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## Incidence and Determinants of Microbial Colonization on Osteosynthesis Implants in Small Animals

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### ABSTRACT

Despite progress in antibiotics, aseptic techniques, and perioperative antimicrobial therapy, post-operative infections remain a common complication of osteosynthesis, causing delayed fracture healing, osteomyelitis, implant loosening, and functional impairment. Osteosynthesis implants are widely used in veterinary medicine, yet the factors influencing microbial colonization remain poorly understood. This study aimed to evaluate the incidence of microbial colonization on implants in small animals and identify potential contributing factors. Seventy-one explants from sixty-five patients were analyzed, with correlations assessed between microbial colonization, patient characteristics, disease progression, radiographic findings, and surgical variables. Factors such as body weight, age, implant type and location, additional injuries (e.g., pulmonary lesions), the surgeon's experience, and the number of personnel present during surgery appeared to influence infection development. Osteolytic changes were observed in 60% of cases, and 73.3% of animals with impaired mobility had infected implants. Although microorganisms were detected in nearly 50% of explants, clinically significant infection occurred in only five animals (7.3%), indicating that the mere presence of microorganisms does not always result in treatment complications. These findings provide important insights into the multifactorial nature of post-operative implant infections in veterinary patients.

**Keywords:** Hardware, Infection, Implants, Cats dogs

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### Introduction

A primary goal of orthopedic surgery is the restoration of normal function through the use of implants. In general, infectious complications following elective orthopedic procedures are relatively rare, with an average infection rate of approximately 5% for osteosynthetic materials in human medicine [1]. Infections are particularly uncommon in closed fractures (1–2%) but may reach up to 30% in open fractures [2]. Despite advances in antibiotics and perioperative antimicrobial protocols, post-operative infections remain among the most frequent surgical complications in humans, often resulting in delayed fracture healing, osteomyelitis, implant loosening, and functional impairment [3,4]. For example, Khan *et al.* [5] reported infections in 6 of 104 patients following osteosynthesis.

The development of surgical infections is influenced by multiple factors, including the formation of microbial biofilms [6]. Biofilms, which can develop on implanted foreign bodies, pose a particular challenge because they persist on the surface and are highly resistant to conventional treatment [7]. Given that implants are routinely used

in veterinary medicine and that bacterial infections can negatively affect fracture healing, understanding microbial colonization in this context is crucial. The present study investigates microbial colonization of osteosynthesis implants in small animals, examining its frequency and identifying factors that contribute to infection. The findings aim to improve our understanding of post-operative complications and inform optimized treatment strategies in veterinary orthopedics.

## Materials and Methods

The objective of this study was to assess bacterial colonization on explanted osteosynthesis plates. The analysis focused on correlations between microbial colonization and patient characteristics, disease progression, and radiographic findings. In addition, potential pathogenetic factors contributing to infection were identified and evaluated to better understand the conditions that promote post-operative implant infections.

### *Patients*

This study included patients presented for explantation procedures at the Small Animal Teaching Hospital of the Freie Universität Berlin (Berlin, Germany) between February 2010 and March 2013.

Data collected for each patient encompassed breed, age, sex, body weight, type and location of injury, presence of additional injuries, time from accident to surgery, duration of hospitalization, previous surgical interventions, antibiotic treatments, type of osteosynthesis plate, presence of additional implants, details of the surgical team, duration of surgery, interval between surgery and implant removal, radiographic findings prior to explantation, and post-removal complications.

Microbiological analysis of explanted plates was conducted at the Institute of Microbiology and Animal Diseases, Freie Universität Berlin. Radiographic images in two perpendicular planes were used to assess injury type and location. For joint involvement, additional stress radiographs were taken to determine dislocation extent and precisely localize lesions, including proximal, distal, epiphyseal, or metaphyseal regions.

Upon presentation, patients underwent systematic examinations to detect additional injuries, such as pneumothorax, other wounds, or abdominal bleeding, using thoracic and abdominal radiographs, as well as sonographic or computed tomographic imaging when indicated. The interval between injury and surgery, prior interventions, and use of antibiotics before, during, or after surgery were documented to evaluate potential effects on healing. Comorbidities, prior surgeries, and potential infection sources, such as pyoderma, were also recorded. Details regarding osteosynthesis implants—including type (DCP, locking plate, T-plate), thickness (2.0, 2.7, 3.5, 4.5 mm), number of plate holes, and any supplementary implants such as cerclages, screws, or Kirschner wires—were documented. Surgical variables analyzed included the surgeon's qualification level (highly qualified: diplomate of the European College of Veterinary Surgery or specialist veterinarian; not yet qualified: resident or in specialist training), duration of surgery, number of assisting personnel, and total hospitalization period. Due to incomplete records, data on the duration or presence of additional personnel during surgery were not always available.

Early post-operative complications were recorded, including wound healing disorders, infections, fistula formation, or implant failures such as loosening or breakage. In patients requiring revision surgery, radiographs taken prior to implant removal were analyzed for implant-related issues (loosening, bending, fracture), bone changes (osteolysis, sequestration, pseudoarthrosis, demineralization, or refracture), and intraoperative complications. Swabs obtained during implant removal were submitted for microbiological analysis at the Institute of Microbiology and Animal Diseases, Freie Universität Berlin.

### *Implants*

All implants evaluated in this study, including plates, screws, and supplementary fixation devices, were manufactured by Königsee Implants (Hamburg, Germany). The study assessed bacterial colonization on locking plates and dynamic compression plates (DCPs) with thicknesses ranging from 2.0 to 4.4 mm and 6 to 16 holes. Additionally, 8-hole T-plates of 2 mm thickness were used, explanted, and analyzed similarly.

Implant removal adhered to standardized surgical protocols for each bone type, as outlined by Piermattei and Flo [8]. During surgery, the connective tissue surrounding the plate was carefully dissected along its base, either cranially or caudally. The tissue was then folded back to expose the screw heads and meticulously separated from any adherent connective tissue.

*Microbiological examination*

Microbiological sampling of explanted implants was conducted using two complementary methods:

1. **Intraoperative Swabbing:** Sterile swabs (Heinz Herenz, Hamburg, Germany) were taken after the implant was exposed but before removal. Each swab was immediately transferred to a sterile transport medium and sent to the Institute of Microbiology and Animal Diseases, Freie Universität Berlin, for further testing.

2. **Direct Culture from Explants:** Implants were directly sampled on various culture media and in broth. For each explant, five different nutrient agar plates and two nutrient broths were employed, including three aerobic media (chocolate agar, Columbia agar, urine chromogenic agar), two anaerobic media (Columbia agar anaerobic, gentamicin agar), and both aerobic and anaerobic brain–heart infusion (BHI) broths.

Once removed, the implants with screws were isolated from surrounding tissue and placed on a sterile surface for processing. Two swabs were collected per implant: one for streaking on culture media to create serial dilution smears, and one for inoculation into nutrient broth. Swab tips were cut using sterile scissors, and the anaerobic plates were sealed in Zeissler pots with AnaeroGen gas (Oxoid, Wesler, Germany) to maintain anaerobic conditions before incubation.

*Statistical analysis*

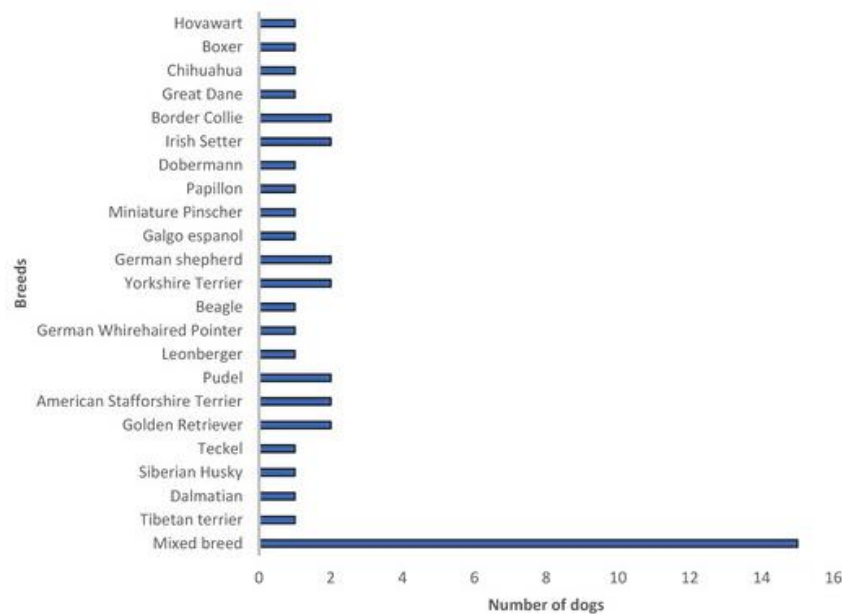
Data analysis was conducted using SPSS (version 22, IBM, Armonk, NY, USA, 2020). Statistical significance was defined as  $p < 0.05$ . Given the non-normal distribution of several variables, non-parametric methods were applied. The relationship between bacterial colonization on explanted implants and other variables—including breed, age, sex, body weight, fracture type and location, associated injuries, prior surgeries, antibiotic use, implant type, additional fixation devices, surgical team composition, duration of surgery and hospitalization, time until implant removal, post-operative complications, and pre-removal radiographic findings—was assessed using chi-square tests, Fisher's exact test, and the Mann–Whitney U test. This approach allowed identification of potential factors influencing microbial colonization.

**Results**

Between February 2010 and March 2013, 65 small animal patients underwent implant removal at the Small Animal Clinic of the Freie Universität Berlin and were included in this study. The cohort consisted of 51 dogs and 14 cats. In six dogs, two plates were removed; for half of these dogs, both implantation and removal occurred during the same procedure, while the remaining three had implants removed during separate surgical events. In total, 71 plate implants were collected from 65 patients across 68 operations and evaluated for microbial colonization.

*Breed, age, sex, and weight*

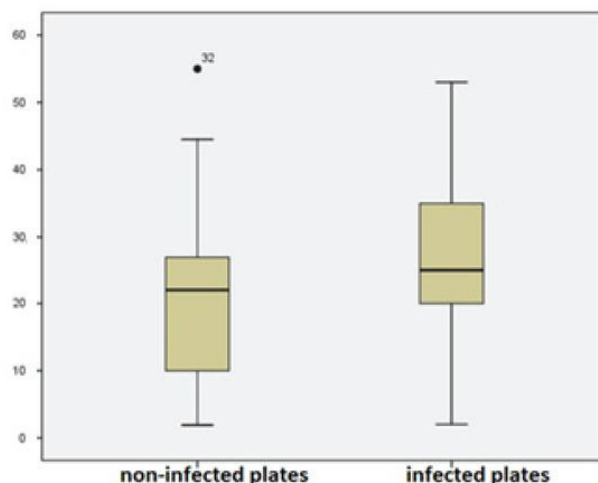
**Figure 1** illustrates the variety of dog breeds included. The 14 cats comprised one Norwegian Forest Cat, one Abyssinian, one Persian, and ten European Shorthairs. Among the 51 dogs, 15 (29.4%) were of mixed breed. Other characteristics such as age, sex, and body weight were documented to explore potential correlations with infection rates.



**Figure 1.** Distribution of Dog Breeds in the Study

The cat cohort ( $n = 14$ ) spanned ages from approximately 8 months to 12 years, averaging 3.9 years, with a median of 2.5 years. Among dogs ( $n = 51$ ), ages ranged from 0.4 to 14 years, with a mean of 4.0 years and a median of 3 years. Most dogs ( $n = 37$ ) were younger than five years, while 14 were older than five. Statistical testing did not reveal a significant link between age and the presence of bacterial colonization on the explanted implants ( $p = 0.511$ ).

In terms of sex, the cat population included two intact males, eight neutered males, two intact females, and two spayed females. The dogs were distributed as follows: 18 intact males, nine neutered males, 18 intact females, and six spayed females. No significant association between sex and implant infection was observed ( $p = 0.726$ ). Weights in cats ranged from 3.0 to 8.5 kg, with an average of 4.7 kg and a median of 4.2 kg. Most cats ( $n = 10$ ) fell into the 3.1–5 kg range, while one cat weighed under 3 kg, one between 5.1–7 kg, and two exceeded 7 kg. Dog body weights were considerably more variable, spanning 1.9 to 55 kg, with a mean of 23.6 kg and a median of 24.2 kg (**Figure 2**).



**Figure 2.** Microbiological findings and its relation to the dogs' body weight

A statistically significant correlation was found between heavier dogs and an explant infection ( $p < 0.05$ ) (**Table 1**).

**Table 1.** Distribution of infected and non-infected explants in the different dogs' body weight groups.

| Weight in Kg | Explant |
|--------------|---------|
|--------------|---------|

|         | Infected | Non-Infected |
|---------|----------|--------------|
| 0–5     | 4        | 4            |
| 5.1–10  | 2        | 2            |
| 10.1–20 | 6        | 5            |
| 20.1–30 | 9        | 11           |
| 30.1–40 | 1        | 6            |
| 40.1–50 | 1        | 2            |
| >50     | 1        | 3            |
| Total   | 24       | 33           |

#### *Indications for surgical intervention*

Arthrodesis using plates and screws was performed in 14 dogs, with the carpal joint being the most frequently treated site ( $n = 5$ ). Among these, three dogs received two plates, while two had a single plate implanted. Two additional cases involved arthrodesis of the shoulder joint, performed due to dysplasia and osteoarthritis. Other arthrodesis procedures addressed two knee joints, two elbow joints, and three tarsocrural joints.

Both knees had previously undergone multiple surgical interventions at another facility for cranial cruciate ligament rupture and were referred for persistent, painful ankylosis. One elbow joint was fused due to severe osteoarthritis, while the second elbow arthrodesis was performed after two unsuccessful surgeries addressing a complicated Y-T humeral condyle fracture. Arthrodesis of the tarsal joint was required in one dog for severe osteoarthritis; this patient had previously undergone two surgeries because the initial plate failed. In two additional dogs, the rationale for tarsal joint arthrodesis was not documented.

Corrective osteotomies were performed in four dogs, three involving the tibia. One tibial osteotomy addressed tibial dysplasia, while the indications for the other two tibial surgeries were not documented. The fourth dog underwent bilateral carpus valgus correction following an old trauma, with two surgeries on the left forelimb. Additionally, two dogs required surgical repair of ruptured long medial tarsal collateral ligaments resulting from car accidents.

Traumatic fractures were diagnosed and treated surgically in 31 of 51 dogs (60.8%). Three of these were grade I open fractures. Among cases where the cause could be identified ( $n = 19$ ), motor vehicle accidents accounted for 73.7%, followed by bite wounds at 10.5%.

Two dogs had previously received total hip endoprostheses for hip dysplasia. In one dog, prosthesis loosening led to intraoperative femur fracture during implant removal, while the second experienced femoral fracture postoperatively due to implant loosening. Both fractures were included in this study, as osteosynthesis with plates and screws was subsequently performed.

Among the 14 cats, all were treated for fractures, with two classified as grade I open fractures. The underlying cause was known in 71.4% of cases, with falls from height being the most frequent etiology (64.3%,  $n = 9$ ).

#### *Fracture localization*

Fracture sites were analyzed in all 65 patients (dogs and cats). Forearm fractures ( $n = 19$ ) and carpal fractures ( $n = 6$ ) were most common, followed by femoral ( $n = 12$ ), lower limb ( $n = 12$ ), and tarsal fractures ( $n = 5$ ). Additional fractures included four humeral, two shoulder, two elbow, two knee, and one ileal fracture. **Table 2** summarizes the incidence of fractures by anatomical location and the distribution of infected versus non-infected plates.

**Table 2.**

| Fracture Localization |                    | Dogs' Incidence | Cats' Incidence | Explants     |          |
|-----------------------|--------------------|-----------------|-----------------|--------------|----------|
|                       |                    |                 |                 | Non-Infected | Infected |
| Forelimb              | Articulatio carpi  | 6               | 4               | 3            | 7        |
|                       | Radius             | 1               | 1               | 2            |          |
|                       | Ulna               |                 | 1               | 1            |          |
|                       | Radius-ulna        | 14              | 2               | 6            | 10       |
|                       | Articulatio cubiti | 2               |                 | 1            | 1        |
|                       | Humerus            | 2               | 2               | 3            | 1        |
| Hindlimb              | Articulatio humeri | 2               |                 | 1            | 1        |
|                       | Articulatio tarsi  | 5               | 1               | 2            | 4        |
|                       | Tibia              | 7               | 1               | 3            | 5        |
|                       | Tibia-fibula       | 2               | 2               | 3            | 1        |
|                       | Articulatio genus  | 2               |                 | 1            | 2        |
|                       | Os femoris         | 7               | 5               | 11           | 1        |

|          |   |   |
|----------|---|---|
| Os ilium | 1 | 1 |
|----------|---|---|

In this study, explants from the forelimbs were more frequently infected than those from the hind limbs, with 21 of 35 forelimb implants (60%) showing colonization, compared to 13 of 33 hind limb implants (39.4%). Notably, infections were more common in distal regions: 85.7% (18/21) of forelimb infections occurred distal to the elbow, while 76.9% (10/13) of hind limb infections were distal to the knee, suggesting that more peripheral structures are at higher risk.

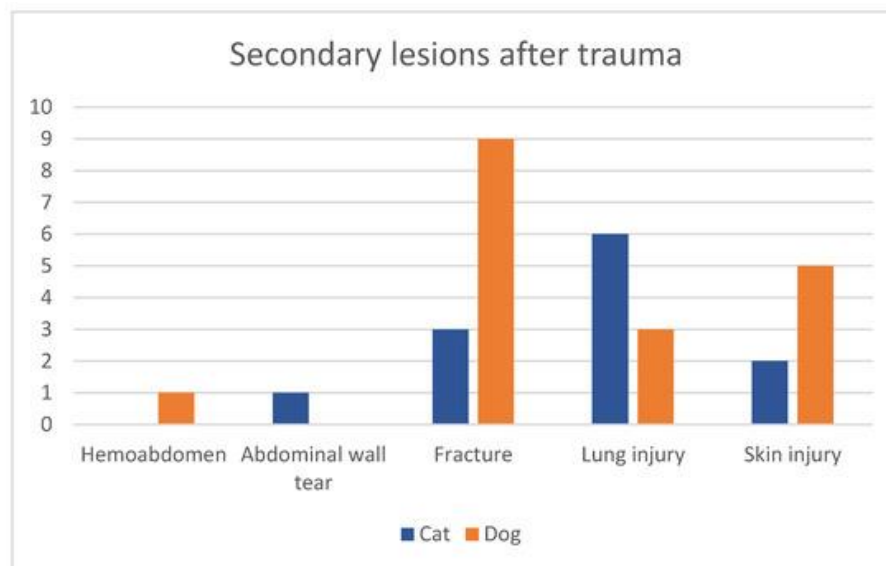
#### *Antibiotic treatment*

Post-operative antibiotic therapy was administered following 30 out of 66 surgeries (45.5%), in addition to pre-operative prophylaxis. For two patients initially treated at another facility, post-operative antibiotic records were unavailable. The most commonly used regimen was amoxicillin combined with clavulanic acid, accounting for 77.4% of cases. Other antibiotics included cefalexin (9.7%), marbofloxacin (6.5%), enrofloxacin (3.2%), and trimethoprim–sulfonamide (3.2%). Only one patient received a combination of two antibiotics post-operatively. Among the 30 patients who received post-operative antibiotics, 17 (56.7%) had infected explants. Comparison between patients treated with and without post-operative antibiotics revealed no statistically significant difference in infection risk ( $p = 0.296$ ).

#### *Additional injuries following trauma*

Nineteen dogs presented for non-traumatic conditions were excluded from this analysis. Of the remaining 32 dogs, 14 (43.8%) had additional injuries documented in their medical records, with three dogs sustaining more than one concurrent injury. Secondary fractures were the most frequently observed additional injury, accounting for 50% of cases (**Figure 3**).

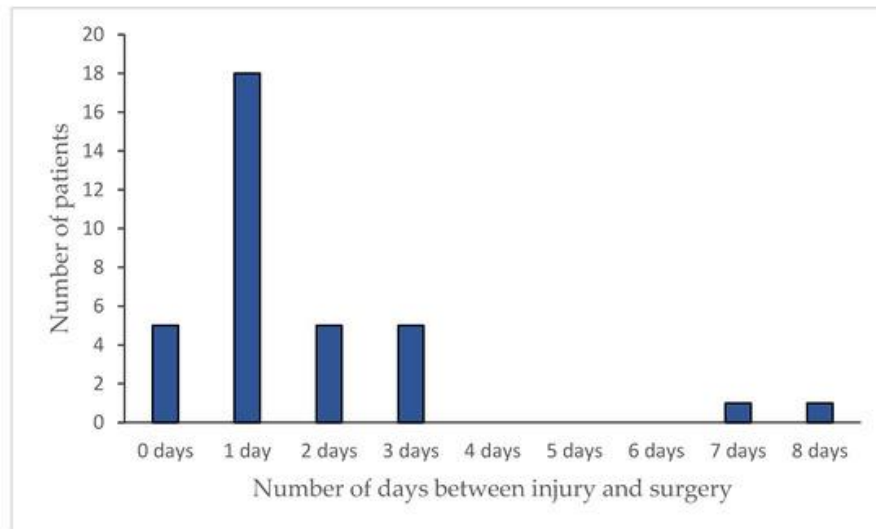
In cats, 10 of 14 (71.4%) had additional injuries, two of which involved multiple concurrent lesions. Lung pathology was the most commonly recorded comorbidity, affecting half of the cats, and was the only type of additional injury significantly associated with explant infection ( $p = 0.028$ ; **Figure 1**).



**Figure 3.** Number of secondary lesions in dogs and cats presented after trauma

#### *Interval between lesion diagnosis and surgical intervention*

For 40 of the 65 patients, medical records contained sufficient information to determine the time elapsed between diagnosis of the lesion and surgical treatment (**Figure 4**). In the remaining 25 cases, this information was unavailable due to incomplete anamnesis. Statistical analysis indicated that the length of this interval did not significantly influence the likelihood of explant infection ( $p = 0.915$ ).



**Figure 4.** Number of patients and number of days between the diagnosis/occurrence of the lesion and surgical treatment

#### *History of prior surgical interventions*

Fifteen animals (23.1%) had undergone previous surgical procedures at other facilities. Of these, seven had been operated on once, four had two prior surgeries, three had three, and one patient had undergone four previous interventions.

#### *Types of plate osteosynthesis*

A total of 71 implants were explanted during the study: 37 locking plates, 28 dynamic compression plates (DCPs), and six T-plates. The specifications of the locking plates and DCPs, including the number of screw holes and plate thickness, are summarized in **Tables 3 and 4**. The T-plates used in this study were uniformly 2 mm thick with eight screw holes.

**Table 3.** Total amount of extracted plates and their respective number of screw holes

| Number of Screw Holes | Number of Locking Plates | Number of Dynamic Compression Plates |
|-----------------------|--------------------------|--------------------------------------|
| 5                     | 0                        | 1                                    |
| 6                     | 6                        | 2                                    |
| 7                     | 0                        | 2                                    |
| 8                     | 9                        | 11                                   |
| 9                     | 0                        | 1                                    |
| 10                    | 9                        | 4                                    |
| 12                    | 7                        | 4                                    |
| 13                    | 0                        | 1                                    |
| 14                    | 3                        | 2                                    |
| 16                    | 3                        | 0                                    |

**Table 4.** Plate thickness and amount of explanted locking plates and DCPs

| Plate Thickness | Number of Locking Plates | Number of Dynamic Compression Plates |
|-----------------|--------------------------|--------------------------------------|
| 2 mm            | 9                        | 9                                    |
| 2.7 mm          | 4                        | 11                                   |
| 3.5 mm          | 15                       | 7                                    |
| 4.5 mm          | 9                        | 1                                    |

Among the 28 explanted DCPs, 11 (39.3%) showed microbial colonization. In comparison, 22 of 37 locking plates (59.5%) and 2 of 6 T-plates (33.3%) were infected. Despite these numerical differences, statistical analysis revealed no significant association between plate type and infection risk ( $p = 0.296$ ).



When considering plate thickness, thinner plates (2–2.7 mm) were associated with a lower infection rate (38.5%) compared to thicker plates (3.5–4.5 mm, 62.5%). However, this difference did not reach statistical significance ( $p = 0.328$ ).

#### *Additional implant materials*

The primary goal of plate osteosynthesis was to achieve load stability, which in some cases required additional fixation devices, particularly in comminuted fractures ( $n = 28$ ). Of these patients, 19 required one supplementary implant, while nine required two. Wire cerclages were the most frequently used adjunct (29.7%), followed by screws (27%), Kirschner wires (16.2%), pins (16.2%), and additional plates (10.8%). Microbiological infection was observed in 50% ( $n = 14$ ) of explants with additional implant material, compared to 47.5% in those without extra fixation. No significant correlation between the use of adjunctive implants and infection was detected ( $p = 0.513$ ).

#### *Surgeons and assistants*

A total of 68 explantations were performed at the clinic by seven surgeons or the referring veterinarians, whose qualifications were not documented. Surgeons at the clinic were categorized by experience, ranging from highly qualified experts (professors or diplomates) to less experienced, in-training veterinarians. The two most experienced surgeons performed 47 procedures, while five less experienced surgeons carried out 18 operations. Three procedures were performed by referring veterinarians.

Assistance during surgery varied: 13 procedures had one assistant, 36 had two, and 16 involved three assistants; the number of assistants for the three procedures by referring veterinarians was unknown. Notably, 14 of the 16 operations with three assistants were conducted by experienced surgeons. Individual surgeons performed between 1 and 45 operations. Infection rates among procedures performed by experienced surgeons were 52% (26/50), compared with 42.9% (9/21) for the less experienced group. This difference was not statistically significant ( $p = 0.582$ ). Interestingly, the highest proportion of infected explants (12/18, 66.7%) occurred in surgeries where three assistants were present.

#### *Surgery duration and hospital stay*

Information regarding the length of surgical procedures was available for 32 of the 68 operations (47.1%) through anesthetic records. Procedures lasted between 30 and 180 minutes, with a mean duration of 87 minutes. Interestingly, shorter procedures (30–80 minutes,  $n = 15$ ) were associated with a higher frequency of implant infection (73.3%) compared with longer surgeries of 90–180 minutes ( $n = 17$ ), which exhibited an infection rate of 47.1%. This counterintuitive pattern suggests that cases requiring less surgical time were more frequently colonized by microorganisms.

Hospitalization duration was recorded for 44 patients, ranging from one to 13 days, with an average stay of 3.1 days. Twenty-one animals were treated on an outpatient basis at the clinic, and three additional patients received outpatient care at referring veterinarians ( $n = 24$ ). While infection risk appeared slightly lower in patients with longer hospital stays, this trend was not statistically significant. No meaningful difference in infection rates was observed between hospitalized and outpatient patients ( $p = 0.802$ ), nor was there a significant association between the total length of hospitalization and the likelihood of explant infection ( $p = 0.563$ ).

**Table 5.** Days of hospitalization in infected and non-infected explants

| Hospitalization in Days | Explants     |          |
|-------------------------|--------------|----------|
|                         | Non-Infected | Infected |
| 0                       | 13           | 13       |
| 1                       | 4            | 6        |
| 2                       | 6            | 6        |
| 3                       | 7            | 2        |
| 4                       | 2            | 3        |
| 5                       | 1            | 2        |
| 6                       | 1            | 1        |
| 8                       | 2            | 0        |
| 13                      | 0            | 2        |
| Total                   | 36           | 35       |



*Complications during healing and timing of implant removal*

Post-operative complications occurred in eight dogs during the healing phase prior to implant removal. These included wound infections ( $n = 5$ ), suture dehiscence ( $n = 1$ ), and fistula formation ( $n = 2$ ). During this period, nine dogs and three cats underwent additional surgeries, either to remove or adjust components of the implants to promote fracture stabilization ( $n = 8$ ), or to combine procedures in a single session, such as cerclage removal with screw replacement. Three patients also required supplemental bone healing support using cancellous bone grafts. In the case of suture dehiscence, wound revision was performed.

The interval from implantation to explantation ( $n = 70$ ) varied widely, ranging from 14 to 1658 days, with a mean of 153.6 days. In one case, the date of implantation by a referring veterinarian was unknown. Among implants removed within 0–120 days, 43.2% were infected, compared with 57.6% of implants removed after longer periods. No statistically significant association was observed between infection rates and the time to implant removal. Notably, seven of eight patients (87.5%) who experienced wound infection, fistula formation, or suture dehiscence during the initial healing phase had infected explants. Additionally, of the 12 patients who required reoperation due to wound healing complications, eight (66.7%) had microbiologically positive implants.

*Radiographic findings prior to explantation*

Pre-removal radiographs were available for 68 operations. Thirty-six images revealed no abnormalities, while 32 showed pathological changes. These included osteolysis around the implant site ( $n = 19$ , 27.9%), localized bone demineralization beneath the plate ( $n = 9$ , 13.2%), broken plates ( $n = 4$ ) or screws ( $n = 3$ ; 10.3%), loose screws ( $n = 6$ , 8.8%), bent plates (2.9%), sequestration (2.9%), or non-union (1.5%). Statistical analysis indicated no significant difference in infection risk between patients with or without radiographic abnormalities ( $p = 0.627$ ).

*Indications for implant removal*

The primary reason for explantation in most cases (72.1%,  $n = 49$ ) was routine removal after uneventful healing of fractures, arthrodeses, or corrective osteotomies. Four patient records lacked documentation regarding the indication. Fifteen implants were removed due to implant-related complications, including severe ( $n = 3$ ), moderate ( $n = 8$ ), or mild ( $n = 1$ ) lameness, pronounced soft tissue swelling with limb misalignment ( $n = 1$ ), or fracture instability ( $n = 1$ ).

Interestingly, implants removed routinely after uncomplicated healing were still infected in 40.8% of cases ( $n = 20$ ). In contrast, implants removed due to functional impairment, limb deformity, or local inflammation exhibited a higher infection rate (73.3%,  $n = 11$ ). Despite these trends, statistical analysis revealed no significant correlation between moderate ( $p = 0.144$ ) or severe ( $p = 0.608$ ) lameness and the presence of infection.

*Post-Explant complications*

Following implant removal, several complications were observed. Wound infections developed within the first 14 days in four animals, while three patients experienced refractures, and two had unstable fractures. All fractures were promptly managed with new osteosynthesis procedures.

All four cases of post-removal wound infection involved previously infected explants ( $p = 0.05$ ). Among the three animals with refractures, two had implants that were colonized with microorganisms ( $p = 0.608$ ). In contrast, the two patients with fracture instability had explants free of infection at the time of removal ( $p = 0.493$ ). Osteomyelitis was diagnosed based on clinical and radiographic findings in five of 51 dogs (9.8%). These dogs were aged 0.5–11 years and weighed between 22 and 53 kg. Two cases involved isolated tibial fractures, while the remaining three involved humeral, tibial, fibular, radial, or ulnar fractures.

Three of these patients required hospitalization for at least one day due to wound infections, necessitating surgical revision or screw replacement. Two dogs developed implant sequestration. Indications for implant removal in these cases included mild ( $n = 2$ ), moderate ( $n = 1$ ), or severe ( $n = 1$ ) lameness, or localized swelling at the implant site ( $n = 1$ ). Microbiological analysis revealed colonization by *Staphylococcus intermedius*, with methicillin resistance detected in two cases. One dog additionally harbored a mixed infection with *Pseudomonas* and *Enterococcus* species.

*Microbiological findings*

Microbial detection was performed using swab samples in 18 cases, while direct smears were prepared for the remaining 53 implants. All 71 samples were analyzed at the Institute of Microbiology and Animal Diseases, Freie

Universität Berlin. Microorganisms were identified in 35 samples (49.3%), including 33 canine and 2 feline implants. Six of the positive samples contained more than one pathogen.

Fungal organisms were detected in two implants: one with *Aspergillus* spp. and one with *Candida* spp., both in mixed infections alongside bacteria, and both cases involved dogs. A total of 42 bacterial isolates were obtained from the 35 positive samples. The most commonly isolated species were *Staphylococcus* spp. (27 isolates) and *Bacillus* spp. (5 isolates), among the 2 feline and 40 canine isolates (**Table 6**).

**Table 6.** Incidence of bacterial species found on the infected explants of dogs and cats

| Bacterial Species              | Incidence |      | N  |
|--------------------------------|-----------|------|----|
|                                | Dogs      | Cats |    |
| <i>Staphylococcus</i> spp.     | 26        | 1    | 27 |
| <i>Bacillus</i> spp.           | 5         |      | 5  |
| <i>Pseudomonas</i> spp.        | 2         |      | 2  |
| <i>Proteus</i> spp.            | 1         |      | 1  |
| <i>Enterococcus</i> spp.       | 1         |      | 1  |
| <i>Providencia</i> spp.        | 1         |      | 1  |
| <i>Enterobacteriaceae</i> spp. |           | 1    | 1  |
| <i>Streptococcus</i> spp.      | 1         |      | 1  |
| <i>Arthrobacter</i> spp.       | 1         |      | 1  |
| <i>Micrococcus</i> spp.        | 1         |      | 1  |
| <i>Paenibacillaceae</i> spp.   | 1         |      | 1  |

## Discussion

Post-operative infections continue to represent one of the most frequent complications in orthopedic surgery, despite advances in antibiotic therapy and peri-operative prophylaxis. Typical consequences include pain, delayed bone healing, osteomyelitis, implant loosening, and compromised implant function [3-5,9]. In the present study, microbiological analysis revealed bacterial colonization in 35 of 71 explants (49.3%). Interestingly, fracture healing proceeded uneventfully in 26 animals, even though 12 of these implants (46.2%) harbored pathogens. Conversely, among the 42 patients who experienced complications, microorganisms were detected in only 21 cases (50%) at the time of explantation. These findings underscore the ongoing debate regarding the clinical significance of bacterial contamination, as some implants may carry microorganisms without eliciting any adverse clinical outcomes.

Our results suggest that only certain bacterial species, under specific conditions, are capable of causing clinically apparent infections. The spectrum of isolated pathogens aligns with previous reports [2,10–14], with *Staphylococcus* spp., particularly *S. intermedius*, being the most frequently detected. Patient factors such as age and body weight appeared to increase the likelihood of infection, which is consistent with observations by Bardet *et al.* [15] and Brown *et al.* [16], though it contrasts with studies reporting hematogenous osteomyelitis predominantly in younger animals [14,17]. Unlike Ethridge *et al.* [18], who found higher infection rates in intact male dogs, our study did not identify sex as a significant risk factor.

Regarding implant type, no statistically significant association with infection risk was observed. Both dynamic compression plates (DCPs) and locking plates showed similar outcomes, although locking plates appeared to have a numerically higher infection rate. This contrasts with previous studies reporting more frequent contamination of DCPs [19, 20]. A possible explanation for this discrepancy may relate to implant dimensions, as thicker plates (3.5–4.5 mm) showed a higher infection rate (62.5%) than thinner plates (2.0–2.7 mm; 38.5%).

Clinically observable impaired wound healing was strongly associated with implant infection, with 87.5% of cases showing colonized explants. It remains unclear whether these infections arose iatrogenically or coincidentally. Similarly, repeated osteosynthesis procedures were associated with a high infection rate (66.7%), potentially reflecting the impact of local soft tissue changes, scar formation, and compromised vascularity, which may facilitate bacterial colonization in subsequent surgeries involving metallic implants.

The effect of surgeon experience on explant infection risk has not been extensively studied. In this investigation, eight surgeons performed the procedures, with two highly experienced surgeons conducting over half of the operations (50/71). No statistically significant association was found between surgeon experience and infection risk, although infection rates were numerically higher in cases managed by the more experienced surgeons. This may reflect the complexity of the cases they handled, including open fractures, comminuted fractures, joint

fractures, and arthrodeses. Additionally, less experienced surgeons often assisted during the latter phases of surgery, such as implant placement or wound closure, which may have contributed to longer procedure times.

The literature indicates that infection risk approximately doubles with every 70 minutes of surgery [21], consistent with findings in human medicine where longer procedures are associated with higher rates of surgical site infection [22]. Interestingly, in our cohort, following observations by Bahn [23], infection rates tended to decrease with longer surgeries, and similar results have been reported by Knobloch [24], who found no significant relationship between surgical duration, individual surgeon, and infection risk.

The number of individuals present during surgery appeared to influence the risk of implant infection. When only one assistant was present, the infection rate was 42.9%, whereas procedures involving two assistants saw an increase to 66.7%. Notably, 14 of 16 surgeries with three assistants were performed by highly experienced surgeons. Overall, the data suggest a trend toward higher infection rates with increasing personnel in the operating room. Carlson [25] reported that both active assistants and passive observers, such as students, can contribute to the risk of post-operative infections, with each additional person potentially raising the risk of wound infection by up to 30% [21, 25]. At our small animal teaching hospital, the standard surgical team comprises at least five individuals: a surgeon, two assistants (including one student), a surgical nurse, and an anesthetist, with up to two additional students observing. Complex procedures are typically assigned to the most experienced surgeons, often necessitating a larger team. Nevertheless, recent human studies indicate that the presence of observers does not significantly increase post-operative complications or infection rates [26], leaving the relationship between operating room personnel and infection risk inconclusive.

Regarding the location of infected explants, 60% (21/35) occurred in the forelimbs, while 40% (14/35) were in the hindlimbs. Forelimb infections included 10/16 radius/ulna fractures and 7/10 carpal joint arthrodeses. The relatively high incidence of infection in radius/ulna fractures may be related to the thin soft tissue coverage in the distal third of the forearm, which accounts for roughly 42.8% of forearm fractures [27]. Limited vascularity in this region may facilitate bacterial colonization. Similarly, infections following carpal joint arthrodesis could be associated with factors such as reduced soft tissue protection, prolonged operative times, extensive articular surface manipulation, autologous cancellous bone grafting, and the use of multiple plates [28]. These same considerations likely apply to tarsal joint arthrodeses, where 4 of 6 explants were infected.

Post-operative antibiotic therapy was continued in 30 patients in addition to standard peri-operative prophylaxis. Despite this, 57% (n = 16) of these cases still developed implant infections. Conversely, 44% of explants (n = 16) became infected after receiving only a single peri-operative antibiotic dose. Analysis revealed that antibiotic regimen, concomitant injuries, time from trauma to surgery, hospitalization duration, or previous procedures did not significantly alter infection risk. Interestingly, 6 of 8 patients with post-operative wound infections had hospital stays of at least one day, a finding supported by prior studies in both human [29] and veterinary patients [21].

Radiographic imaging alone was insufficient to reliably detect implant colonization. Early signs, such as localized soft tissue opacities and persistent gas inclusions, have limited sensitivity (63%) and specificity (57%) [30, 31]. Around one week after infection, subtle periosteal reactions, osteolysis, sequestration, and ill-defined radiodense zones may appear; however, distinguishing these from non-infectious changes remains challenging [32]. In this study, radiographic abnormalities without clinical significance were observed in 47% of cases (32/68). Implants associated with evident fracture healing complications, screw loosening, or implant breakage were infected in 60% of cases, rising to 73% in patients exhibiting functional musculoskeletal impairments or malalignment of the healed limb. These findings are consistent with Dvořák *et al.* [33], who demonstrated that radiographic signs of disturbed fracture healing do not always correlate with clinical symptoms.

Bacterial osteomyelitis remains a diagnostic challenge [17] and was identified in 9.8% of the dogs included in this study. This aligns with previous reports, which have documented post-operative osteomyelitis rates ranging from 0.6% to 14.8% [11, 34, 35]. Affected dogs in this cohort had a mean age of 6.1 years (median: 7 years) and a mean and median body weight of 35.8 kg and 39.9 kg, respectively, consistent with literature indicating a higher prevalence of osteomyelitis in medium- to large-breed dogs [15, 36]. Radiographically, the most frequent findings included osteolysis in all affected dogs, with two cases exhibiting sequestration and one showing localized bone loss. As Walker *et al.* [37] noted, radiographic changes may not always be apparent in patients with osteomyelitis. Microbiologically, *Staphylococcus intermedius* was the predominant pathogen isolated, corroborating earlier studies identifying staphylococci as common causative agents [11, 17, 38, 39]. Advances in molecular diagnostics

now allow the identification of fastidious organisms, such as *Kingella kingae*, suggesting that previous prevalence estimates of *Staphylococcus aureus* may require reevaluation [14].

Several limitations of this study should be acknowledged. Data accuracy was dependent on medical records, and the sample size was relatively small. These preliminary findings highlight the need for larger, prospective studies in which microbial samples are collected not only during explantation but also during implantation and prior to wound closure, to better differentiate between contamination and true infection.

Currently, implant removal in both human and veterinary medicine is generally indicated for complications such as implant-associated pain, mechanical failure, metal hypersensitivity, risk of peri-prosthetic fractures, functional impairment, or infection [40–43], yet standardized guidelines are lacking [44]. In this study, microbial colonization was observed in nearly 50% of explants, but clinically significant infection was present in only five animals (7.3%). This suggests that bacterial colonization alone may not constitute a definitive indication for implant removal in the absence of clinical signs. The prevalence of bacteria on metallic plate implants appears considerably higher than the occurrence of osteomyelitis, with most animals remaining clinically unaffected. Nevertheless, this balance can shift, and timely explantation is advisable when clinical conditions warrant [45].

## Conclusions

This study represents the first systematic investigation in small animal orthopedic surgery evaluating risk factors associated with explant infection. Factors including patient body weight and age, implant location and type, presence of concomitant injuries such as pulmonary lesions, surgeon experience, and the number of personnel in the operating room appear to influence infection development. Osteolytic changes were observed in 60% of animals, and 73.3% of patients with impaired mobility had an infected implant.

Although microorganisms were detected in nearly half of all explants, clinically significant infection occurred in only a small fraction (7.3%). These findings underscore the importance of further research into the microbial ecology of implants, including the roles of commensal flora, local oxygenation, pH, temperature, anatomical variability, surgical hygiene, and antibiotic usage, all of which may impact the balance between colonization and pathogenicity.

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