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Prevalence of *Staphylococcus aureus* and methicillin resistant *Staphylococcus aureus* (MRSA) in the oral cavity

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ABSTRACT

Objective: To assess the prevalence of *Staphylococcus aureus* and methicillin resistant *Staphylococcus aureus* (MRSA) in plaque and tongue samples from systemically healthy subjects with periodontal health, gingivitis or chronic periodontitis.

Methods: After screening 720 potentially eligible subjects, 154 systemically healthy participants were ultimately enrolled in the current study. Subgingival samples were taken from the first molars and the tongue and analyzed for the presence of *S. aureus* and MRSA by polymerase chain reaction (PCR), using primers and conditions previously described in the literature. In addition, samples were taken from deep periodontal pockets of chronic periodontitis patients. Statistical analysis was performed by applying non-parametric tests (Kruskal–Wallis for clinical parameters, and z-test with Bonferroni corrections for distributions of assessed parameters). All comparisons were set at the 0.05 significance level.

Results: *S. aureus* was detected in 18% of all participants and in 10% of the samples tested. No significant differences were found in its distribution among the three investigated groups (z-test for proportions with Bonferroni corrections, $p > 0.05$). The *mecA* gene was not present in any of the *S. aureus* found.

Conclusions: *S. aureus* can be found in the oral environment regardless of the periodontal conditions and therefore should be considered as a member of the transient flora not participating in periodontal pathology. Subgingival sites and tongue surfaces seem to be an unusual habitat of MRSA.

1. Introduction

The prevalence of *Staphylococcus* spp. in the oral cavity and their contribution to periodontal infections have not been extensively investigated. Although they have been isolated in the oral cavity and are correlated with conditions such as suppurative parotitis, angular cheilitis, denture stomatitis and acute dentoalveolar infections, they are generally regarded as transient members of the oral flora.[1–3] Recent studies also

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suggest that *Staphylococci* can be frequently isolated from the oral cavity of specific patient groups such as newborns in hospital units, the elderly, patients with malignancies as well as subjects with immunocompromising systemic conditions such as rheumatoid arthritis.[4–6] Last but not least they are considered as aetiological factors of prosthetic valve endocarditis.[7]

The relationship between periodontal disease and *Staphylococci* is currently not well understood. In a number of studies, using cultural techniques, they have been isolated from plaque samples, however a wide range for their prevalence (5.6–75%) has been reported. In studies including more than 500 subjects with periodontitis, it has been observed that approximately half harbour *Staphylococcus* spp. with *S. epidermidis* and to a lower extent *S. aureus* being the predominant species,[1,8–11] but data comparing their prevalence with samples from periodontally healthy controls have not shown differences between these groups.[3]

Although usually a harmless colonizer of the skin and the nasopharynx in 25–35% of healthy individuals, on some occasions *S. aureus* is known to cause severe infections.[12] One of the concerns with *S. aureus* is the current extent of the prevalence of methicillin resistant *Staphylococcus aureus* (MRSA) which was initially isolated 50 years ago, only two years after the introduction of methicillin in clinical practice and has developed into a major global health issue due to its pathogenic potential to cause bloodstream infections, pneumonia as well as surgical site infections.[13] Resistance of *S. aureus* to β-lactam antibiotics is acquired by the exogenous *mecA* gene, which encodes a modified form of Penicillin Binding Protein (PBP2a), that does not allow proper binding and thus prevents the inhibition of cell wall synthesis that this class of antibiotics cause.[14,15]

Healthcare-associated MRSA (HA-MRSA) is related to prolonged length of hospital stay and is currently one of the most frequently identified pathogens in hospitals in many parts of the world.[16] Furthermore, Community acquired MRSA (CA-MRSA) has demonstrated increasing trends, hence guidelines for prevention and surveillance have been issued by several healthcare officials.

Thus, MRSA in the oral cavity could potentially be disseminated by carriers to the environment or to other individuals.[17]

The aim of the present study is to investigate the prevalence of *S. aureus* and the *mecA* gene encoding for MRSA in the oral cavity of Greek subjects with various periodontal conditions.

2. Materials and methods

2.1. Patient selection

720 subjects attending the Clinic of Periodontology at 251 Air Force Hospital, Athens, Greece and the Clinic of the Department of Preventive Dentistry, Periodontology and Implant Biology, Dental School, Aristotle University of Thessaloniki, Greece, were screened in order to be enrolled in the study from September 2011 to April 2014.

Subjects were deemed eligible as long as they fitted the following criteria: age >30 years, absence of systemic diseases or medications known to affect periodontal tissues, without infectious conditions (hepatitis, HIV) or pregnancy and lactation, who did not receive periodontal treatment or antibiotics within the last six months. They were also required to have at least 20 teeth present and meet the criteria of one of three periodontal conditions (healthy, gingivitis or periodontitis). Periodontally healthy subjects would have no periodontal pockets measuring more than 3 mm and bleeding on probing would be less than 10%. Gingivitis subjects had no periodontal pockets measuring more than 4 mm or attachment loss of more than 3 mm and bleeding on probing should exceed 20%, without radiographic bone loss. Moderate or advanced periodontitis subjects had ≥30% of teeth with proximal attachment loss ≥5 mm, and presented radiographic bone loss which exceeded 30%.[18] Out of the 720 subjects initially screened, 156 fulfilled the inclusion criteria and 154 agreed to participate in the study.

The study was conducted according to the protocol outlined by the Research Committee, Aristotle University of Thessaloniki, Greece and approved by the Ethical Committee of the School of Dentistry (#120), in compliance with the ethical principles of the World Medical Association Declaration of Helsinki. All patients read and signed an appropriate informed consent document prior to the participation in the study.

2.2. Study design and clinical protocol

The present cross-sectional study, included three groups as follows: (a) periodontal health ($n = 50$), (b) gingivitis ($n = 52$), and (c) moderate or advanced chronic periodontitis ($n = 52$). Clinical recordings were performed at six points of all teeth present at the dentition (mesio-buccal, buccal, disto-buccal, mesio-lingual, lingual, distolingual). Recordings included the following parameters: Probing Pocket Depth (PPD), Recession, Clinical Attachment Level (CAL) and Bleeding on Probing (BOP). Recordings were performed by a calibrated examiner (GK) using an automated probe (Florida Probe, Florida Probe Corporation, Gainesville, FL, USA).

All subjects were interviewed by one of the authors (DS) and completed a questionnaire regarding the following: smoking, frequency of antibiotic intake for medical and dental reasons, class of antibiotics used 6–12 months before the interview as well as within the 5-year period prior to the interview, whether they have ever obtained antibiotics without prescription, whether they have antibiotics available at home and whether they are aware of the phenomenon of antibiotic resistance. In order to avoid false reporting by participants, the class of antibiotics that they had used was recorded based on their personal National Health Record.

2.3. Sampling and analysis

Two clinical samples were collected from each patient: a pooled subgingival plaque sample from the mesiobuccal surface of the four first molars (or premolars when molars were missing) taken with sterile Gracey curettes, and a sample collected from the dorsal surface of the tongue with a sterile straight surgical bone curette, after applying three consecutive strokes. All samples were immediately placed in 200 μl of TE