The effect of human periodontal pathogenic bacteria on immediate basal implant placement: A comparative study in beagle dogs

Ali Dakheel**, Ismaeil Mammani², Jiyar Naji³

Abstract
This study can clarify and compare two lines of the same design of basal implants based on microbiological criteria. Ten beagle dogs were involved in the first stage of the study when swabs from the gingival sulci were collected before extraction of the first and second premolars in both maxillary and mandibular segments of the left side of the mouth, followed by immediate implant placement of bicortical screws and compression screws of the basal implants. A waiting period of four months was followed by swab collection from the peri-implant areas of the successful implants of eight dogs. The swabs were sent for a qualitative polymerase chain reaction (PCR) technology to detect five periodontopathogens. In the first stage of the study, detecting some bacterial species can be correlated to the surgical results of the failure rate already detected. In the second stage, the prevalence of specific bacterial species was more significant compared to the first stage of the study, and the detected bacterial percentage was also higher. Periodontopathogens play an important role in peri-implant postoperative infection and subsequent failure, whether individually or even together.

Keywords: Basal implant, Screw-designed basal implants, Compression screw, Bicortical screw, Bacteria, Infection, Periodonto-pathogen, Periodontics.

Introduction
Basal implant dentistry divides the jawbone into two parts: The tooth-bearing alveolar or crestal component and the basal component. The characteristic histology of the crestal part is less thick. It is more susceptible to infections caused by tooth-borne diseases, traumas, or iatrogenic causes, leading to a greater percentage of marginal bone loss. In contrast, the basal part is densely cortical and is less susceptible to infection and bone loss. Because of its highly cortical structure, the basal bone may support the implants. In comparison, the load-bearing capability of the basal bone is considerably higher than that of the more cancellous crestal part. This reasoning derives from orthopedic surgery and the observation that cortical regions are critical because they are robust against bone resorption; consequently, basal implants are also referred to as orthopedic implants (Vijayebenezer et al., 2021). Basal implants can be presented with different designs, one of which is screw form type basal implants, which include compression screw form (with the rough surface of the screw) and bi-cortical screw form (with the smooth surface of the screw) with different peri-implant tissues responses to each form of which because of the different surface topographies in between (Mustilwar & Johnson, 2022).

More recent studies about dental implants’ correlation with the bacterial population in the mouth of patients treated with multiple dental implants found that those patients had significantly higher bacterial colonization than people without any implant treatment (Sharafuddin et al., 2023), a finding that can help to conclude that the main reason for the peri-implant diseases is the microbial contamination of the implant surface, implant-abutment
interface and biofilm of the natural teeth with biofilm attached to the implant surface (Daubert & Weinstein, 2019).

Peri-implant diseases after successful osseointegration of endosseous implants are caused by an imbalance between bacterial activity and host response (Romanos et al., 2015).

The pathological bacteria observed in the oral microflora in the peri-implant lesions were identified to be the red complex types (Porphyromonas gingivalis, Treponema denticola, Tannerella forsythia) similar to those of the periodontitis and orange complex species (Fusobacterium and Prevotella intermedia) (Ata-Ali et al., 2011), but the problem that other researchers found that even in the case of successful implants, periodontal pathogens were isolated from healthy peri-implant area (Casado et al., 2011). A claim that was explained by other researchers’ findings that a history of periodontitis had a greater impact on the peri-implant microbiota than implant loading time. The major influence on the peri-implant microbiota was, however, the microbiota on the remaining teeth. P. gingivalis and T. forsythia, red complex periodontal pathogens, colonised several implants, although all implants were successfully osseointegrated (Jakobi et al., 2015). Such opinions could help to conclude the significance of the microbial role in postoperative infection in dental implantology (Esposito et al., 2013), a role that represents one of the possible reasons for implant failure whether early or late failure (Chaushu et al., 2023).

One of the vitally investigated parameters is the bacterial infection of the dental implant that can lead to implant failure, not to forget about the level of the abutment in the case of basal implants (which would expose the implant to the oral environment from the first moment of placement). The purpose of this study is to provide a thorough understanding and a reliable comparison of the rough-surface compression screw and the smooth-surface bicortical screw; just to afford a piece of detailed information about the interrelation between those two kinds of basal implants and the associated bacterial effects.

**Materials and Methods**

As soon as agreement from the Ethical Board of the University of Duhok had been given, the first stage of the study was started using ten healthy, adult Beagle dogs. They were brought from the Dogs’ yard at the municipality of Zakho and were fitted in the Animals Investigation facility at the College of Veterinary Medicine/University of Duhok. The animals were maintained in individual cages in a (12:12 lightness/darkness series) and treated with anti-helminths drugs, vaccinated with antivirals, and provided with three meals of mixed diet, dry food and three times a day changes of tap water.

The implants were bought from Demirtas implant company, the DE|TECH screw-designed basal implants, the length of the fixture was 12 mm, and the abutment was 7.5 mm. The diameter of the compression screw implants was 3.5 mm while the diameter of the bicortical screw basal implants was 3.6 mm.

A split-mouth study technique was followed when one study quadrant in each jaw (one in the maxilla and the other in the mandible) on the same side of the mouth was involved, this way each study quadrant contained two implants, one of each type.

**Stage One**

The surgical operations were performed in the morning, and the animals to be operated on were fasting for 12 hours. The surgery was carried out under general anaesthetic effects. The anaesthetic treatment was as follows: The dogs were initially premedicated with ketamine (15 mg/kg/i.m.) and their pain was managed with dipyrone (500 mg/dog/i.m.). The dogs were subsequently administered xylazine (2 mg/kg), atropine (0.04 mg/kg/i.m.) and kept on a 2.5 to 4% isoflurane dosage during the operation, using an orotracheal tube size 7.5 for this purpose. Throughout the anaesthesia, the dogs were monitored. Cardiac frequency, respiratory frequency, oxygen saturation, expired carbon dioxide, and arterial pressure were all assessed.

The next step was swab collection for molecular bacteriology diagnostic purposes with sterile paper point sizes ranging from 25 to 40 according to the related gingival crevice size. The swabs were collected from the maxillary and the mandibular gingival crevice of the second premolar teeth on the left side, Figure 1.

The first and second premolars in the left side of the maxilla and mandible were extracted. That was followed by irrigation of the sockets with chlorhexidine, then drilling with copious irrigation of normal saline and finally implant placement immediately in the sockets. The compression screws were placed in the first premolar area and the bicortical screws in the second premolar area in each jaw.

![Figure 1: Preoperative swab collection from (a) The mandible and (b) The maxilla](image_url)
All the implants were placed in a more lingual position to bypass the inferior dental nerve and with a high torque (more than 50 Ncm) to guarantee good primary stability, with the abutments left out of occlusion at the trans gingival level.

A waiting period of four months was taken when dogs were kept under observation.

Two of the elderly dogs developed worm infections during this period when one of them responded to the anthelmintics while the other passed on.

**Stage Two**

The same stage one anesthetic procedure were followed during stage two of the operation. The implants were checked for any mobile implants that failed to osseointegrate into the surrounding tissues. The failed implants were excluded since our research is about the successfully osseointegrated implants only.

The swabs were collected from the compression screw and the bicortical screw implants’ surfaces with sterilized paper points and immersed immediately into the broth, Figure 2.

**Bacteriology**

The vials of bacteria in nutrient broth were sent directly to the laboratory for incubation for 24 hours at 37°C.

The examination procedures were carried out in two stages. The first stage involved the natural teeth preoperatively, whereas the second stage involved the successful implants postoperatively. The procedures were carried out separately and each stage of is whole process will be explained distinctly.

All the vials ended in successful growth after incubation, Figure 3.

Each selected microbe was arranged for its related primer. The primers were ordered from the Macrogen company in South Korea for a molecular diagnostic approach. The primers used in this work are shown in Table 1 and were produced according to the technique described in the datasheet. To create primer stock (100 Pmol/µL), 300 to 320 µL (depending on the primer type) of free nuclease water (ddH$_2$O) was mixed with 30 nmol of primer origin.

DNA was extracted from bacterial samples following the boiling method. Overnight cultivated nutrient broth bacteria (500 µL of broth) were collected and blended in a 1.5 mL tube with 200 µL of sterile double distilled water. The combination was then heated at 95°C for 10 minutes after the vortex for 15 seconds; the samples were iced immediately with ice, and the frozen suspension was centrifuged. Lastly, 150 µL of supernatant was employed as a DNA template for qualitative polymerase chain reaction (PCR). The purity and concentration of extracted DNA were detected using a nanodrop (Thermo Scientific, USA) to determine the quality and amount of all samples.

About 25 µl PCR mixture was made up of 12.5 µL of hot start premix (Addbio, Korea), 1-µL of reverse and forward primer (10 pmol), 4 µL of sample DNA, and the rest was filled with nuclease-free water (Qiagen, Germany). Polymerase chain reaction amplification was performed in a PCR system 9700 GeneAmp (applied bio-system, USA) with a 5-minute pre-PCR heating at 95°C, followed by 35 cycles (30 seconds at 94°C, 30 seconds at 62°C, 30 seconds at 72°C), and a final 5-minute cycle at 72°C.

The amplified PCR products were separated by agarose gel electrophoresis as recommended by (Nair et al., 2015)

- Agarose gel was created by dissolving 1.5 g of agarose powder in 100 mL of 1X TAE buffer.
- To ensure good mixing, the mixture was spun and heated to boiling point.
- The liquid was allowed to cool at 45 to 50°C before inserting and thoroughly mixing 5 µL of premier-safe dye.

<table>
<thead>
<tr>
<th>Table 1: Bacteria and primers’ sequence</th>
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<tbody>
<tr>
<td><strong>Bacterial isolates</strong></td>
</tr>
<tr>
<td>T. denticola</td>
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<tr>
<td></td>
</tr>
<tr>
<td>P. intermedia</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Tannerella forsythia</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>P. gulae</td>
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<tr>
<td></td>
</tr>
<tr>
<td>Campylobacter rectus</td>
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</table>
• Prime-safe dye (5 µL/100 mL) was put into gels at 50°C for staining, then spun to thoroughly mix.
• Prior to pouring the gel, a comb was placed into the gel plate.
• The gel was carefully applied to the plate to minimize bubble formation and then allowed to set for 15 to 20 minutes before gently separating the comb from the formed gel.
• The gel tank received one X Tris-acetate DETA (TAE) buffer.
• The 100 bp marker was placed in the first well, the positive control (if available) was placed in the second well, and the negative control was placed in the third well.
• Ten microliters of each sample’s PCR products were carefully placed into the gel wells next to the negative control.
• The power supply was turned on at 80V for 45 minutes to run DNA samples and DNA bands were observed under UV light.

The vials were examined in two stages for further diagnosis to have 240 reactions.

Results
As previously mentioned, ten dogs were included in the first stage, but two dogs were excluded (one of which was passed on and the other had total implant failure) to have eight dogs in the second stage of the study.

Out of 32 implants of both types, only 20 were successfully integrated into the jawbone. About 14 compression screw implants out of 16 were successfully osseointegrated, whereas six out of 16 of the bicortical basal implants were integrated into the jawbones.

All of the eight mandibular compression screws were successfully osseointegrated, while six of the eight compression screws were successful in the maxilla.

The mandibular bicortical basal implants also reported six of eight implant success rates, while no successful bicortical screws in the maxilla were detected.

Five species of pathogenic oral bacteria were investigated and were all positively diagnosed in qualitative PCR tests, see Figures 4-8.

Starting with the left side, which included the two quadrants of the mandible and maxilla. In the first preoperative stage, the swabs of 20 natural teeth showed positive results for all the suggested bacteria. The positive
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The time differences among different species’ percentages in both stages were more noticeable, whether in the periodontal or the peri-implant areas, Figure 9. The highest percentage was for *C. rectus* in both implant and teeth areas. In contrast, *P. intermedia* was the lowest in the first and second stages of the study.

**Discussion**

Talking about microbial involvement of the bacteria in periodontal diseases in canines is important enough to mention the related bacteria diagnosed in periodontal diseases in the case of humans and canines to be assured that the bacteria involved in periodontal diseases and peri-implantitis is surprisingly similar in both cases, not only as we could find in our research, but also by other researchers that could find the same bacteria in both cases. The proportion of the diagnosed bacteria was different. Still, the bacteria itself was the same with some other researchers who went to compare human and dog oral microbes those that also had an exception to the rule which was the *P. gulae*, not the *P. gingivalis* which was detected in a low rate in dogs (Yukio Kato, 2011), with some differences regarding the species in the case of *P. gulae* and *P. gingivalis*. However, there was a cross-reaction between the two species during the

**Table 2: Bacterial isolates in the 1st stage with the bacteria/tooth percentage**

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Mandible positive isolates &amp; percentage</th>
<th>Mandible</th>
<th>Maxilla</th>
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</thead>
<tbody>
<tr>
<td><em>C. rectus</em></td>
<td>18 (90%)</td>
<td>8 (80%)</td>
<td>10 (100%)</td>
</tr>
<tr>
<td><em>Tannerella forsythia</em></td>
<td>10 (50%)</td>
<td>6 (60%)</td>
<td>4 (40%)</td>
</tr>
<tr>
<td><em>T. denticola</em></td>
<td>12 (60%)</td>
<td>2 (20%)</td>
<td>10 (100%)</td>
</tr>
<tr>
<td><em>P. gulae</em></td>
<td>12 (60%)</td>
<td>6 (60%)</td>
<td>6 (60%)</td>
</tr>
<tr>
<td><em>P. intermedia</em></td>
<td>4 (20%)</td>
<td>2 (20%)</td>
<td>2 (20%)</td>
</tr>
<tr>
<td>P-value</td>
<td>0.06</td>
<td>0.29</td>
<td></td>
</tr>
</tbody>
</table>

The bacterial detection of 56 can be related to 20 teeth from which swabs were collected to produce a tooth/bacteria percentage of 35.7%, Table 2.

After osseointegration and a waiting period of four months, the second stage was started and a swab collection process was done for 18 of the successfully integrated implants of both types. Table 3 shows every species with the related results and percentages. With a bacterial detection of 40 out of 18 implants from which the swabs were collected to have an implant/bacteria percentage of 45%.

**Table 3: Bacterial isolates in the 2nd stage of both implant types, with the bacteria/implant percentage**

<table>
<thead>
<tr>
<th>Bacterial</th>
<th>Total results and bacteria/ implant percentage</th>
<th>Mandibular bicortical screws (6 implants)</th>
<th>Mandibular compression screws (8 implants)</th>
<th>Maxillary compression screws (4 implants)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. rectus</em></td>
<td>18 (100%)</td>
<td>6 (100%)</td>
<td>8 (100%)</td>
<td>4 (100%)</td>
</tr>
<tr>
<td><em>Tannerella forsythia</em></td>
<td>8 (44.4%)</td>
<td>2 (33.3%)</td>
<td>4 (50%)</td>
<td>2 (50%)</td>
</tr>
<tr>
<td><em>T. denticola</em></td>
<td>4 (22.2%)</td>
<td>2 (33.3%)</td>
<td>2 (25%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td><em>P. gulae</em></td>
<td>10 (55.6%)</td>
<td>4 (66.6%)</td>
<td>4 (50%)</td>
<td>2 (50%)</td>
</tr>
<tr>
<td><em>P. intermedia</em></td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>p-value</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>0.02</td>
</tr>
</tbody>
</table>

The results and the related bacteria/tooth percentage are shown in Table 2. A total bacterial detection of 56 can be related to 20 teeth from which swabs were collected to produce a tooth/bacteria percentage of 35.7%, Table 2.

![Figure 8: Molecular results of *P. intermedia*](image)

![Figure 9: Comparison between bacteria/tooth (implant) percentages in both stages](image)
diagnosis (Hamada et al., 2008) that could support the similar pathogenesis in case of periodontal diseases despite the difference on the species level. However, other researchers could confirm the exact similarity on virulence factor point in between those two species of Porphyromonas which would certify the periodontopathogens identical behavior (Kačírová et al., 2021).

The comparison between the results of bacterial detection in the two stages of the study helped to support the idea that bacterial species can be similar in teeth and implant sites, as suggested by (Retamal-Valdes et al., 2019), but this should not interfere with the result of having higher bacterial percentage collected from the peri-implant areas in the second stage in comparison with the results of periodontal swabs collected from the study side in the first stage, or even the control side in the second stage of the study. This is a detail that agrees with other researchers’ opinions who had a human study about the same problem of research (Sharafuddin et al., 2023) and would not agree with the findings of (Rismanchian et al., 2012) that followed a different and less advanced investigation technique in comparison with the formerly compared studies. A further conclusion can be made about the bacterial transmission from the natural teeth to the implants causing implant infection and loss, which was investigated in some other studies that researched the bacterial existence, abundance and composition on the implant and the related tissues (Aoki et al., 2012; Siddiqi et al., 2016).

The diversity of bacteria species was not significantly variant in the first stage of the treatment when it came to the periodontal swabs, while significant statistical differences were detected between the bacteria species in the second stage when swabs were collected from the peri-implant areas. These findings would go against (Daubert & Weinstein, 2019) opinion about the bacteria diversity in the peri-implant area in comparison with the periodontal area.

When it comes to the first stage of our study, the higher bacterial population in the maxilla in comparison with the mandible would possibly support the claim of (Daubert & Weinstein, 2019) that bacteria in the biofilm play an important role in the peri-implant infection process; since we had the higher implant failure rate in the maxilla in comparison with the mandible, that had the lower bacterial population. This can agree with (Naert et al., 2012) that the bacterial role is still important to be marked as a vital role in implant failure in comparison with the biomechanical factor when it would be placed in a transgingival level, especially when we can mention that we used the same implants, followed the same surgical protocol with high primary stability and offered the same treatment criteria in both jaws in every single animal; which would eliminate the other related possible causative factors of implant failure.

Even when we study the infective bacterial species separately the highest detected species was C. rectus in both jaws, whether in the first or the second stage of the study. Such diagnosis can’t be correlated with a possible pathogenic role of this species of bacteria, which was already diagnosed as a periodonto-pathogen by (Ihara et al., 2003) and (Macuch & Tanner, 2000) when they could isolate this bacterium from diseased periodontal tissues using classical approaches or even with more advanced molecular technologies like (Swetalin Das, 2016) and was noticed to have changes in the levels of this bacterium from healthy to diseased states in the peri-implant area in (Canullo et al., 2015) which will not be consistent with our observations about the high level of this bacterium in both different stages of the research. Specific research was done by Bostanci et al. to investigate the virulence of P. gingivalis and C. rectus. When those two periodontal pathogens combine in the tissues then they can impair host immune defenses and inhibit pro-inflammatory cytokines, which may lead to further pathogenic developments in periodontal infection. The same researchers found that C. rectus alone was a less aggressive periodonto-pathogen. This finding can be correlated to implant failure in our study when all of the six quadrants that were positive for C. rectus and P. gulae in stage one of the study had at least one implant failure in stage two (Bostanci et al., 2007).

The significant prevalence of T. denticola in the maxilla in comparison with the mandible in the first stage of the study, together with the detection of this bacterium in two of the mandibular quadrants with failed bicortical screws could indicate a highly significant correlation between this species and implant infection -and subsequent failure, that would be explained with the conclusion of the (Petain et al., 2021) about the pathogenic role of T. denticola in periodontal and peri-implant diseases, and controversially agree with some other researchers point about the elevated level of some species (including T. denticola) in peri-implant areas in diseased conditions (Alves et al., 2022; Ata-Ali et al., 2015); since that may not be totally consistent with our detection of this species as the only investigated microbiome with such a high correlation with the failure sites, but we were not the first researchers who could draw attention to T. denticola as the only significantly correlated bacteria to peri-implant infections when (Wang et al., 2016) noticed that also.

P. gulae represented the second most suspicious bacterium in our study when detected in 50% of the preoperative swabs collected from the quadrants with postoperative failures. This can be similar to the related aggressive peri-implant behavior mentioned in (Monje et al., 2021) research and be correlated to the P. gingivalis role in peri-implant infections mentioned by (Ata-Ali et al., 2015) in his human study or (Tzach-Nahman et al., 2017) in his animal study, which will support (Lenzo et al., 2016) claim about the high level of similarity at the immunological and virulence levels between those two different species of the same genus.
Conclusion
Within the limits of this study, we could draw the following conclusions:

- The detection of human periodontopathogens in the peri-implant and periodontal sulci can show the reliability of using dogs as a good model for the peri-implant and periodontal infections studies.
- Periodontal pathogenic bacteria play an important role in basal implant postoperative infection because of the one-piece design that leads to instant direct exposure to the oral environment and subsequent microbial infectious effects.
- The definite role of *T. denticola* in postoperative infection and subsequent implant failure was obvious.
- The synergism of virulence of the *P. gulae* and *C. rectus* in combination and the notable failure rate in those quadrants could support the same claim about synergism of virulence of *P. gingivalis* and *C. rectus* when detected in combination.
- *P. gulae* (*P. gingivalis* in case of humans) may have a vital role in postoperative infection and subsequent failure of basal implants.

Recommendations
Further investigations about the peri-implant infections of basal implants are necessary to have a better understanding of this restricted field.

Acknowledgments
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