistant CoNS (MRCoNS) among healthcare workers (HCWs) is a significant concern, particularly in nasal carriers. Routine screening for methicillin and mupirocin resistance in CoNS is essential, along with decolonization strategies to prevent healthcare-associated infections. The high prevalence of CoNS nasal colonization among dental students further emphasizes the need for ongoing surveillance and prompt control strategies to curb infection transmission in healthcare teaching institutions. Prompt detection of nasal carriers of *mupA* positive CoNS among HCWs would enable judicious administration of the decolonizing agent for the treatment of nasal carriers.

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