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The condition of periodontal tissues and selected microorganisms presence around implants and natural teeth

Original Study

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Abstract

Introduction. Periodontitis is a disease of the tissues surrounding the tooth, leading to tooth loss. Missing teeth are often restored with fixed prostheses supported by implants. The aim of this study was to analyze subgingival microbiota in periodontal pockets and around implants, as well as to assess the correlation of periodontal parameters and risk factors for periodontitis with the presence of selected bacteria.
Materials and Methods. The study included 33 patients of both sexes, aged 39 to 79 years, having their own dentition and intraosseous implants with healthy periodontium or reversible gingivitis. The patients underwent periodontal examination and bacteriological analysis with the use of a PET RT-PCR kit (MIP Pharma, Germany).
Results. Less plaque was observed on implant surfaces than around natural teeth (28.7% vs. 39.8%). On the other hand, significantly deeper pockets (2.9 vs. 2.3 mm), and a slightly higher gingival bleeding index (27.9 vs. 22.6%) were noted around implants.
Conclusions. There were no significant differences in the overall presence and number of studied pathogens between the natural teeth and implants. In the case of natural teeth, the presence of *Porphyromonas gingivalis*, *Treponema denticola*, *Tannerella forsythia* and *Prevotella intermedia* showed correlation with clinical status: the amount of plaque (Td, Tf), bleeding (Td, Tf, Pi) and probing depth (Pg, Td, Tf, Pi). Such relationships were not observed in the case of implants. Bacteria from periodontal pockets also demonstrated more correlation with the general condition compared to bacteria residing in tissues around implants.

Keywords

periodontitis • peri-implantitis • bacteria • microbiota • implants

1. Introduction

About 700 different species of bacteria have been identified residing in the oral cavities of healthy people [1]. Gram-positive Cocci are mainly observed in healthy tissues surrounding natural teeth and implants, and 80% of them are bacteria of the genera: *Veilonella, Streptococcus, Peptostreptococcus* [1,2,3]. It has been proven that bacterial flora varies between individuals, in particular depending on the time of collecting sample material for tests, the patient's age, diet, and even sex or the level of education of the examined person. In addition, it has been shown that different species of bacteria have predilections for different sites within the oral cavity [2]. Bacteria of the genus *Veilonella* spp. and *Streptococcus salivarius* predominate on the surface of the tongue, *Actinomyces* spp., *Streptococcus mutans* and *Streptococcus sanguinis* are more frequently detected on the surface of teeth, while *Fusobacterium* spp. and anaerobic spirochetes demonstrate a preference for the gingival fissure [3]. The diverse distribution of individual species of bacteria in the oral cavity is related to the presence of specific adhesins on their bacterial surfaces, which have an affinity for specific receptors within the oral cavity. In addition to bacteria, fungi also belong to the residual microbiota [4]. Such a diversified environment, and above all, maintenance of an appropriate balance and interactions between residual microorganisms, all protect against invasion of unwanted pathogens [5].

Bacteria residing in the fissures around teeth and implants initiate the inflammatory process. Inflammation is a basis for development of periodontitis and peri-implantitis. In individuals suffering from periodontitis, a higher number of anaerobic and gram-negative bacteria were observed compared to healthy volunteers [6,7]. Socransky et al. divided pathogens isolated from gingival pockets into 5 complexes: red, orange, yellow, purple and green [8]. This division was made on the basis of the observation that bacteria from individual complexes usually coexist with each other. This is called "community theory".

The authors observed that certain individual complexes were associated with a more severe or less severe course of periodontitis. Pathogens classified as red and orange complexes are usually responsible for more severe periodontal disease, with probing depth (PD) of pockets above 4 mm, while a lower PD (less than 3 mm) is typically observed in the course of inflammation caused by bacteria from the green or yellow complexes. However, not all literature confirms that severity of disease is associated with pathogens isolated from the oral cavity of patients.

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According to studies, the most frequently isolated pathogenic strains in cases of peri-implantitis are *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia, Fusobacterium nucleatum, Campylobacter* spp., *Peptostreptococcus micros,* and *Tannerella forsythia* [9,10]. Fungi of the *Candida* genus may also appear in peri-implant lesions.

Currently, it is believed that the pathomechanism of the development of peri-implantitis is complex and depends in part upon the properties of the implant material and its design. In the surface of the implant material, it has been found that the most important factors are the physico-chemical properties and surface topography, which can promote bacterial adhesion to the implant surface [11,12].

Diagnostics of both periodontal and peri-implant diseases is based on specific symptoms such as bleeding, soft tissue swelling (mucositis, gingivitis and alveolar bone loss visible in x-rays (periimplantitis, periodontitis), as well as microbiological tests [13].

Peri-implantitis development may also be influenced by the presence of periodontal disease and its characteristic pathological microbiota. This is of great concern due to the possibility of transmission of bacteria from periodontal pockets to the fissure around the implant. Numerous studies have confirmed a higher incidence of peri-implantitis in patients who had lost teeth as a result of periodontitis before implant treatment compared with those who had not had periodontal disease [14,15,16].

The primary aim of the study was to analyze the presence and quantity of subgingival bacterial flora in periodontal pockets and around implants. The secondary aim was to assess the correlation of periodontal parameters with the presence of selected bacteria around implants and in periodontal pockets. The authors also attempted to correlate the presence of bacteria with the risk factors of periodontal and peri-implant disease.

2. Materials and Methods

The study included 33 patients of both sexes, aged 39 to 79 years, having their own dentition and intraosseous implants with a stable situation within periodontal tissues (healthy periodontium or reversible gingivitis). The patients underwent periodontal examination and bacteriological analysis with the use of a commercial PET RT-PCR kit (MIP Pharma, Germany).

The study was approved by the Ethics Committee of the Medical University of Warsaw KB/53/2019, and all study participants gave informed, voluntary and written consent for their participation in the study.

2.1. Study group

Inclusion criteria:

- Presence of at least one intraosseous implant in the oral cavity burdened with prosthetic reconstruction for at least three years.
- Presence of at least 12 natural teeth.
- Stable situation within periodontal tissues—no progressive loss of periodontal tissues in clinical and radiological examination.

All implants used in the study were titanium implants with internal conical connections. Two implant brands were used: Astra Tech TX (Dentsply Implants, Sweden) and Neodent Implants (Straumann, Switzerland). All implants were located in molar and premolar areas of upper or lower jaw.

Exclusion criteria:

- Systemic or local antibiotic therapy in the oral cavity less than six months prior to the examination.
- Periodontal treatment less than six months prior to the examination.
- Professional hygiene treatments less than six months prior to the examination.
- Use of preparations with chlorhexidine less than six months prior to the examination.
- History of unregulated systemic diseases like diabetes, stroke, cardiovascular diseases, osteoporosis and past neoplastic diseases within the last five years.

In the clinical portion of the study, the patients were subjected to periodontal examination, which included assessment of clinical parameters at six measurement points for each tooth (mesio-buccal, buccal, disto-buccal, mesio-lingual, lingual and disto-lingual) using a WHO 621 manual periodontal probe. During the examination, PD and presence of bleeding 30 seconds after probing (BOP acc. to Ainamo and Bay [17]) were recorded. Presence of plaque was also assessed on four surfaces of each tooth. On this basis, plaque index (PI) according to O'Leary [18] and approximate plaque index (API) according to Lange [19] were calculated.

The bacteriological examination was performed using a commercial PET RT-PCR kit (MIP Pharma, Germany). Samples were taken with the use of paper points from a pocket next to an implant, and from a pocket of a natural tooth with comparable PD value. Whenever possible, more than one implant-tooth pair meeting this condition was chosen. In total, the study included 40 implant-tooth pairs.

The examined pocket was isolated and plaque was removed from the gingival margin using a cotton swab. The collection area was then dried. Sterile paper points were placed in the pocket to the full depth for 20 s, then packed in plastic containers and delivered to the laboratory.

The laboratory part of the bacteriological test was performed on the basis of the molecular-biological Real-Time PCR method for qualitative and quantitative determination of pathogens in gingival pockets and fissures around implants. The PET test was conducted at MIP-Pharma Laboratory in Germany using the method described by Kotsilkov et al. [20]. The Plus version

of the test includes the following pathogens: *Aggregatibacter actinomycetemcomitans (Aa), Porphyromonas gingivalis (Pg), Treponema denticola (Td), Tannerella forsythia (Tf), Prevotella intermedia (Pi), Peptostreptococus micros (Pm), Fusobacterium nucleatum (Fn), Eubacterium nodatum (En),* and *Capnocytophaga gingivalis (Cg).*

3. Statistical analysis

Statistical analyses were performed using PQStat v. 1.4.4 software. (PQStat Software, Poznań, Poland). For quantitative variables, an assessment comparing the consistency of distribution with normal distribution was carried out using the Kolmogorov-Smirnov test. Due to the lack of normal distribution for some quantitative data (number of implants, years of smoking, number of cigarettes smoked, hygiene around the examined teeth and implants, and number of bacteria), non-parametric tests were used for these variables.

Descriptive statistics are presented in the form of:

- For qualitative variables: number and percentage
- For quantitative discrete variables: median and range
- For quantitative continuous variables showing normal distribution: mean and standard deviation (SD)
- For quantitative continuous variables not showing normal distribution: median and range

For comparative analyses, the following were used:

- For dichotomous variables (in the case of tooth-implant comparisons in one patient): Z-test for two dependent proportions
- For quantitative variables with normal distribution: Student's t-test for independent variables or Student's t-test for dependent variables (in the case of tooth-implant comparisons in one patient)
- For quantitative variables not showing normal distribution: Mann-Whitney U test for independent groups or Wilcoxon test for dependent groups (in the case of tooth-implant comparisons in one patient)

For variables not showing normal distribution: Spearman's rank correlation analysis was used to assess the correlation between quantitative variables. A significance level of 0.05 ($p < 0.05$) was adopted in all analyses. The strength of the association, expressed by Spearman's coefficient, was classified as very weak ($r = 0–0.19$), weak (0.2–0.39), moderate (0.40–0.59), strong (0.60–0.79), or very strong (0.80–1.00).

4. Results

General characteristics of the study group, as well as data on periodontal parameters (hygiene status, bleeding index and mean probing depth), are presented in Table 1. The study included 33 patients of both genders (7 men and 26 women), aged 39 to 79 years, with both natural dentition and intraosseous implants. The mean age was 57.1 years. The median number of natural teeth and implants were 25 teeth and 2 implants, respectively.

Table 1. General characteristics, whole-mouth hygiene status, bleeding index, and probing depth in the study group

Variable	Characteristics (n = 33)
Age (mean ± SD)	57.1 ± 10.9
Number of teeth (median, range)	25 (13–27)
Number of implants (median, range)	2 (1–9)
Body Mass Index (mean ± SD)	24.3 ± 4.0 underweight (BMI < 18.5): 1 (3.0%) normal weight (18.5–24.9): 19 (58.0%) overweight (25.0–29.9): 10 (30.0%) obesity (BMI ≥ 30): 3 (9.0%)
Cigarette smoking (n, %)	9 (27.3%)
PI (mean ± SD)	38.4% ± 19.2 p.p.
API (mean ± SD)	68.2% ± 29.8 p.p.
BOP (mean ± SD)	23.2% ± 14.1 p.p.
Mean PD (mean ± SD)	2.4 ± 0.4 mm

SD – standard deviation
p.p. – percentage points
PI – plaque index
API – approximal plaque index
BOP – bleeding on probing
PD – probing depth
Tiv – Student's t-test for independent variables

The periodontal status of the subjects was stable. The mean probing depth was 2.4 mm. Most of the patients demonstrated good or average oral hygiene (PI < 40%, API < 70%) and the mean bleeding index was 23.2%.

The comparison of hygiene and inflammation indices (Table 2) around natural teeth and implants showed that, on the one hand, there was much less plaque on the implant surfaces than around the natural teeth (25% vs. 45%). On the other hand, however, pockets around the implants were significantly deeper (2.9 vs. 2.3 mm), and bleeding index around the implants was slightly higher (27.9 vs. 22.6%).

Table 2 shows the total number of bacteria, the percentage of teeth/implants host to specific species of bacteria, and the quantitative evaluation of individual pathogens in the periodontal pocket in each tested tooth and in the fissures around the implants. No significant differences between the tooth-implant pair in individual patients for any of the assessed parameters were observed.

The analysis of the correlation between the local condition of tissues surrounding the tooth/implant and the presence of individual bacterial species revealed significant differences between the two environments (Table 3). In the case of natural teeth, Pg, Td, Tf and Pi all showed correlation of varied strength with clinical status. Strong correlations were observed between

Table 2. Comparison of local hygiene status, bleeding, probing depth, and qualitative and quantitative assessment of bacteria between implants and the natural teeth

Variable	Teeth	Implants	Comparison
PI (median, range)	45% (0%–71%)	25% (0%–88%)	p = 0.006 [W]
BOP (mean ± SD)	22.6% ± 14.2 p.p.	27.9% ± 23.3 p.p.	p = 0.091 [Tdv]
Mean PD (mean ± SD)	2.3 ± 0.4 mm	2.9 ± 0.7 mm	p < 0.001 [Tdv]
Number of bacteria in total (median, range [mln])	11 (0.1–5900)	28 (0.4–2600)	p = 0.174 [W]
Aa – percentage of teeth/implants (mean ± SD)	5.0% ± 22.1 p.p.	5.0% ± 22.1 p.p.	p = 1.000 [Z]
Aa – number (median, range)	0 (0–2700)	0 (0–52 000)	p = 0.855 [W]
Aa – number per million bacteria in total (median, range)	0 (0–192.9)	0 (0–14 444.4)	p = 0.584 [W]
Pg – percentage of teeth/implants (mean ± SD)	30.0% ± 46.4 p.p.	27.5% ± 45.2 p.p.	p = 0.564 [Z]
Pg – number (median, range)	0 (0–470 000)	0 (0–230 000)	p = 0.263 [W]
Pg – number per million bacteria in total (median, range)	0 (0–48 611)	0 (0–25 275)	p = 0.442 [W]
Td – percentage of teeth/implants (mean ± SD)	45.0% ± 50.4 p.p.	40.0% ± 49.6 p.p.	p = 0.480 [Z]
Td – number (median, range)	0 (0–140 000)	0 (0–11 000)	p = 0.211 [W]
Td – number per million bacteria in total (median, range)	0 (0–18 056)	0 (0–57 303)	p = 0.867 [W]
Tf – percentage of teeth/implants (mean ± SD)	40.0% ± 49.6 p.p.	30.0% ± 46.4 p.p.	p = 0.317 [Z]
Tf – number (median, range)	0 (0–23 000)	0 (0–15 000)	p = 0.119 [W]
Tf – number per million bacteria in total (median, range)	0 (0–9444)	0 (0–58 427)	p = 0.603 [W]
Pi – percentage of teeth/implants (mean ± SD)	10.0% ± 30.4 p.p.	7.5% ± 26.7 p.p.	p = 0.655 [Z]
Pi – number (median, range)	0 (0–190 000)	0 (0–180 000)	p = 0.528 [W]
Pi – number per million bacteria in total (median, range)	0 (0–370.8)	0 (0–12 857.1)	p = 1.000 [W]
Pm – percentage of teeth/implants (mean ± SD)	17.5% ± 38.5 p.p.	25.0% ± 43.9 p.p.	p = 0.366 [Z]
Pm – number (median, range)	0 (0–12 000)	0 (0–2600)	p = 0.975 [W]
Pm – number per million bacteria in total (median, range)	0 (0–21.8)	0 (0–453.1)	p = 0.108 [W]
Fn	Absent in all the specimens	Absent in all the specimens	NA
En – percentage of teeth/implants (mean ± SD)	7.5% ± 26.7 p.p.	5.0% ± 22.1 p.p.	P = 0.317 [Z]
En – number (median, range)	0 (0–4200)	0 (0–7500)	p = 0.423 [W]
En – number per million bacteria in total (median, range)	0 (0–280)	0 (0–500)	p = 0.423 [W]
Cg – percentage of teeth/implants (mean ± SD)	80.0% ± 40.5 p.p.	70.0% ± 46.4 p.p.	p = 0.157 [Z]
Cg – number (median, range)	375 (0–51 000)	1070 (0–89 000)	p = 0.421 [W]
Cg – number per million bacteria in total (median, range)	46.7 (0–3200)	37.5 (0–24 722)	p = 0.775 [W]

SD – standard deviation
p.p. – percentage points
PI – plaque index
BOP – bleeding on probing
PD – probing depth
W – Wilcoxon test for dependent groups
Tdv – Student's t-test for dependent variables
*Aa – **Aggregatibacter actinomycetemcomitans***
*Pg – **Porphyromonas gingivalis***
*Td – **Treponema denticola***
*Tf – **Tannerella forsythia***
*Pi – **Prevotella intermedia***
*Pm – **Peptostreptococus micros***
*Fn – **Fusobacterium nucleatum***
*En – **Eubacterium nodatum***
*Cg – **Capnocytophaga gingivalis***
W – Wilcoxon test for dependent groups
Z – Z-test for two dependent proportions
NA – not applicable

Table 3. Correlations between the local condition of tissues around teeth or implants and the presence of bacteria (Spearman's rank correlation analysis)

	Number of surfaces with plaque		Number of bleeding points		Max probing depth	
	Natural teeth	Implants	Natural teeth	Implants	Natural teeth	Implants
Number of bacteria in total	r = 0.150 p = 0.405	r = 0.179 p = 0.318	r = 0.034 p = 0.852	r = 0.154 p = 0.391	r = -0.012 p = 0.947	r = -0.331 p = 0.060
Aa number	r = 0.130 p = 0.472	r = 0.274 p = 0.122	r = -0.047 p = 0.797	r = -0.029 p = 0.874	r = 0.050 p = 0.781	r = -0.173 p = 0.336
Aa number per million bacteria in total	r = 0.130 p = 0.472	r = 0.274 p = 0.122	r = -0.047 p = 0.797	r = -0.029 p = 0.874	r = 0.050 p = 0.781	r = -0.173 p = 0.336
Pg number	r = 0.277 p = 0.118	r = 0.229 p = 0.200	r = 0.155 p = 0.388	r = 0.190 p = 0.290	r = 0.395 p = 0.023	r = 0.020 p = 0.910
Pg number per million bacteria in total	r = 0.278 p = 0.118	r = 0.193 p = 0.282	r = 0.154 p = 0.392	r = 0.184 p = 0.306	r = 0.368 p = 0.035	r = 0.019 p = 0.916
Td number	r = 0.659 p < 0.001	r = 0.156 p = 0.387	r = 0.541 p = 0.001	r = 0.320 p = 0.070	r = 0.435 p = 0.011	r = -0.036 p = 0.844
Td number per million bacteria in total	r = 0.691 p < 0.001	r = 0.129 p = 0.473	r = 0.609 p < 0.001	r = 0.305 p = 0.084	r = 0.412 p = 0.017	r = -0.028 p = 0.875
Tf number	r = 0.576 p < 0.001	r = -0.008 p = 0.964	r = 0.615 p < 0.001	r = 0.061 p = 0.737	r = 0.406 p = 0.019	r = -0.045 p = 0.802
Tf number per million bacteria in total	r = 0.593 p < 0.001	r = -0.036 p = 0.844	r = 0.637 p < 0.001	r = 0.046 p = 0.801	r = 0.328 p = 0.063	r = -0.028 p = 0.877
Pi number	r = 0.285 p = 0.107	r = -0.163 p = 0.366	r = 0.400 p = 0.021	r = -0.029 p = 0.874	r = 0.347 p = 0.048	r = -0.173 p = 0.336
Pi number per million bacteria in total	r = 0.305 p = 0.084	r = -0.162 p = 0.366	r = 0.402 p = 0.021	r = -0.029 p = 0.874	r = 0.349 p = 0.047	r = -0.173 p = 0.336
Pm number	r = 0.051 p = 0.778	r = -0.226 p = 0.206	r = -0.021 p = 0.908	r = 0.095 p = 0.600	r = -0.033 p = 0.853	r = -0.086 p = 0.633
Pm number per million bacteria in total	r = 0.061 p = 0.736	r = -0.246 p = 0.167	r = 0.065 p = 0.720	r = 0.069 p = 0.704	r = 0.002 p = 0.993	r = -0.012 p = 0.947
En number	r = 0.235 p = 0.188	r = 0.090 p = 0.619	r = 0.306 p = 0.083	r = 0.185 p = 0.302	r = 0.041 p = 0.823	r = -0.100 p = 0.580
En number per million bacteria in total	r = 0.243 p = 0.173	r = 0.090 p = 0.619	r = 0.306 p = 0.083	r = 0.185 p = 0.302	r = 0.037 p = 0.836	r = -0.100 p = 0.580
Cg number	r = 0.268 p = 0.132	r = 0.274 p = 0.123	r = 0.003 p = 0.986	r = -0.033 p = 0.854	r = -0.146 p = 0.419	r = -0.101 p = 0.576
Cg number per million bacteria in total	r = 0.321 p = 0.068	r = 0.337 p = 0.055	r = 0.064 p = 0.722	r = 0.002 p = 0.992	r = 0.032 p = 0.856	r = 0.176 p = 0.328

*Aa – **Aggregatibacter actinomycetemcomitans***
*Pg – **Porphyromonas gingivalis***
*Td – **Treponema denticola***
*Tf – **Tannerella forsythia***
*Pi – **Prevotella intermedia***
*Pm – **Peptostreptococus micros***
*Fn – **Fusobacterium nucleatum***
*En – **Eubacterium nodatum***
*Cg – **Capnocytophaga gingivalis***

Td number and plaque index, as well as between the Td and Tf numbers and the bleeding index. Other statistically significant correlations, presented in detail in Table 3, were either moderate or weak. Relationships between bacteria number and intraoral indices were not observed in the case of implants.

Also, an analysis of the relationship between the number of individual species and the presence of general risk factors and health condition of the entire oral cavity showed differences when examined around natural teeth and around implants. Bacteria residing in periodontal pockets showed greater correlation with the general condition, as compared to bacteria in implant-surrounding tissues (Tables 4 and 5). The presence of species such as Pg, Td, Tf, Pi, Pm, and Cg were correlated with overall oral hygiene, bleeding index, and mean probing depth. The numbers of Aa and Pg were significantly higher in the group of smokers, and Td, Pi and En were more numerous in men.

In the case of tissues around implants, the spectrum of bacteria correlating with the general condition of the oral cavity was narrower (Table 5). Hygienic negligence as well as inflammation were associated with greater prevalence of Pg, Td and Cg. In tissues around implants in smokers, higher levels of Aa, Pg and Td were observed. The number of Pg and Td in tissues around implants also increased with age.

Table 4. Number of specific bacteria in periodontal pockets versus presence of risk factors and whole-mouth indices of plaque, bleeding, and pocket depth (only species for which at least one statistically significant difference/correlation was obtained)

		Bacteria in periodontal pockets (median, range)								
		Number of bacteria in total [mln]	Aa	Pg	Td	Tf	Pi	Pm	En	Cg
Age		r = -0.071 p = 0.663 S	r = -0.081 p = 0.619 S	r = 0.279 p = 0.081 S	r = 0.398 p = 0.011 S	r = 0.196 p = 0.226 S	r = 0.154 p = 0.343 S	r = 0.218 p = 0.177 S	r = 0.056 p = 0.734 S	r = -0.139 p = 0.392 S
Sex	Women	9 (0–1000)	0 (0–150)	0 (0–41 000)	0 (0–120 000)	0 (0–17 000)	0 (0)	0 (0–12 000)	0 (0–4200)	225 (0–51 000)
	Men	132 (7–5900)	0 (0–2700)	6000 (0–470 000)	28 560 (0–140 000)	345 (0–23 000)	650 (0–190 000)	0 (0–10 000)	0 (0–310)	1550 (140–40 000)
	Comparison	p = 0.013 M	p = 0.283 M	p = 0.058 M	p = 0.033 M	p = 0.093 M	p < 0.001 M	p = 0.592 M	p = 0.050 M	p = 0.228 M
Body Mass Index		r = 0.237 p = 0.141 S	r = 0.053 p = 0.747 S	r = 0.305 p = 0.055 S	r = 0.109 p = 0.503 S	r = 0.066 p = 0.687 S	r = 0.210 p = 0.193 S	r = 0.065 p = 0.691 S	r = 0.084 p = 0.605 S	r = 0.125 p = 0.440 S
Cigarette smoking	Yes	4 (0–240)	0 (0–2700)	12 000 (0–470 000)	340 (0–140 000)	270 (0–23 000)	0 (0–89 000)	0 (0)	0 (0)	140 (0–40 000)
	No	14 (1–5900)	0 (0)	0 (0–11 000)	0 (0–120 000)	0 (0–17 000)	0 (0–190 000)	0 (0–12 000)	0 (0–4200)	1000 (0–51 000)
	Comparison	p = 0.126 M	p = 0.022 M	p = 0.008 M	p = 0.066 M	p = 0.225 M	p = 0.309 M	p = 0.082 M	p = 0.289 M	p = 0.014 M
PI		r = 0.506 p < 0.001 S	r = 0.028 p = 0.865 S	r = 0.435 p = 0.005 S	r = 0.608 p < 0.001 S	r = 0.593 p < 0.001 S	r = 0.477 p = 0.002 S	r = 0.452 p = 0.003 S	r = 0.248 p = 0.123 S	r = 0.205 p = 0.204 S
API		r = 0.514 p < 0.001 S	r = 0.070 p = 0.667 S	r = 0.227 p = 0.159 S	r = 0.414 p = 0.008 S	r = 0.278 p = 0.082 S	r = 0.221 p = 0.170 S	r = 0.507 p < 0.001 S	r = 0.155 p = 0.341 S	r = 0.321 p = 0.044 S
BOP		r = 0.236 p = 0.142 S	r = 0.004 p = 0.978 S	r = 0.282 p = 0.078 S	r = 0.574 p < 0.001 S	r = 0.453 p = 0.003 S	r = 0.408 p = 0.009 S	r = 0.141 p = 0.387 S	r = 0.308 p = 0.053 S	r = 0.161 p = 0.321 S
PD mean		r = 0.014 p = 0.932 S	r = 0.100 p = 0.541 S	r = 0.603 p < 0.001 S	r = 0.696 p < 0.001 S	r = 0.507 p < 0.001 S	r = 0.389 p = 0.013 S	r = 0.086 p = 0.598 S	r = 0.098 p = 0.548 S	r = -0.181 p = 0.263 S

PI – plaque index
API – approximal plaque index
BOP – bleeding on probing
PD – probing depth
*Aa – **Aggregatibacter actinomycetemcomitans***
*Pg – **Porphyromonas gingivalis***
*Td – **Treponema denticola***
*Tf – **Tannerella forsythia***
*Pi – **Prevotella intermedia***
*Pm – **Peptostreptococus micros***
*En – **Eubacterium nodatum***
*Cg – **Capnocytophaga gingivalis***
M - Mann-Whitney U test for independent groups
S – Spearman's rank correlation

5. Discussion

In this study, the total number of bacteria and quantitative composition of individual pathogens in the fissures around the examined teeth and implants were analyzed. On the basis of the observations, no significant differences were found between the total number of bacteria or the presence and number of tested pathogens.

The research of Jakobi et al. indicated that both in healthy patients and in those who had symptoms of periodontitis/peri-implantitis, a similarly rich variety of bacterial flora was found in all examined sites around natural teeth and implants [6]. This may suggest cross-contamination of the implant area, which was also found by other researchers. The hypothesis is also confirmed by the finding that in patients who have both implants and preserved natural teeth, there was a greater number of pathogenic bacteria in the vicinity of implants than in patients who have completely lost their own teeth and use only restorations based on implants.

Yu et al. conducted a study in which they assessed microbiological composition of subgingival plaque samples collected from the same patients from implant areas, from teeth surrounded by healthy tissues, and from areas affected by peri-implantitis and periodontitis [7]. However, the study did not confirm the cross-infection hypothesis because significantly fewer similarities were found between flora collected from different parts of the oral cavity, both in healthy subjects and those with lesions.

Out of the large number of bacteria detected, only *Porphyromonas gingivalis, Enterococcus italicus, Bacillus sp. Taxon B77,* and *Bacillus sp. Taxon C43* species were present in all samples collected from individuals with lesions. In one patient, *Enterococcus sp. taxon A43* bacteria were present both

Table 5. Number of specific bacteria in tissues around the implants versus presence of risk factors and whole-mouth indices of plaque, bleeding, and pocket depth (only species for which at least one statistically significant difference/correlation was obtained)

		Bacteria in tissues around the implants (median, range)				
		Number of bacteria in total [mln]	Aa	Pg	Td	Cg
Age		r = 0.113 p = 0.488 S	r = 0.189 p = 0.243 S	r = 0.368 p = 0.020 S	r = 0.320 p = 0.044 S	r = -0.129 p = 0.426 S
Sex	Women	23 (0–2600)	0 (0–52 000)	0 (0–230 000)	0 (0–11 000)	1070 (0–89 000)
	Men	360 (1–1700)	0 (0)	160 (0–37 000)	140 (0–6800)	2740 (0–44 000)
	Comparison	p = 0.088 M	p = 0.502 M	p = 0.190 M	p = 0.504 M	p = 0.391 M
Body Mass Index		r = 0.167 p = 0.304 S	r = -0.358 p = 0.023 S	r = 0.120 p = 0.462 S	r = -0.028 p = 0.863 S	r = -0.185 p = 0.252 S
Cigarette smoking	Yes	41 (0–1700)	0 (0–52 000)	320 (0–37 000)	3800 (0–11 000)	230 (0–13 000)
	No	26 (0–2600)	0 (0)	0 (0–230 000)	0 (0–6800)	1900 (0–89 000)
	Comparison	p = 0.928 M	p = 0.023 M	p = 0.028 M	p = 0.017 M	p = 0.231 M
PI		r = 0.485 p = 0.001 S	r = 0.060 p = 0.715 S	r = 0.279 p = 0.081 S	r = 0.339 p = 0.032 S	r = 0.307 p = 0.054 S
API		r = 0.486 p = 0.001 S	r = 0.100 p = 0.541 S	r = 0.109 p = 0.505 S	r = 0.277 p = 0.083 S	r = 0.405 p = 0.009 S
BOP		r = 0.216 p = 0.180 S	r = -0.030 p = 0.855 S	r = 0.146 p = 0.370 S	r = 0.366 p = 0.020 S	r = 0.185 p = 0.253 S
PD mean		r = 0.133 p = 0.414 S	r = 0.199 p = 0.219 S	r = 0.501 p < 0.001 S	r = 0.545 p < 0.001 S	r = -0.007 p = 0.966 S

PI – plaque index
API – approximal plaque index
BOP – bleeding on probing
PD – probing depth
*Aa – **Aggregatibacter actinomycetemcomitans***
*Pg – **Porphyromonas gingivalis***
*Td – **Treponema denticola***
*Cg – **Capnocytophaga gingivalis***
M – Mann-Whitney U test for independent groups
S – Spearman's rank correlation

at an implant with soft tissue lesions and on the adjacent tooth. In some other patients, both at the implant and at the tooth with the greatest probing depth, the following were identified: *Enterococcus casseliflavus, Porphyromonas gingivalis, Bacillus cellulosilyticus* and *Veillonella* spp. (one species for each patient).

No significant differences were found between the bacterial flora around implants and those around natural teeth, which is consistent with the results of our own study. This was also observed by Menini et al. Tamrakar et al., who found a similarity between microflora of the implant area and that of preserved natural teeth [9,10]. Most of the bacteria identified by them belonged to the gram-negative group, including *Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Enterobacter* spp. The first two of the mentioned species were observed more often around implants than natural teeth.

Other results were obtained by Heuer et al., who observed that there is a much greater diversity of bacterial flora around teeth with gingivitis compared to mucositis lesions [21]. Similarly, Kumar et al. described much more diverse bacterial flora around natural teeth compared to that around implants, both in patients with healthy periodontium and with lesions [22].

Cortelli et al. conducted a study to verify whether a comparable frequency of selected bacterial species can be observed in tissues in a similar clinical condition around implants and periodontal tissues around teeth [23]. This study assessed the presence of *Porphyromonas gingivalis, Tannerella forsythia, Campylobacter rectus, Prevotella intermedia, Treponema denticola,* and *Aggregatibacter actinomycetemcomitans* in cases of healthy peri-implant tissues (n = 53), healthy periodontium (n = 53), mucositis (n = 50), gingivitis (n = 50), peri-implantitis (n = 50) and periodontitis (n = 50). The pattern of incidence of bacteria in the vicinity of the implant was as expected (peri-implantitis> mucositis> healthy periodontium around implants). With the exception of *P. intermedia* ($p > 0.05$), the incidence of individual bacteria was higher in peri-implantitis than in normal periodontal tissues ($p < 0.05$). The incidence of *P. gingivalis* and other red-complex bacteria was higher in peri-implantitis than in periodontal pockets ($p < 0.05$). *T. forsythia* and *T. denticola* were identified in samples from periodontal pockets, confirming the

more frequent presence of these bacteria in periodontitis as compared to gingivitis. The incidence of *C. rectus* and *T. forsythia* was higher in shallow pockets and gingivitis, respectively, than in healthy peri-implant/mucositis tissues ($p < 0.05$). The respective incidence of *P. gingivalis* and *A. actinomycetemcomitans* were similar for periodontitis and peri-implantitis ($p > 0.05$), while in all other species, the incidence was higher in periodontitis than in peri-implantitis ($p < 0.05$). Based on these results, the authors found a tendency for higher incidence of bacteria in periodontal pockets compared to material obtained from fissures around implants.

Although in our study no differences were found between the composition and number of bacteria around natural teeth and implants, the analysis of relationships between the identified species and clinical parameters yielded slightly different results. In the case of natural teeth, a significant correlation was observed between the presence of four species: *T. denticola, T. forsythia, P. intermedia, P. gingivalis*, and the PD, and for the first three of the mentioned species also the presence of bleeding upon probing. A weaker correlation with the occurrence of bleeding was found for *E. nodatum*. The relative abundance of *Capnocytophaga gingivalis* in relation to the total bacteria showed a close statistical correlation with the number of sites with plaque around the examined tooth.

In the case of implants, the clinical condition of peri-implant tissues basically did not correlate with presence of bacteria studied. Only the presence of bleeding on probing showed a negligible correlation with the amount of *T. denticola. C. gingivalis*, as in the case of natural teeth, constituted a higher percentage of total bacteria in regions with higher amount of plaque. Moreover, an inverse correlation was found, close to statistical significance, between the total number of bacteria and probing depth around the implants.

It seems that the observed differences in correlations between the presence of specific bacteria and severity of symptoms of periodontitis may have significant clinical implications and provide insight into the pathogenesis of tissue lesions around implants. Our findings indicate that mucositis and peri-implantitis lesions may have a slightly different pathomechanism than in the case of gingivitis and periodontitis. Although in both cases the same species of bacteria were isolated in a similar number, only their presence around natural teeth was correlated with clinical conditions, including symptoms of inflammation (bleeding, deepened pockets).

On the other hand, this may also mean that it is not possible to directly transfer therapeutic strategies for periodontal diseases to treatment of peri-implant lesions, especially when it comes to causal treatment such as antibiotics.

A similar analysis of relationships between the clinical condition of tissues around implants and the presence of periopathogens in patients with permanent restorations based on implants was carried out by Menini et al. [9]. They also observed a relationship between the amount of *T. denticola* and bleeding. Additionally, this parameter correlated with the amount of *T. forsythia*. The amount of *T. denticola* was also higher for implants with more plaque. In a study carried out on dogs in which peri-implantitis was experimentally induced, Zhu et al. found that the number of *T. forsythia* was correlated with probing depth [24]. Ardila et al. also conducted a study on the microflora of patients treated with implants, including in their research only opportunistic bacteria, and reported a relationship between an increase in probing depth, clinical attachment loss and bleeding on probing index and the presence of gram-negative intestinal bacilli and *Pseudomonas* spp. [25] .

A study by Monje et al [26] found interesting information on relationships between the microbiota and change in the clinical condition of tissues around implants. The authors induced peri-implantitis in dogs that had previously had implants. They observed that the degree of marginal bone loss correlated with the total number of bacteria in gingival crevicular fluid samples ($r = 0.21$; $p = 0.009$), as well as with *Porphyromonas gulae* and *Tannerella forsythia* species. The amounts of these bacteria also correlated with the presence of purulent exudate and with the value of the bleeding index ($p < 0.05$).

6. Limitations of the study

Limitations of this study included a lack of microbiological analysis of a control group of patients with no dental implants or implants with various types of connections. Also, the two included implant brands could not be directly compared due to the insufficient subgroup size. The correlations observed between the presence and/or quantity of specific bacteria species and local or general health cannot be interpreted as a cause-and-effect relationship—such a hypothesis would require further experimental studies.

7. Conclusion

There were no significant differences between the tooth-implant pair in terms of the total number of bacteria or the presence and number of studied pathogens. In the case of natural teeth, the presence of *Porphyromonas gingivalis*, *Treponema denticola*, *Tannerella forsythia* and *Prevotella intermedia* showed correlation with clinical status: the amount of plaque (*T. denticola, T. forsythia*), bleeding (*T. denticola, T. forsythia, P. intermedia*) and probing depth (*P. gingivalis, T. denticola, T. forsythia, P. intermedia*). Such relationships were not observed in the case of implants. Furthermore, bacteria from periodontal pockets demonstrated more associations with general condition compared to bacteria residing in tissues around implants.

Authors' contribution

Magdalena Jaszczak-Małkowska – Research concept and design, Carrying out the experiments, Acquisition of data, Writing - Original Draft Preparation, Literature review

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Bartłomiej Górski – Research concept and design, Supervising the project, Writing - Review and Editing
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Ethics approval

The study was approved by the Ethics Committee of the Medical University of Warsaw (KB/53/2019).

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