

# Identification of *Staphylococcus aureus* at the internal and external implant surfaces in individuals with periimplant disease: A cross-sectional study

## Abstract

### Objective

The objective of this study was to investigate the prevalence of *Staphylococcus aureus* (*S. aureus*) at internal and external dental implant surfaces in patients with periimplant disease.

### Materials and methods

Samples for microbiological analysis were obtained from four types of sites in the following order: (1) the periimplant sulcular fluid (PISF) of each implant; (2) the gingival sulcus (GS) of the adjacent teeth; (3) the implant–abutment connection and abutment inner portions (IIP) of each implant; and (4) the oropharyngeal complex (OF)—oral, tongue and pharynx swabs were also collected.

Quantitative real-time polymerase chain reaction assays were carried out for total bacterial counts. The Kruskal–Wallis test was used to compare the *S. aureus* levels at the various sites.

### Results

Mean bacterial counts of *S. aureus* were as follows: GS =  $5.02 \times 10^2$ ; PISF = 0, IIP = 0 and OF = 0. A positive value was found for one out of the 35 sites for each group, but under the limit of quantification. For GS, one out of the 35 sites presented with a total bacterial count of  $2.11 \times 10^4$ . No statistically significant differences were found among groups regarding site location ( $p = 0.40$ ).

### Conclusion

Within the limits of this study, *S. aureus* could not be quantified in the PISF and inside the IIP affected by periimplantitis.

### Keywords

Periimplantitis, periimplant disease, microbiological analysis, opportunistic pathogens, implant connection, *S. aureus*.

Luigi Canullo,\*  
Paulo Henrique Orlato Rossetti,<sup>†</sup>  
Marco Tallarico\*  
& David Peñarrocha Oltra<sup>‡</sup>

\*Independent researcher, Rome, Italy

<sup>†</sup>Independent researcher, Bauru, Brazil

<sup>‡</sup>Oral Surgery Unit, Department of Stomatology,  
Faculty of Medicine and Odontology,  
University of Valencia, Valencia, Spain

### Corresponding author:

**Dr. Luigi Canullo**

Via Nizza 46  
00198 Rome  
Italy

T +39 347 620 1976

F +39 06 841 1980

luigicanullo@yahoo.com

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## Introduction

Dental implantology is a central part of modern dentistry concerned with the replacement of missing teeth in various clinical situations. In the past 30 years, the materials and methods of implant dentistry have undergone a substantial process of development and evolution. Implant surface, macrodesign and type of implant–abutment connection have been found to be of major relevance to initial healing and long-term stability.<sup>1–3</sup> Since the number of implants placed has increased in the last ten years, optimal maintenance has become increasingly important.<sup>4,5</sup> While in many cases, it has been reported that dental implants are a safe and predictable treatment method with high survival rates, they are not immune from biological and iatrogenic complications associated with improper treatment planning, surgical and prosthetic execution, or material failure, as well as maintenance problems.<sup>5</sup> Also, the biological complications of periimplant mucositis and periimplantitis, which may result in soft- and hard-tissue defects, have been suggested to be relevant for later marginal bone loss.<sup>6</sup>

Several approaches have been followed in seeking to understand the pathomechanism of periimplantitis. According to a consensus conference of the American Academy of Periodontology, bacterial colonization of the implant surface and the occurrence of bone loss indicate the point of no return in periimplantitis.<sup>7</sup> Periimplantitis is characterized by an inflammatory process around an implant that includes both soft-tissue inflammation and progressive loss of periimplant supporting bone. Periimplantitis occurs primarily as a result of overwhelming periodontal insult and subsequent immune response.<sup>7</sup> The connection to periodontitis as an infectious disease with comparable symptoms and outcomes suggests that investigating the associated local bacteria is fundamental to establishing the pathomechanism of periimplantitis.

The implant surface may be colonized with different pathogens other than periodontal bacteria.<sup>8</sup> According to Albertini et al., opportunistic pathogens such as *Pseudomonas aeruginosa*, *Staphylococcus aureus* (*S. aureus*) and *Candida albicans* may be associated with implant failure.<sup>9</sup>

As suggested in an American Academy of Periodontology report, secondary diagnostic measures, that is, bacterial culturing, inflammatory markers and genetic factors, may be useful

in the diagnosis of periimplant disease.<sup>7</sup> According to Canullo et al., bacterial agglomerates around dental implants and their prosthodontic adjacent structures have been identified.<sup>10</sup> These results suggested that all of the connections were contaminated after five years of functional loading; thus, the implant–abutment connection design might influence bacterial activity levels qualitatively and quantitatively, especially inside the implant connection.<sup>10</sup> Furthermore, Cosyn et al. found that intracoronal compartments of screw-retained fixed restorations were heavily contaminated.<sup>11</sup> Further investigations have shown that the restorative margin is the principal pathway for bacterial leakage and contamination of abutment screws, and bacteria most likely pass from the periimplant sulcus through the implant–abutment and abutment–prosthesis interfaces.<sup>10</sup>

With the aim of identifying the pathogens that contribute toward the development of periimplantitis defects, different working groups have reported a cluster of bacteria, including *Treponema forsythia* and *S. aureus*, associated with periimplant disease.<sup>12</sup>

The presence of *S. aureus* as an opportunistic pathogen in the early stage of active periimplantitis in patients has also been confirmed by Mombelli and Décaillot.<sup>13</sup> In addition, Salvi et al. reported that detection or lack of *S. aureus* at implant sites at 12 weeks resulted in the highest positive (i.e., 80%) and negative (i.e., 90%) predictive values for the incidence of periimplantitis, respectively.<sup>14</sup> Moreover, Canullo et al. showed that *S. aureus* is present on the external and internal abutment surfaces if these are not cleaned before screwing.<sup>15</sup>

The aim of the present study is to investigate the prevalence of *S. aureus* in the oral cavity of patients with active periimplantitis. This study followed the Strengthening the Reporting of Observational Studies in Epidemiology guidelines.<sup>16</sup>

## Materials & methods

### Study design

This cross-sectional study evaluated data collected from 51 consecutive, partially edentulous patients of both sexes, aged 18 or older (mean age of 54.2), who had been treated with a single implant-supported, cemented or screw-retained restoration functionally loaded for at least 12

months, with adjacent healthy teeth, but presenting signs of periimplant disease according to Mombelli and Décaillet.<sup>13</sup> The patients were invited to participate and were enrolled after being given a detailed explanation of the study protocol. Written informed consent was obtained for each patient. All of the patients were recruited from the Department of Oral Surgery, University of Valencia, Spain, between September and December 2013. The investigation was conducted according to the principles outlined in the Declaration of Helsinki of 1975 for biomedical research involving human subjects, as amended in 2008. All patients were evaluated clinically and radiographically, and their medical histories were recorded. Bone volumes were analyzed using periapical radiographs.

The inclusion criteria were:

- presence of periimplant disease with a vertical bone defect of > 3 mm after implant integration according to Mombelli and Décaillet<sup>13</sup>
- age > 18
- no relevant medical conditions.

The exclusion criteria were:

- pregnancy or lactation
- known systemic disease or metabolic disorders (e.g., HIV) treated with medication detrimental to soft tissue and/or bone healing (e.g., high-dose steroid therapy, systemic treatment with tetracycline or tetracycline analogs, bone therapeutic levels of fluorides, bisphosphonates, medication affecting bone turnover, antibiotics for more than seven days or any investigational drug)—topical application of steroids and steroid application through inhalation were not exclusion criteria
- malignant diseases or other diseases treated with radiotherapy or chemotherapeutic agents (chemotherapy) during the past five years
- a history of head and neck radiation treatment owing to certain medical conditions
- a suspected allergy or incompatibility with any of the bone graft substitute components (calcium phosphates, PLGA, NMP)
- inability to comply with the protocol requirements, including severe alcohol or drug user
- involvement in any other clinical trial during the course of the present trial, or within a period of 30 days prior to its beginning or after its completion
- acute abscesses localized in the proximity of the prospective surgical field.

After full screening, 16 patients were to be excluded: 13 had taken systemic antibiotics during the three months prior to the microbiological sampling, two were pregnant, and one refused to participate. The final sample consisted of 35 individuals (20 male, 15 female) and 63 affected dental implants.

#### Microbiological sampling

Samples for microbiological analysis were obtained from four sites in each patient in the following order: **(1)** the periimplant sulcular fluid (PISF) of each implant; **(2)** the gingival sulcus (GS) of the adjacent teeth, used as control group; **(3)** the inner portions of the implant connection and the abutment of each implant (IIP); and **(4)** the oropharyngeal complex (OF). In all of the groups, the microbiological samples were taken using the GUIDOR Perio-Implant Diagnostic Kit (Sunstar Iberia, Sant Just Desvern, Spain), consisting of five sterile absorbent paper tips and an empty sterile 2 ml microtube.

Prior to collection of the subgingival plaque, supragingival plaque was eliminated from implants and teeth using a cotton tip, without penetrating the GS. OptraGate (Ivoclar Vivadent, Schaan, Liechtenstein) was used to retract the lips and cheeks completely and to ensure relative isolation. After light drying of the area with an air syringe, paper tips were inserted into the periimplant or periodontal sulci for 30 s. The samples from the inner surfaces of the implant–abutment complex were obtained after careful removal of both the restorations and the abutments, seeking to avoid contamination. One drop of RNA- and DNA-free water (Water Molecular Biology Reagent, W4502, Sigma-Aldrich, St. Louis, Mo., U.S.) was placed inside the implant connection and three paper tips were inserted for 30 s. The inner surface of the abutment was wet with a drop of RNA- and DNA-free water and smeared with two paper tips. The paper tips were placed into the microtubes and sent for microbiological analysis to the Institut Clinident laboratory (Aix-en-Provence, France) in the provided mailing envelopes. Finally, an oral environment analysis was performed using sterile cotton swabs collected from the cheeks, tongue, throat and pharynx of each patient.

After sample collection, the inner part of the implants and the abutment–restoration complex were cleaned with a 5% chlorhexidine

Table 1

Sites	Positive sites/ number of sites	TBC	Mean bacterial counts
GS	1/35	$2.11 \times 10^4$	$5.02 \times 10^2$
PISF	1/35	1 positive case, but below level of quantification	0
IIP	1/35	1 positive case, but below level of quantification	0
OF	1/35	1 positive case, but below level of quantification	0

GS: gingival sulcus of the adjacent teeth; PISF: periimplant sulcular fluid; IIP: inner parts of connection; OF: oropharyngeal complex.

Table 1

Investigated sites and bacterial counts.

solution in an ultrasonic bath for 10 min. Afterward, a new abutment screw was connected using a torque wrench (Torq Control, Anthogyr, Sallanches, France) until it reached a torque of 30 N cm, according to the manufacturer’s instructions. In order to verify proper fit between the dental restoration and the implant, standardized digital periapical radiographs were taken using a dental radiographic film holder (Rinn Centrator Bite, DENTSPLY RINN, Elgin, Ill., U.S.) and the paralleling technique.

Quantitative real-time polymerase chain reaction assays

Quantitative real-time polymerase chain reaction (PCR) assays were carried out for total bacterial counts (TBCs) for each target species,<sup>17,18</sup> in a volume of 10 µL composed of 1× QuantiFast SYBR Green PCR (Qiagen, Hilden, Germany), 2 µL of DNA extract, and 1 µM of each primer. The species-specific PCR primers used in this study were provided by Institut Clinident and manufactured by metabion (Martinsried, Germany). Assays were carried out on the Rotor-Gene Q thermal cycling system (Qiagen) with the following program: for TBC, 95 °C for 30 s, followed by 40 cycles of 10 s at 95 °C, 10 s at 60 °C, and 35 s at 72 °C; for *S. aureus*, 95 °C for 5 min, followed by 40 cycles of 10 s at 95 °C, 10 s at 66 °C, and 35 s at 72 °C. A final melting curve analysis (70–95 °C in 1 °C steps for 5 s increments) was performed. Fluorescence signals were measured every cycle at the end of the extension step and continuously during the melting curve analysis. Serial dilutions of standard DNA, provided by Institut Clinident, were used in each reaction as external standards for absolute quantification of the target pathogen. Finally, the data were analyzed using Rotor-Gene Q Series Software (Qiagen).

Statistical analysis

The mean and standard deviations for TBCs at each inspected site (PISF, GS, IIP, OF) were recorded and analyzed according to a pre-established analysis plan. A bio-statistician with expertise in dentistry analyzed the data using statistical software (SigmaPlot, Version 13, Systat Software, San Jose, Calif., U.S.). Before running the statistical analysis, the TBCs for each site were transformed (log transformation [ $\log_{10}$ ]) in an attempt to render less skewed distributions, making the data more interpretable and helping to meet the assumptions of inferential statistics. As the normality test failed, a nonparametric test (Kruskal–Wallis) was used. The level of significance was set at  $\alpha = 0.05$ .

**Results**

No implants were lost, and all of the prostheses were *in situ* at the time of examination. At the end of the study, just one site (out of 35) in the GS of the adjacent teeth presented a TBC of  $2.11 \times 10^4$ . The mean bacterial count of *S. aureus* was  $5.02 \times 10^2$ ; therefore, this value was taken as control. Conversely, in the PISF of each implant, the IIP and the OF complex, the mean bacterial counts of *S. aureus* were 0, with only one site (out of 35) positive, but below the level of quantification. The data are reported in **Table 1**. No statistically significant differences were found among groups regarding site location (Kruskal–Wallis test;  $p = 0.40$ ).

**Discussion**

Currently, there are neither standardized antibiotic prophylactic regimens for dental implant place-

ment nor universally accepted treatment for periimplantitis. The treatment of infected implants is difficult and usually requires removal.<sup>18</sup> However, it has become clear that therapy of periimplant mucositis should be considered a preventive measure for the onset of periimplantitis. Completion of active periodontal therapy should precede implant placement in periodontally compromised patients.<sup>19</sup>

*S. aureus* is a facultative coccus and Gram-positive bacterium normally associated with surgical wounds in orthopedic patients.<sup>20</sup> Part of this can be explained by the impedance seen on cultured osteoblasts, with *S. aureus* surviving up to 48 h after internalization by those bone cells and still eliciting interleukin 6 and interleukin 8 responses,<sup>21</sup> which have pro-inflammatory effects and are involved in osteoclastogenesis<sup>22</sup> and foreign body reactions.<sup>23</sup> In addition, *S. aureus* has the ability to form a biofilm and lead to chronic infection.<sup>24</sup>

A retrospective study has demonstrated that patients capable of maintaining high immunoglobulin G antibody titers to *S. aureus* had successful implants compared with nonosseointegrated fixtures.<sup>25</sup>

In the present study, the lack of significance regarding the bacterial counts of *S. aureus* at IIP and PISF must be considered, since *in vitro* this pathogen has shown an affinity for titanium surfaces,<sup>26</sup> and two studies have related its levels to deep periimplant pockets with bleeding on probing.<sup>27, 25</sup> One study has demonstrated that the bacterial counts of *S. aureus* increase from 5% to 15% at implant sites 12 weeks after surgery.<sup>28</sup> However, another study pointed out that even after seven years of follow-up the presence of *S. aureus* at tooth sites could be indicative of the presence of the same pathogen at implant sites,<sup>25</sup> while another study indicated that the lack of *S. aureus* at implant sites after 12 weeks demonstrated a high negative predictive value after 12 months.<sup>29</sup> More recently, an article demonstrated that regardless of health status, periodontal and periimplant sites harbored *S. aureus* cells, being the highest load of all six species analyzed.<sup>30</sup>

## Conclusion

Within the limits of this study, *S. aureus* could not be quantified inside and around dental implants in detectable limits. However, clinicians must bear in mind that, in the early stage of healing, this pathogen can influence the immune response and lead to periimplant bone loss.

## Competing interests

The study was supported by Sweden & Martina (Due Carrare, Italy), which paid for the kits, and by the Institut Clinident, which performed the analysis free of charge. The authors declare that they have no competing interests related to this study.

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