Research article

A preliminary study of *mecA* gene expression and methicillin resistance in staphylococci isolated from the human oral cavity

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Abstract

Introduction: Staphylococci are common human commensals that acquire methicillin resistance via the *mecA* gene. Methicillin resistance in staphylococci from various clinical sources has been assessed using cefoxitin disc diffusion test (CDDT) and PCR detection of the *mecA* gene. However, oral staphylococci have been studied less frequently compared with other clinical sources. There are no previous studies on methicillin resistance in oral staphylococci in Sri Lanka.

Objective: This study aimed to demonstrate methicillin resistance in staphylococci isolated from the human oral cavity using CDDT and PCR detection of *mecA* gene.

Materials and methods: Twenty-five oral isolates of staphylococci were selected after confirming their identity using colony morphology, Gram stain, catalase test, and the coagulase test. Further authentication of identity was obtained using amplification of the 16S rRNA gene. Methicillin resistance was demonstrated using CDDT and PCR detection of the *mecA* gene.

Results: There were 7 (28%) isolates of coagulase positive (presumed *S. aureus*) and 18 (72%) of coagulase negative staphylococci (CoNS). All the coagulase positive isolates were methicillin sensitive. Within the 18 CoNS, 2 (11%) were methicillin resistant and were found to carry the *mecA* gene using PCR.

Conclusion: Coagulase positive and negative staphylococci with or without methicillin resistance may colonize the human oral cavity. Coagulase negative staphylococci were the majority in this limited study. Further studies are warranted to

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determine the incidence of staphylococci in the oral cavity and their antimicrobial sensitivity.

***Key words**: mecA gene, Methicillin resistance, Oral cavity, Staphylococci*

Introduction

Staphylococci are important human commensals inhabiting the skin, nasal mucosa and the oral mucosa.[1-4] Staphylococci are notorious opportunistic pathogens that are responsible for the majority of hospital acquired infections worldwide.[5,6] While *S. aureus* is the leading pathogenic species, other species of coagulase negative staphylococci (CoNS) have also emerged as pathogens, especially in immunocompromised patients and patients with prosthetic devices.[7]

Several investigations support the fact that staphylococci are human oral colonizers both in health and disease. For instance, staphylococci have been abundantly isolated from the subgingival biofilm collected from patients with chronic periodontitis as well as from healthy individuals.[8-10] A recent analysis of subgingival biofilm collected from patients with chronic periodontitis as well as from healthy individuals identified both *S. aureus* and CoNS including *S. auricularis, S. epidermidis* and *S. saprophyticus* as oral microorganisms.[8] Staphylococci are also found to colonize removable partial dentures along with *Candida* and enteric bacilli.[11,12]

Development of antimicrobial resistance in staphylococci is a serious challenge faced by the clinicians.[5,6] In addition to the production of beta-lactamase, staphylococci generate antimicrobial resistance through the *mecA* gene that encodes penicillin binding protein-2a (PBP-2a) responsible for methicillin resistance.[13,14] PBP-2a has lower affinity for β-lactam antibiotics compared to the typical penicillin binding protein-2 (PBP2) produced by methicillin susceptible *S. aureus* (MSSA) as it blocks the active site from binding β lactams.[15,16] Consequently, staphylococci which carry chromosomally confined *mecA* gene are considered highly virulent due to their resistance to all β-lactam antibiotics.

Methicillin resistance in staphylococci is often detected by antimicrobial disc diffusion or broth dilution methods whereas detection of the *mecA* gene by PCR is a rapid and far more reliable technique.[17-20] Although methicillin resistance in staphylococci isolated from different human sources has been studied extensively[5], there are very few studies on methicillin resistance in oral staphylococci.[9] The purpose of this study therefore was to investigate methicillin resistance in oral staphylococci, using the CDDT and PCR for the detection of the *mecA* gene.

Materials and methods

Isolates of staphylococci

A total of 25 *Staphylococcus* isolates collected from the oral cavities of patients attending the Dental (Teaching) Hospital, Peradeniya, Sri Lanka were used for the study. These isolates were collected in an earlier study during which patients' informed consent was obtained to use such organisms for future research. The isolates

included 9 samples collected by subgingival plaque sampling and 16 samples collected using the concentrated oral rinse technique.[8] None of the samples were identifiable by the personal details of the patient. Ethical approval was obtained from the ethics review committee of the Faculty of Dental Sciences, University of Peradeniya.

Freeze-stored bacteria samples were recovered by culture on blood agar at 37 °C for 24-48 h. Identity of the bacteria was reconfirmed by cultural characteristics on blood agar, Gram stain, catalase and coagulase tests.

Cefoxitin disc diffusion test (CDDT)

The antibiotic sensitivity of staphylococci was tested using the CDDT following the Clinical and Laboratory Standards Institute (CLSI).[21] Standard suspensions of bacteria (0.5 McFarland) were prepared and inoculated onto Muller Hinton Agar (MHA) plates. After placing cefoxitin 30µg discs in the center of the plates, they were incubated at 37 ºC for 18-24h and the zones of inhibition were measured. For coagulase positive staphylococci (*S. aureus*), an inhibition zone diameter of ≤ 21mm was considered as methicillin resistant and ≥ 22mm was considered as methicillin sensitive whereas for CoNS, inhibition zone diameter of ≤ 24mm was considered as methicillin resistant and ≥ 25mm was considered as methicillin sensitive (CLSI M100[21]).

Extraction of DNA

The species characterization and demonstration of *mecA* gene in the genomic DNA of staphylococci were performed according to a method described previously with minor modifications.[22]

All 25 staphylococcal isolates, standard isolates of MSSA (ATCC 25923) and MRSA (ATCC 43300) were subjected to DNA extraction. Bacterial DNA was extracted from fresh bacterial cultures grown overnight on blood agar medium. From the fresh bacterial cultures, 3 to 4 loopfuls were harvested into 10mM TE buffer (10mM Tris-HCl pH, 7.5 /25mM EDTA) and subsequently washed twice with 10mM TE buffer. The resultant pellet after centrifugation was suspended in 0.6ml of 10mM TE buffer followed by addition of 10-20µl of lysozyme (50mg/ml) to the cell suspension and incubated at room temperature for 30min. The suspension was mixed gently after addition of 20µl of proteinase K (10mg/ml) and 60µl of SDS (10%) and the final suspension was incubated at 50 °C for 1h. The suspension was then mixed well with 0.6ml of phenol/chloroform and centrifuged at 13000rpm for 15min. 30µl of 5M NaCl was added to the aqueous layer extracted from the centrifuged product. This phenol/chloroform step was repeated once more with 10 min centrifugation and the resulting aqueous solution mixed with two volumes of absolute ethanol and centrifuged at 10000rpm for 5min. The supernatant was discarded, and the pellet washed with 70% ethanol. Finally, the DNA pellet was dried and dissolved with 50-100µl of TE buffer and stored at -20 ºC. The quality of the DNA was assessed by electrophoresis in 1% agarose gel.

Species characterization and the detection of *mecA* gene by multiplex PCR

16S rRNA gene amplification was performed as an internal control using the primers given in Weisburg *et al*.[23] Accordingly, FD1 (5'- AGAGTTTGATCCTGGCTCAG -3') and RD1 (5'- AAGGAGGTGATCCAGCC -3') primers were used to amplify the region of *16S rRNA* gene with the amplicon size of 1500bp.

For the amplification of *mecA* gene with the amplicon size of 532bp PCR was performed using the primers described previously.[17,22] The *mecA* locus was amplified using forward and reverse primers, (5'-AAAATCGATGGTAAAGGTTGG-3'/5'-AGTTCTGCAGTACCGGATTTGC-3') respectively. The amplifications were performed in 15µl reaction volumes each with 5µl of Taq mix (2X GoTaq Green® master mix reaction buffer [pH,8.5] with 400µM dATP, 400µM dATP 400µM dGTP, 400µM dTTP, 400µM dCTP, and 3mM $MgCl_2$), 0.5µl of each primer, 6ng templates DNA and nuclease free water. The reactions were carried out in a thermal cycler using the following program. Initial denaturation at 94 ºC for 5min, followed by 35 cycles of 1min of denaturation at 94 ºC, 1min of annealing temperature 55 ºC, 30 seconds of extension at 72 ºC and final extension at 72 ºC for 10min. The amplified PCR products were subsequently visualized on 1.5 % agarose gel stained with ethidium bromide (1µg/ml) for confirmation of PCR amplification. Finally, PCR products were visualized under UV and photographed.

Results

Identification of staphylococci

All 25 staphylococcus isolates were Gram positive, catalase positive cocci arranged in clusters. There were 7 (28%) coagulase positive staphylococcal (presumed *S. aureus*) isolates and the remaining 18 (72%) were coagulase negative staphylococci (CoNS). *16S rRNA* gene amplification results confirmed that all the isolates were staphylococci (Figure1). Both isolates which were identified as methicillin resistant by CDDT demonstrated the *mecA* gene by PCR whereas the remaining 23 isolates were negative for the *mecA* gene (Figure1).

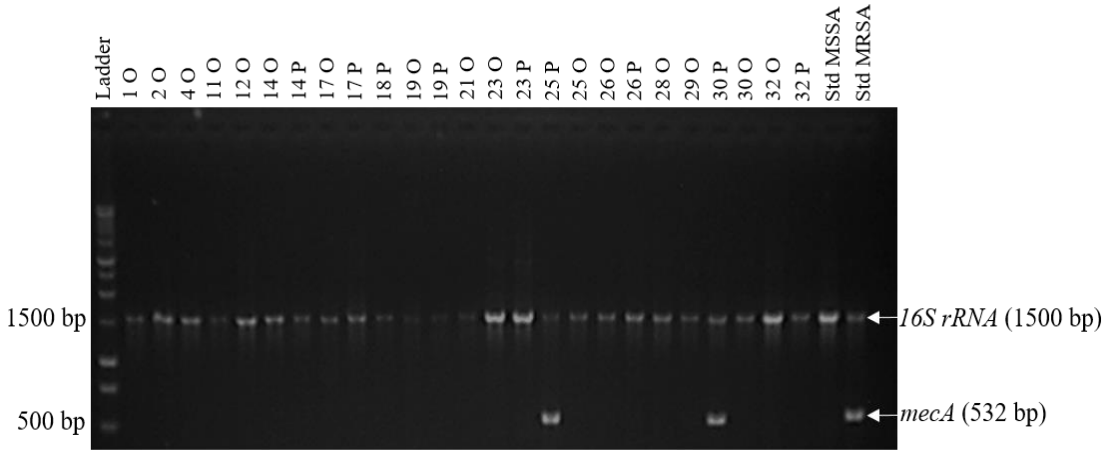

Figure 1 Gel image of the multiplex PCR using primer pairs for the amplification of *mecA* locus and the internal control *16S rRNA* locus, performed on all staphylococcal isolates, standard susceptible and resistant isolates.

Discussion

Although staphylococci are known to be frequent colonizers of the oral cavity, the incidence of methicillin resistance in oral staphylococci is poorly studied.[1,2,24] Hence, the current study investigated methicillin resistance and the responsible *mecA* gene in oral staphylococci isolated from a group of Sri Lankan patients.

Although a very limited study, the present study showed that the majority (72%) of oral staphylococci were CoNS supporting the previous findings of Loberto *et al*.[8] Only two of the coagulase negative staphylococci and none of the coagulase positive staphylococci in the present study were methicillin resistant. The present study shows that methicillin resistant staphylococci are found in the oral cavity of patients presenting to the Dental (Teaching) Hospital, Peradeniya, Sri Lanka. A retrospective analysis of data relevant to diagnostic oral microbiology in the UK showed that a small proportion (5%) of *S. aureus* isolated from oral specimens were MRSA.[2] However, these investigators did not report on the methicillin resistance of CoNS isolates. In contrast, another study that compared oral colonization of opportunistic pathogens including staphylococci in elderly Japanese patients with oral cancer and a healthy group showed that a large proportion, 9 of 13 oral *S. aureus* (69.2%) were MRSA. These investigators also demonstrated that 1 of 9 oral CoNS isolates (11.1%) were methicillin resistant.[25] Data obtained in the current study should be carefully interpreted due to the limited number of samples used in the analysis. Further studies using a larger sample would be beneficial to confirm the incidence of oral staphylococci and their antimicrobial resistance.

Both methicillin resistant isolates in the current study were collected from subgingival plaque samples of patients with chronic periodontitis lesions. Although some investigators[8-10] have isolated staphylococci from the subgingival biofilm collected from patients with chronic periodontitis as well as from healthy individuals, antimicrobial resistance of those staphylococci has not been adequately studied. Therefore, the detection of methicillin resistant isolates in subgingival plaque samples of patients with chronic periodontitis lesions warrants further investigations of antimicrobial resistance in staphylococci associated with periodontitis lesions.

As a phenotypic method for the detection of MR in staphylococci, the disc diffusion test was carried out using cefoxitin which is considered as the most reliable antibiotic for this purpose at present. Multiplex PCR assay was used to demonstrate the *mecA* gene in staphylococci. It has already been suggested that the detection of *mecA* gene with PCR offers rapid, simple, and accurate identification of methicillin resistance in staphylococci.[19] PCR and CDDT corroboration in this very limited study agrees with the previous reports that CDDT is in concordance with the PCR for demonstration of *mecA* gene.[20]

In conclusion, *S. aureus* and CoNS with or without methicillin resistance may colonize the human oral cavity as discussed above. Therefore, further studies with an increased sample size are warranted to confirm the exact prevalence of methicillin resistance in oral staphylococci.

Conflicts of interest: There are no conflicts of interest

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