

Cluster of Bacteria Associated with Peri-Implantitis

G. Rutger Persson, DDS, PhD;* Stefan Renvert, DDS, PhD†

ABSTRACT

Background: Information on the microbiota in peri-implantitis is limited. We hypothesized that neither gender nor a history of periodontitis/smoking or the microbiota at implants differ by implant status.

Materials and Methods: Baseline microbiological samples collected at one implant in each of 166 participants with peri-implantitis and from 47 individuals with a healthy implant were collected and analyzed by DNA–DNA checkerboard hybridization (78 species). Clinical and radiographic data defined implant status.

Results: Nineteen bacterial species were found at higher counts from implants with peri-implantitis including *Aggregatibacter actinomycetemcomitans*, *Campylobacter gracilis*, *Campylobacter rectus*, *Campylobacter showae*, *Helicobacter pylori*, *Haemophilus influenzae*, *Porphyromonas gingivalis*, *Staphylococcus aureus*, *Staphylococcus anaerobius*, *Streptococcus intermedius*, *Streptococcus mitis*, *Tannerella forsythia*, *Treponema denticola*, and *Treponema socranskii* ($p < .001$). Receiver operating characteristic curve analysis identified *T. forsythia*, *P. gingivalis*, *T. socranskii*, *Staph. aureus*, *Staph. anaerobius*, *Strep. intermedius*, and *Strep. mitis* in peri-implantitis comprising 30% of the total microbiota. When adjusted for gender (not significant [NS]), smoking status (NS), older age ($p = .003$), periodontitis history ($p < .01$), and *T. forsythia* (likelihood ratio 3.6, 95% confidence interval 1.4, 9.1, $p = .007$) were associated with peri-implantitis.

Conclusion: A cluster of bacteria including *T. forsythia* and *Staph. aureus* are associated with peri-implantitis.

KEY WORDS: DNA analysis, microbiota, peri-implantitis

INTRODUCTION

The diversity of bacteria in the oral cavity is large.¹ Peri-implantitis may have an infectious etiology.² It remains, however, unclear if there is a specific cluster of bacteria that can be associated with, or explanatory to peri-implantitis. It has been suggested that the bacterial biofilm on implant and on tooth surfaces is similar.² Recent data suggest that the microbiota in peri-implantitis is a polymicrobial anaerobic infection and not fully corresponding to the disease severity.³ Thus,

the infection at dental implants is more complex than what has been demonstrated in periodontitis.⁴ Notwithstanding, bacteria associated with periodontitis are commonly found in peri-implantitis including: *Bacteroides*, *Campylobacter*, *Eubacterium*, *Fusobacterium*, and *Treponema* species.⁵ Higher counts of *Aggregatibacter actinomycetemcomitans*, *Prevotella intermedia*, *Porphyromonas gingivalis*, *Treponema denticola*, and *Tannerella forsythia* have been reported in peri-implantitis in comparison with findings at teeth with periodontitis.^{6,7}

Within an hour after the installation of dental implants, bacteria can be identified and a complex biofilm is formed within 2 weeks.^{8,9} Data have shown that if *Staphylococcus aureus* is part of the early colonizing bacteria, *Staph. aureus* is also predictably present 1 year later.¹⁰ In addition, others have reported on the presence of *Staph. aureus* and enteric rods in cases with peri-implantitis.^{3,11} Failing dental implants have been associated with low antibody titer and avidity levels to *Staph. aureus*.¹² In vitro studies have demonstrated that *Staph. aureus* has a strong affinity to titanium surfaces.¹³

*Professor, Department of Oral Sciences, Kristianstad University, Kristianstad, Sweden, Department of Periodontology, University of Bern, Bern, Switzerland, and Departments of Periodontics and Oral Medicine, University of Washington, Seattle, WA, USA; †professor, Department of Oral Sciences, Kristianstad University, Kristianstad, Sweden, School of Dental Sciences, Trinity College, Dublin, Ireland, and Blekinge Institute of Technology, Karlskrona, Sweden

Reprint requests: Professor Rutger Persson, Kristianstad University, SE-29188 Kristianstad, Sweden; e-mail: rutger.persson@hkr.se

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Thus, *Staph. aureus* infection may be of importance in the development of peri-implantitis induced by bacterial infection.

In order to identify the characteristic bacterial profile for implants with healthy or inflamed conditions, bacterial samples from many subjects with either peri-implantitis or healthy implant conditions using a methodology providing information on a variety of pertinent bacteria should be employed. Many studies on the microbial composition at dental implants have evaluated small samples and/or few bacteria.

The primary aim of the present study was to assess the presence of 78 bacterial species using the checkerboard DNA–DNA hybridization method at implants with either a diagnosis of peri-implantitis or being defined as healthy implant. In addition, we studied if the microbiota at dental implants with or without peri-implantitis could be associated with the age of the individual, with gender, and with a history of smoking and/or periodontitis.

MATERIALS AND METHODS

The present study is a retrospective analysis of subject-based clinical and microbiological data.

Individuals

The Regional Ethics Review Board at Lund University, Sweden, approved the study. All enrolled individuals signed written informed consent. The present retrospective clinical study was based on material and data collected between 2007 and 2011 at the University of Kristianstad, Sweden, the Specialty Clinic for Periodontology, Region Halland, Halmstad, Sweden, and at the Uppsala Käkkirurgiska Centrum, Uppsala, Sweden.^{14–18} Routine data were obtained from all participants including data on past history of smoking, periodontitis, age, and gender. Probing pocket depths (PPDs) at all implants were measured using a standardized probing force of 0.2 N and with the same probe design (Hawe Click-Probe, Hawe Neos Dental, Switzerland). Bone loss was assessed from digital intraoral (26 × 37 mm) radiographs using OSIRIX open source software 4.0 for MAC 10.6 (Pixmeo Sari, Geneva, Switzerland). The same examiner (GRP) measured the distance between bone to implant contact and implant platform level of all study implants.

None of the participants had received antibiotics during the preceding 6 months. None of them had been

treated for peri-implantitis. Those individuals who presented with chronic periodontitis had received treatment and were in remission. None of the subjects had been diagnosed with aggressive periodontitis, or necrotizing gingivitis/periodontitis. Such information was also obtained from review of existing dental records and current clinical examination. Information on smoking habits was obtained through questionnaire.

All microbiological samples were analyzed at the Oral Microbiology Laboratory, School of Dentistry, and University of Bern, Switzerland, and supervised by the same laboratory director (GRP).

Definition of Implants with Healthy Conditions and Implants with Peri-Implantitis

Among participants with a diagnosis of peri-implantitis, and with more than one implant, only the implant with the worst clinical conditions was studied. If individuals without implants with peri-implantitis had more than one implant with healthy conditions, the clinical data at the implants and the microbial samples were collected from the implant that was best suited for sampling. Implants with healthy conditions were defined as those with no bleeding on probing (BOP), or with only a point of bleeding at one surface. No suppuration and no bone loss ≥ 2.0 mm could be present. Implants must have been in function for at least 2 years. Implants with peri-implantitis were defined in accordance with the guidelines recently provided.¹⁹ Thus, implants with peri-implantitis must have evidence of a vertical distance of ≥ 2 mm from the expected marginal bone level following remodeling post-implant placement. At the time of examination, BOP or suppuration must also be present.

Microbiological Sampling, Analysis, and Enumeration of Organisms Using DNA–DNA Probes

The same process was used for the collection of the bacterial samples. All bacterial samples were taken prior to the measurements of BOP and PPD of the implants. The implant site with the deepest PPD (previously identified) represented the site from which the microbiological samples were taken. At sampling, implants were isolated with cotton rolls to prevent saliva contamination. Supragingival plaque was removed with sterile cotton pellets. Two paper points (Dentsply Maillefer size 55, Ballaigues, Switzerland) were inserted into the selected pocket until resistance was met and left in situ

during 20 seconds, placed in labeled Eppendorf tubes (1.5 mL natural flat cap microcentrifuge tubes, Starlab, Ahrensburg, Germany), and stored in a freezer at -79°C within 30 minutes after sampling. All samples were analyzed at the Oral Microbiology Laboratory, School of Dentistry at the University of Bern, Switzerland. After thawing of the samples, 0.5 mL NaOH and 0.15 mL TE (10 mM Tris-HCl, 1 mM EDTA, pH 7.6) was added to the samples. The checkerboard DNA–DNA hybridization process was performed as described elsewhere.²⁰ In the present study, 79 bacterial species (Table 1) were included in the assay.^{21,22} The same microbiology laboratory technicians performed all the laboratory procedures (MW and RH-I) and with the same laboratory director (GRP). Presence or absence of bacteria was defined at two cutoff levels ($\geq 1.0 \times 10^4$ bacterial cells, and $\geq 1.0 \times 10^5$ bacterial cells).

Statistical Analysis

The Kolmogorov–Smirnov test was used to identify if the microbiological data presented with a normal distribution pattern or not. The following statistical methods were used to study the data: descriptive statistics, independent t -tests, Mann–Whitney U tests, Pearson χ^2 tests, multinomial logistic regression analysis, and analysis of receiver operating curves (ROC). An algorithm provided by the PASW/SPSS version 18.0 for non-parametric assumption was used to calculate the area under the curve. Given the large number of microbiological variables studied, α was set at 0.001. For all other data α was defined at 0.05. The PASW/SPSS 18.0 statistical software package (IBM/SPSS, Armonk, NY, USA) was used for the analyses.

RESULTS

Characteristics of the Study Participants

Clinical and microbiological data from 166 individuals with peri-implantitis and from 47 individuals with healthy dental implant conditions were included. The characteristics of the study participants are presented (Table 2). The age range varied between 18 and 88 years of age, and 46.4% of the subjects were below age 67.

Analysis by independent t -test (equal variance not assumed) identified that independent of implant status, women (mean age 67.0, standard deviation [SD] ± 12.1) were older than men (mean age 60.3, SD ± 15.8) ($p < .001$). Individuals with a history of periodontitis

were also older (mean age 66.9, SD ± 12.0) than individuals without a history of periodontitis (mean age 59.4, SD ± 16.4) ($p < .001$). Statistical analysis failed to demonstrate a difference in age by smoking status ($p = .98$). Analysis by Pearson χ^2 also failed to demonstrate a gender difference in smoking habit. This was the case regardless whether the individuals had peri-implantitis or not.

Clinical Conditions at Dental Implants

The mean PPD of the implants with peri-implantitis ($n = 166$) was 5.9 mm (SD ± 1.5). The mean PPD of the implants with healthy conditions ($n = 47$) was 4.1 mm (SD ± 1.1) (PPD mean difference 1.8 mm, standard error [SE] of difference 0.2, 95% confidence interval [CI] 1.4, 2.2, $p < .001$). According to the definition, all implants with healthy conditions had a distance between the implant platform and bone level < 2.0 mm and with a mean value of 1.3 mm (SD ± 0.3 , range 0.8–1.8) as assessed from digitized radiographic images. The mean radiographically assessed distance between the implant platform level and the osseous defect depth at implants with peri-implantitis was 5.4 mm (SD ± 1.9) (Table 2).

Microbiological Results

Analysis by Kolmogorov–Smirnov test failed to identify a normal distribution pattern for all the bacterial species studied. This was the case at both implants with healthy conditions and implants diagnosed with peri-implantitis. At implants with peri-implantitis, analysis by Mann–Whitney U tests identified higher bacterial counts for 19/78 bacterial species from implants with a diagnosis of peri-implantitis in comparison to implants with healthy conditions including the following species: *Actinomyces odontolyticus*, *A. actinomycetemcomitans* (a), *Campylobacter gracilis*, *Campylobacter rectus*, *Campylobacter showae*, *Helicobacter pylori*, *Haemophilus influenzae*, *Leptothrichia buccalis*, *P. intermedia*, *Propionibacterium acnes*, *Porphyromonas endodontalis*, *P. gingivalis*, *Staph. aureus*, *Staph. anaerobius*, *Streptococcus intermedius*, *Streptococcus mitis*, *T. forsythia*, *T. denticola*, and *Treponema socranskii*. The distribution of implants with a positive identification of bacterial cells defined at the $\geq 1.0 \times 10^4$, and at the $\geq 1.0 \times 10^5$ bacterial cells are presented for these species (Table 3).

Further analysis by Mantel–Haenszel unadjusted odds identified that at the $\geq 1.0 \times 10^4$ Cutoff level the odds ratio of bacterial counts greater than the cutoff levels and

TABLE 1 Bacteria Included in the Checkerboard DNA–DNA Hybridization Assays

Bacteria	Collection	Bacteria	Collection
<i>Aggregatibacter actinomycetemcomitans</i> (a)	ATCC29523	<i>Actinomyces neuui</i>	GUH550898
<i>Aggregatibacter actinomycetemcomitans</i> (Y)	ATCC43718	<i>Aerococcus christensenii</i>	GUH070938
<i>Actinomyces israelii</i>	ATCC 1201	<i>Anaerococcus vaginalis</i>	GUH290486
<i>Actinomyces naeslundii</i>	ATCC121045	<i>Atopobium parvulum</i>	GUH160323
<i>Actinomyces odontolyticus</i>	ATCC17929	<i>Atopobium vaginae</i>	GUH010535
<i>Capnocytophaga gingivalis</i>	ATCC33612	<i>Bacteroides ureolyticus</i>	GUH080189
<i>Capnocytophaga ochracea</i>	ATCC33596	<i>Bifidobacterium biavatii</i>	GUH071026
<i>Capnocytophaga sputigena</i>	ATCC33612	<i>Bifidobacterium bifidum</i>	GUH070962
<i>Campylobacter gracilis</i>	ATCC33236	<i>Bifidobacterium breve</i>	GUH080484
<i>Campylobacter rectus</i>	ATCC33238	<i>Bifidobacterium longum</i>	GUH180689
<i>Campylobacter showae</i>	ATCC451146	<i>Corynebacterium nigricans</i>	GUH450453
<i>Eikenella corrodens</i>	ATCC238345	<i>Corynebacterium aurimucosum</i>	GUH071035
<i>Eubacterium saburreum</i>	ASTCC33271	<i>Dialister</i> sp.	GUH071035
<i>Fusobacterium nucl. naviforme</i>	ASTCC49256	<i>Enterococcus faecalis</i>	GUH170812
<i>Fusobacterium nucl. nucleatum</i>	ATCC25586	<i>Enterococcus faecalis</i>	ATCC29212
<i>Fusobacterium nucl. polymorphum</i>	ATCC10953	<i>Echerichia coli</i>	GUH070903
<i>Fusobacterium periodonticum</i>	ATCC33993	<i>Gardnerella vaginalis</i>	GUH080585
<i>Lactobacillus acidophilus</i>	ATCC11975	<i>Haemophilus influenzae</i>	ATCC49247
<i>Leptothrichia buccalis</i>	ATCC14201	<i>Helicobacter pylori</i>	ATCC43504
<i>Neisseria mucosa</i>	ATCC33270	<i>Lactobacillus crispatus</i>	GUH160342
<i>Parvimonas micra</i>	ATCC19696	<i>Lactobacillus gasseri</i>	GUH170856
<i>Prevotella intermedia</i>	ATCC25611	<i>Lactobacillus iners</i>	GUH160334
<i>Prevotella melaninogenica</i>	ATCC25845	<i>Lactobacillus jensenii</i>	GUH160339
<i>Prevotella nigrescens</i>	ATCC33563	<i>Lactobacillus vaginalis</i>	GUH0780928
<i>Porphyromonas gingivalis</i>	ATCC33277	<i>Mobiluncus curtisii</i>	GUH070927
<i>Propionibacterium acnes</i>	ATCC11827/28	<i>Mobiluncus mulieris</i>	GUH070926
<i>Selenomonas noxia</i>	ATCC43541	<i>Peptoniphilus</i> sp.	GUH550970
<i>Streptococcus anginosus</i>	ATCC33397	<i>Peptostreptococcus anaerobius</i>	GUH160362
<i>Streptococcus constellatus</i>	ATCC27823	<i>Porphyromonas endodontalis</i>	ATCC35406
<i>Streptococcus gordonii</i>	ATCC10558	<i>Prevotella bivia</i>	GUH450429
<i>Streptococcus intermedius</i>	ATCC27335	<i>Prevotella disiens</i>	GUH190184
<i>Streptococcus mitis</i>	ATCC49456	<i>Proteus mirabilis</i>	GUH070918
<i>Streptococcus mutans</i>	ATCC25175	<i>Pseudomonas aeruginosa</i>	ATCC33467
<i>Streptococcus oralis</i>	ATCC35037	<i>Staphylococcus aureus</i>	ATCC25923
<i>Streptococcus sanguinis</i>	ATCC10556	<i>Staphylococcus aureus</i> yellow strain	GUH070921
<i>Tannerella forsythia</i>	ATCC43037	<i>Staphylococcus aureus</i> white strain	GUH070922
<i>Treponema denticola</i>	ATCC354405	<i>Staphylococcus epidermis</i>	DSMZ20044
<i>Treponema socranskii</i>	D40DR2	<i>Staphylococcus haemolyticus</i>	DSMZ20263
<i>Veillonella parvula</i>	ATCC10790	<i>Streptococcus agalactiae</i>	GUH230282
		<i>Varibaculum cambriense</i>	GUH070917

ATCC, American Type Culture Collection, LGC Standards S.a.r.l. Molsheim Cedex, France; D, sample from Forsyth Institute, Boston, MA; DSMZ, German Collection of Microorganisms and Cell Cultures, Braunschweig Germany; GUH, Ghent University Hospital Collection, Ghent, Belgium.

a clinical diagnosis of peri-implantitis was identified (OR and *p* values) for the following species: *T. forsythia* (OR 4.7, *p* = .001), *T. denticola* (OR 4.6, *p* = .001), *C. rectus* (OR 4.2, *p* = 0.001), *T. socranskii* (OR 3.5,

p = 0.002), *P. gingivalis* (OR 3.3, *p* = .001), *Staph. aureus* (OR 3.2, *p* = 0.003), *C. gracilis* (OR 3.2, *p* = .003), and *P. intermedia* (OR 3.1, *p* = .003). At the $\geq 1.0 \times 10^5$ cutoff level, only *T. forsythia* had a significant OR in

TABLE 2 Characteristics of the Study Participants

Variable	Peri-Implantitis	Healthy Conditions	p Value
Female/male	62.5%/37.5%	55.3%/44.7%	0.017
Age (mean value and SD)	67.0 ± 9.7	53.7 ± 18.8	0.001
Smoking habit: current smoker	47.1%	23.4%	0.002
Tooth loss: periodontitis	81.3%	36.2%	0.001
Other causes	18.7%	63.8%	
Probing depth at sites sampled (mm) (mean value and SD)			0.001
Range	5.9 ± 1.5	4.1 ± 1.1	
1–3	0.0%	31.9%	
4	19.8%	19.1%	
5	23.7%	48.9%	
6–7	39.9%	0.0%	
≥8	16.6%	0.0%	
Radiographic distance between implant platform to bone to implant contact (mm) (mean value and SD)	5.4 ± 1.9	1.3 ± 0.3	0.001

relation to peri-implantitis (OR 5.4, 95% CI 2.3, 12.8, $p < .001$).

The Impact of a History of Periodontitis on the Microbiota at Dental Implants

A history of periodontitis as the cause of tooth loss and implant placement was identified in 36.2% of the individuals with healthy implants. A history of periodontitis was the cause of tooth loss in 81.3% of the individuals with peri-implantitis ($p < .001$).

When only the individuals with healthy implants were studied, the statistical analysis failed to demonstrate differences in the microbiota at the implants based on periodontal status of the individuals. Analysis by Mann-Whitney U test identified that independent of implant status, the following species were found at higher counts in individuals with a history of periodontitis ($p < .001$): *Actinomyces naeslundii*, *C. rectus*, *Fusobacterium nucleatum* sp. *naviforme*, *Fusobacterium nucleatum* sp. *nucleatum*, *Parvimonas micra*, *Staphylococcus haemolyticus*, *T. forsythia*, and *T. denticola*.

The Impact of a History of Smoking on the Microbiota at Dental Implants

A history of smoking was significantly more prevalent among subjects with a diagnosis of peri-implantitis ($p = .002$). When only the individuals with healthy implants were studied, the statistical analysis failed to demonstrate differences in the microbiota at the

implants based on smoking status of the individuals. The statistical analysis identified that at the implants from individuals with peri-implantitis and with a history of smoking, the following bacterial species were found at higher counts ($p < .001$): *C. rectus*, *F. nucl* sp. *naviforme*, *F. nucl* sp. *nucleatum*, *Fusobacterium nucleatum* sp. *polymorphum*, *F. periodonticum*, and *Veillonella parvula*. Independent of implant status, the following species were found at higher counts in individuals with a smoking history: *C. rectus*, *F. nucl* sp. *naviforme*, *F. nucl* sp. *nucleatum*, and *F. nucl* sp. *polymorphum*.

Assessments of Bacterial Cluster and Risk for Peri-Implantitis

The 19/78 bacteria that differed by the Mann-Whitney U test analysis described previously were included in a further analysis of ROC. ROC curves including the bacteria that distinguished peri-implantitis from healthy implants are graphically presented with ROC curves (Figure 1). The respective area under the curve, SE, and 95% CI are presented (Table 4). The area under the curve analysis confirmed that the bacterial counts that differed by implant status included the following cluster of bacteria: *T. forsythia*, *P. gingivalis*, *T. socranskii*, *Staph. aureus*, *Strep. intermedius*, *Strep. mitis*, and *H. influenzae*. These species were found at significantly higher levels in peri-implantitis and different from findings at healthy implants. In relation to the total bacterial load of the 78 species, these seven species comprised

TABLE 3 Prevalence Rates of Implants with Implant Health or a Diagnosis of Peri-Implantitis with Bacteria Present as Detected by Two Cutoff Levels ($\geq 1.0 \times 10^4$ Cells and $\geq 1.0 \times 10^5$ Cells) Including Those Species That Differed by Implant Conditions Assessed by Mann-Whitney *U* Tests

Bacterial Species	Implant Health	Peri- Implantitis	Implant Health	Peri-Implantitis
	$\geq 1.0 \times 10^4$ Cells (%)	$\geq 1.0 \times 10^4$ Cells (%)	$\geq 1.0 \times 10^5$ Cells (%)	$\geq 1.0 \times 10^5$ Cells (%)
<i>Actinomyces odontolyticus</i>	17.0	37.3	8.5	4.8
<i>Aggregatibacter actinomycetemcomitans</i> (a)	17.0	38.0	10.6	27.7
<i>Campylobacter gracilis</i>	21.3	46.4	14.9	10.8
<i>Campylobacter rectus</i>	27.7	61.4	10.6	21.1
<i>Campylobacter showae</i>	46.8	66.3	21.3	27.2
<i>Fusobacterium nucleatum</i> sp. naviforme	40.4	58.4	19.1	31.3
<i>Fusobacterium nucleatum</i> sp. nucleatum	40.4	64.5	23.4	26.5
<i>Fusobacterium nucleatum</i> sp. polymorphum	38.3	57.8	17.0	18.7
<i>Fusobacterium periodonticum</i>	40.4	57.2	14.9	17.5
<i>Haemophilus influenzae</i>	12.8	19.3	6.4	7.8
<i>Helicobacter pylori</i>	23.4	44.6	6.4	15.1
<i>Parvimonas micra</i>	34.0	54.0	14.9	18.7
<i>Prevotella intermedia</i>	21.3	45.8	14.9	15.7
<i>Porphyromonas gingivalis</i>	27.7	56.0	8.5	18.7
<i>Pseudomonas aeruginosa</i>	21.3	44.0	12.8	25.3
<i>Staphylococcus anaerobius</i>	19.1	42.2	6.4	8.4
<i>Staphylococcus aureus</i>	19.1	43.4	6.4	10.8
<i>Staphylococcus haemolyticus</i>	44.7	59.6	17.0	16.3
<i>Streptococcus intermedius</i>	25.5	49.4	8.5	15.7
<i>Streptococcus mitis</i>	21.3	46.4	8.5	7.2
<i>Tannerella forsythia</i>	25.5	61.4	14.5	48.8
<i>Treponema denticola</i>	14.9	45.2	6.4	11.4
<i>Treponema socranskii</i>	19.1	45.2	10.6	18.1
<i>Veillonella parvula</i>	36.2	58.4	21.3	19.3

30.2% from implants with peri-implantitis and 14.1% from healthy implants. The mean value of the bacterial load of these seven species was 6.5×10^5 bacterial cells for peri-implantitis and 1.8×10^5 bacterial cells for healthy implants (mean difference 4.7×10^5 , 95% CI 2.7 to 6.7×10^5 cells, $p < .001$ [equal variance not assumed]). The distribution of these seven bacteria at implants with either healthy conditions or a diagnosis of peri-implantitis is presented in a boxplot diagram (Figure 2). These species were also present at higher counts in subjects with a history of periodontitis (independent of implant status) ($p < .001$). The statistical analysis failed to demonstrate that these seven bacterial species were present at higher counts in subjects with a history of smoking (independent of implant status).

Backward stepwise (Wald) binary regression analysis demonstrated the best goodness of fit by Hosmer–Lemeshow test for the seven bacterial species as the only

variables included in the model ($\chi^2 = 15.8$, $p = .027$) with significant values for *Staph. aureus*, *Strep. intermedius*, and *T. forsythia* (Table 5). When adding age (continuous data), gender, smoking history, and periodontal disease history as dichotomous data, the best goodness of fit ($\chi^2 = 15.8$, $p = .027$) was obtained for a model including age ($p < .001$), gender ($p = .18$), smoking ($p = .24$), *Staph. aureus* ($p = .06$), *Strep. mitis* ($p = .06$), and *T. forsythia* ($p = .13$) (Table 6).

DISCUSSION

Our study identified that bacteria commonly associated with periodontitis were highly prevalent in peri-implantitis. This finding is consistent with other studies.^{5,23,24} Thus, specifically *T. forsythia* was found in 49% at implants with peri-implantitis and at 15% at implants with healthy conditions (cutoff level 1.0×10^5 cells). The present study also identified that a cluster of

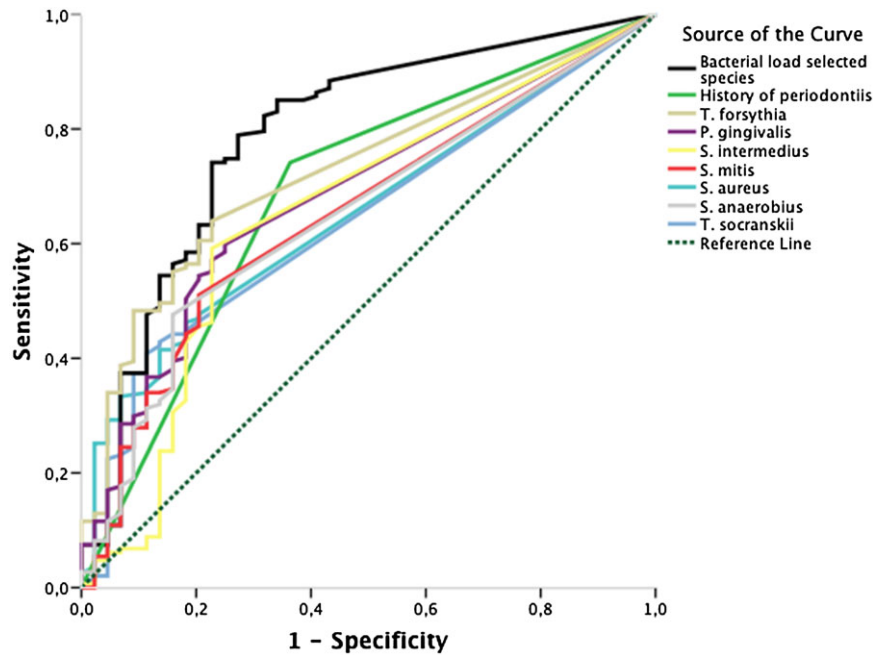


Figure 1 Receiver operating characteristic (ROC) curves identifying bacteria that distinguish between implant health and disease. The area under the curve for each of the bacterial species is presented in Table 4.

seven bacterial species could be associated with peri-implantitis. The microbiological data also identified that the total bacterial load in peri-implantitis for these seven species (*T. forsythia*, *P. gingivalis*, *T. socranskii*, *Staph. aureus*, *Staph. anaerobius*, *Strep. intermedius*, and *Strep. mitis*) was approximately four times higher than at healthy implants. Thus, the bacterial burden as such may be an important factor in peri-implantitis.

The present data suggested that peri-implantitis is a polymicrobial infection.

We recognize that the present study is a cross-sectional study without a longitudinal follow-up. In a recent study with focus on long-term outcome following implant placement with bacterial samples analyzed at the same laboratory and with the same methodology the authors concluded that also at healthy implants, higher levels of some bacteria associated with periodontitis could be found than at contra-lateral teeth.²⁵ The microbiological data in the present study from the healthy implants are consistent with the findings by Dierens and colleagues.²⁵

TABLE 4 Area under the Curve and Statistical Data for the Seven Bacterial Species That Distinguished between Peri-Implantitis and Healthy Implant Status

Test Result	Area under the Curve	SE*	Asymptotic Sign†	Asymptotic 95% Confidence Interval	
				Lower Level	Upper Level
Periodontal status	0.69	0.05	0.001	0.60	0.78
Bacterial load selected species	0.78	0.04	0.001	0.70	0.87
<i>Tannerella forsythia</i>	0.73	0.04	0.001	0.65	0.81
<i>Porphyromonas gingivalis</i>	0.68	0.04	0.001	0.60	0.77
<i>Streptococcus intermedius</i>	0.66	0.05	0.001	0.56	0.75
<i>Streptococcus mitis</i>	0.65	0.05	0.002	0.56	0.74
<i>Staphylococcus aureus</i>	0.65	0.04	0.002	0.58	0.74
<i>Staphylococcus anaerobius</i>	0.65	0.05	0.002	0.56	0.74
<i>Treponema socranskii</i>	0.64	0.04	0.001	0.56	0.73

In addition, dichotomous scoring for periodontal status is also included (*nonparametric assumption; †null hypothesis true area: 0.5).

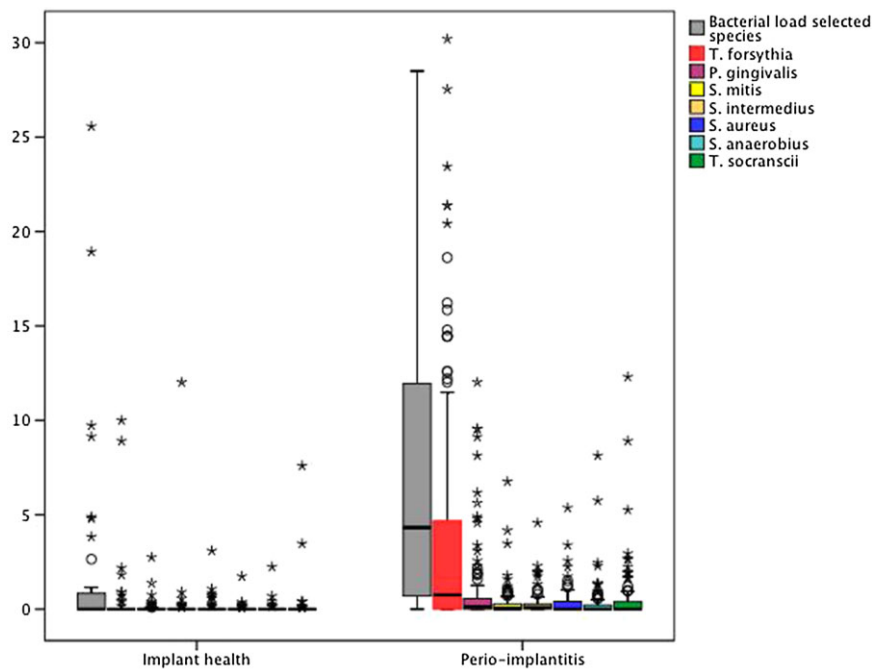


Figure 2 Boxplot diagram illustrating median, 25 and 75 percentiles as well as outlier values for counts of the seven bacterial species defined by the ROC analysis with significant differences by implant status. In addition, dichotomous status for periodontitis is included as a reference in the ROC curve analysis.

It appears that titanium dental implants provide a suitable environment for the development of a complex microbial biofilm. Dental implant design and surface chemistry may also have an impact on the invasion of oral microorganisms into the fixture-abutment

interface.^{26,27} This may partly explain the differences of bacterial counts at implants and teeth.

Data have demonstrated the presence of several Archaea species at dental implants with peri-implantitis.²⁸ Thus, *Methanobrevibacter oralis* is known

TABLE 5 Bacteria Included in Final Model Assessing Bacteria in Cluster Defining Microbiological Differences by Implant Status (Peri-Implantitis versus Health)						
Variable	Regr. Coeff.	SE	Wald	Likelihood Ratio	95% Confidence Interval	Sign.
<i>Tannerella forsythia</i>	0.3	0.1	6.0	1.3	1.1, 1.6	0.01
<i>Staphylococcus aureus</i>	2.5	1.1	4.9	11.8	1.1, 53.0	0.03
<i>Treponema socranskii</i>	−0.2	0.2	11.1	0.8	0.6, 1.2	0.19
<i>Porphyromonas gingivalis</i>	0.5	0.4	1.6	1.6	0.8, 1.7	0.21

TABLE 6 Factors Distinguishing between Peri-Implantitis and Healthy Implant Conditions in Individuals with Dental Implants						
Variable	Regr. Coeff.	SE	Wald	Likelihood Ratio	95% Confidence Interval	Sign.
Age	0.1	0.0	14.4	1.1	1.0, 1.1	0.001
Periodontitis	−1.1	0.1	6.8	0.4	0.2, 0.7	0.01
<i>Staphylococcus aureus</i>	2.7	0.4	3.4	1.2	1.0, 160.0	0.02
<i>Streptococcus intermedius</i>	−1.4	0.6	4.6	0.3	0.1, 0.9	0.03
<i>Tannerella forsythia</i>	0.2	0.1	3.4	1.2	1.0, 1.5	0.07
<i>Treponema socranskii</i>	−0.2	0.2	1.3	0.8	0.6, 1.1	0.82

to produce methane gases.²⁸ This may, in part, explain the high prevalence of other methane gas-producing bacteria in peri-implantitis (i.e., *Treponema* sp. and *T. forsythia*) as identified in the present study.

In vitro studies have shown that staphylococci species have a high affinity to titanium surfaces.¹³ This may explain why, in the present study, *Staph. aureus* and *Staph. anaerobius* were associated with peri-implantitis. Staphylococci may also be involved in peri-implantitis due to immunity factors as subjects with failing dental implants appear to lack efficient antibodies to *Staph. aureus* and *T. forsythia*, but also that antibodies to *Strep. intermedius* may be explanatory and supported by the elevated findings of these bacteria.^{12,29}

The present study identified that the bacterial load of seven identified species was significantly higher among individuals with peri-implantitis. It must be recognized that it is not only the total bacterial counts that should be considered in assessing the pathogenic microbiota. The presence of highly pathogenic strains in small numbers may be sufficient to establish a severe host inflammatory response.

A history of periodontitis has been considered as a risk factor for future peri-implantitis.³⁰ Thus, it has been demonstrated that patients with a past history of either moderate or advanced periodontitis are at greater risk for peri-implantitis than periodontally healthy patients.³¹ Others have also identified those patients with a history of periodontitis more commonly also develop peri-implantitis.^{32,33} Data also suggest that patients treated for periodontitis and on maintenance care but with residual probing depths at teeth ≥ 5 mm have an elevated risk for peri-implantitis and implant loss.³⁴ Our data support the conclusions made by other studies. In the present study, we also identified that a cluster of specific bacteria is associated with peri-implantitis. The results of the present study are also consistent with other observations suggesting that specific clusters of bacteria in periodontal pockets depend on genetic factors and may therefore explain the enhanced susceptibility to infection and peri-implantitis.³⁵

The association between a smoking habit and peri-implantitis may primarily be driven by other factors than the infectious etiology. Other studies have also failed to demonstrate that smoking is a significant risk factor for peri-implantitis, while subject age was a factor.^{36,37} The impact of smoking on the risk for

peri-implantitis is controversial and there is also evidence that a smoking habit is a risk factor for peri-implantitis.³⁸ In the present study, the odds that smoking was associated approached significance when considered alone ($p = .07$). When studied with other covariates, that is, gender, and history of periodontitis smoking did not remain as a statistically significant factor. Nevertheless, the present study demonstrated that specifically *Fusobacterium* species were identified at higher levels in individuals with a smoking habit. Smoking may be associated with a risk for peri-implantitis in subjects who are positive for interleukin 1 gene polymorphism.³⁹ Thus, it is possible that there might be an elevated risk for infection at implants with *Fusobacterium* species given specific genetic conditions.

The present study identified that subject age is an important comorbidity factor in peri-implantitis. This is consistent with other studies.^{36,40} In contrast, another study failed to demonstrate that older age was not identified as a risk for peri-implantitis in older subjects.⁴¹ There are many studies suggesting that poor oral hygiene can be associated with an elevated risk of peri-implantitis. Assuming that older patients are having more problems with oral hygiene, it seems reasonable that older age could be linked to an increased risk for peri-implantitis. Declining periodontal health in older subjects has been associated with elevated levels of *T. forsythia* in periodontal pockets.⁴² Thus, the finding that *T. forsythia* is associated with peri-implantitis may specifically be a link to the increased risk for peri-implantitis. The present study identified that within the cluster of seven bacterial species, *Staph. aureus* and *T. forsythia* may be key putative pathogens.

In conclusion, a distinctive bacterial profile was found at implants with peri-implantitis including: *P. gingivalis*, *Staph. aureus*, *Staph. anaerobius*, *Strep. intermedius*, *Strep. mitis*, *T. forsythia*, and *T. socranskii*. The bacterial load of these species was significantly higher at samples from individuals with peri-implantitis. Independent of implant status, several bacteria associated with periodontitis were found at elevated levels in individuals with a history of periodontitis. Older age was also associated with an increased risk for peri-implantitis.

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CONFLICT OF INTEREST

None of the authors has a conflict of interest.

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