Are atrophic long-bone nonunions associated with low-grade infections?

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Abstract: Impaired fracture healing, especially when associated with bacterial infection, is a severe complication following long-bone fractures and requires special treatment. Because standard diagnostic techniques might provide falsely negative results, we evaluated the sonication method for detection of bacteria on implants of patients with fracture nonunions. A total of 49 patients with a nonunion (group NU) and, for comparison, 45 patients who had undergone routine removal of osteosynthetic material (group OM), were included in the study. Five different diagnostic methods (culture of tissue samples, culture of intraoperative swabs, histopathology of tissue samples, culture of sonication fluid, and 16S ribosomal DNA polymerase chain reaction of sonication fluid) were compared and related to clinical data. Among the diagnostic tests, culture of sonication fluid demonstrated by far the highest detection rate of bacteria (57%) in group NU, and rather unexpectedly 40% in group OM. Culture of sonication samples also revealed a broad spectrum of bacteria, in particular Propionibacterium spp. In conclusion, our results indicate that more bacteria can be detected on implants of patients with atrophic nonunions of long-bone fractures by means of the sonication procedure, which provides a valuable additional diagnostic tool to decide on a surgical procedure (eg, two-step procedure) and to further specify antimicrobial therapy.

Keywords: sonication, osteosynthetic material, osteomyelitis

Background

Fracture nonunions caused by bacterial infections still pose one of the most feared complications in the field of orthopedic surgery.1 Therapy frequently consists of repeated and extensive debridement accompanied by prolonged antibiotic treatment, which puts patients at high risk of associated complications, reduces quality of life due to functional disabilities, and results in high socioeconomic costs.2–7

In particular, patients with open fractures are at risk of infection, but also patients with closed fractures and severe soft-tissue damage develop infections following osteosynthetic procedures.8,9 Staphylococcus aureus has been described as the main causative agent in approximately 50%–60% of cases,10–12 but recently other species also, such as Propionibacterium spp., have been linked to implant-associated infections.13,14

Infections of the bone are particularly difficult to treat, since bacteria form so-called biofilms on the implant surface, which make them more resistant to antibiotics and biocides.15–18 The diagnosis of biofilm infections by standard microbiological diagnostics (cultures of tissue samples or swabs) often yields falsely negative results, and it has been argued that up to 40% of cases are falsely deemed aseptic.19–21

Sonication of removed implants has led to an improved detection rate, because the majority of bacteria are attached to the implant surface, and thus can be gently removed.22,23 Aim of this study was to apply sonication to implants from patients with
atrophic nonunions and compare the results with standard diagnostic techniques (culture of tissue samples, intraoperative swabs, histopathology of tissue samples). We hypothesized that more nonunions are associated with an infection and that sonication might demonstrate a broader spectrum of bacteria that so far has gone undetected. We also compared the results with implants from patients who had undergone routine removal of osteosynthetic material.

Materials and methods

Patient-derived material

From March 2014 to September 2014, 94 patients were included in this study at the Clinic for Orthopedics and Trauma Surgery, Heidelberg University Hospital. A total of 49 patients were scheduled for surgery due to long-bone nonunion, and underwent implant removal or exchange (an example of a patient treated for fracture nonunion is shown in Figure 1). Diagnosis of nonunion was made due to patients’ complaints, clinical examination, and by conventional X-ray and/or computed tomography scan. The diagnosis of an infectious nonunion was based on clinical evaluation (reddening, swelling, hyperthermia, pain, pus intraoperatively, existence of a sinus tract) and laboratory results (elevated C-reactive protein [CRP] concentration and white cell count).

The criteria for an infection were a sinus tract, pus intraoperatively, or at least three positive signs just mentioned. During surgery, an intraoperative swab of the wound, as well as one to three tissue samples, were taken directly adjacent to the

Figure 1  Treatment of an infectious nonunion by means of the Masquelet technique.

Notes: Due to a Staphylococcus warneri infection (as detected by culture of sonication fluid), the patient showed impaired fracture healing of the distal tibia and fibula 20 months after fracture (A). According to Masquelet step 1, osteosynthetic material was removed and extensive debridement performed, followed by implantation of a PMMA-spacer and application of an external fixer (B, C). Due to impaired wound healing and persistent exudation, the PMMA spacer was exchanged and debridement performed 2 months later. After 4 months, the wound had healed and Masquelet step 2 was performed. The PMMA spacer was removed, followed by osteosynthesis using an Expert Tibial Nail Protect; BMP-7 and cancellous bone of the femur (using the reamer–irrigator–aspirator system) were implanted into the fracture gap. The patient showed improved fracture healing 26 months after fracture (D).

Abbreviation: PMMA, polymethyl methacrylate.
implant, and if possible each sample was divided and sent to
the Microbiology Department and the Pathology Department
for examination. In cases of very small tissue specimens, the
entire sample was sent to the Microbiology Department.

Additionally, 45 patients who had undergone routine
removal of osteosynthetic material were included in this
study. There were no clinical or laboratory signs of an infec-
tion, and X-rays showed satisfactory fracture healing. Swabs
tissue samples were collected as described earlier, though
due to small surgical incisions, only a limited number of tis-
sue samples were available for histopathological analysis.

To test the sonication procedure for possible contami-
nation, ten randomly chosen sterile screws were collected
during different surgical procedures and treated accord-
ing to the sonication protocol. All of these samples tested
negative for bacterial contamination. All of the sonication
samples were handled by one person only. The study was
approved by the ethics committee of the Faculty of Medicine
of Heidelberg University, and written informed consent was
obtained from the patients.

**Patient data**

A total of 49 patients with nonunions (group NU) and 45 patients
undergoing routine removal of osteosynthetic material (group
OM) were included in the study. The patients’ average age
was 49.3 (22–81) years for group NU and 45.2 (18–80) years
for group OM. There were 15 female and 34 male patients in
group NU, and 22 females and 23 males in group OM. None
of the patients was under immunosuppressive therapy; 20.4%
of group NU and 2.2% of group OM were diagnosed with
diabetes (clinical data are summarized in Table 1).

**Sonication of implants**

The removed osteosynthetic materials (nails, screws, plates)
were placed into sterile plastic boxes in various sizes depend-
ing on implant size (Bandelin Electronic GmbH & Co KG,
Berlin, Germany) in the operating room. Enough Ringer’s
solution to cover the implant was added (50–200 mL). They
were then placed in an ultrasound bath (Ultrasonic TI-H 20;
Elma Schmidbauer GmbH, Singen, Germany) and treated
with ultrasonic power of 100% (250 W) and ultrasonic
frequency of 45 kHz for 1 minute. These settings have been
shown to detach bacteria from the implant surface while
ensuring bacterial viability.24 Following sonication, 10 mL of
the fluid was placed into each aerobic and anaerobic blood-
culture bottle (Bactec plus aerobic/anaerobic; BD, Franklin
Lakes, NJ, USA) (Figure 2). The bottles were incubated at
36°C until positive or for a maximum of 14 days in a Bactec
FX (BD). Positive bottles were subcultured on Columbia 5%
sheep-blood agar (BD), chocolate agar, MacConkey agar,
and *Streptococcus*-selective (SCS) agar (all BioMérieux SA,
Marcy-l’Etoile, France) until positive. All positive samples
showed growth on the subculture the next day. Bacteria were
identified by a matrix-assisted laser desorption/ionization
time-of-flight (MALDI-TOF) mass spectrometer (Microflex;
Bruker Corporation, Billerica, MA, USA). Susceptibility
testing was done using the Vitek® 2 microbial identification
system (BioMérieux). Additionally, eu bacterial 16S ribo-
sonal DNA (rDNA) polymerase chain reaction (PCR) was
performed as previously described.25

**Diagnostic procedures**

Tissue samples were processed according to the following
protocol. After arrival at the lab, the tissue was ground using
a porcelain mortar, followed by the addition of 1 mL of 0.9%
NaCl. This suspension was inoculated onto Columbia 5%
Sheep blood agar (BD), chocolate agar, MacConkey agar,
SCS agar, Schaedler Neo Vanco +5% sheep blood (SNVS)
agar (all BioMérieux), and thioglycolate broth (BD), and
then Gram staining was performed. Plates and broth were
incubated until positive or for a maximum of 5 days at 36°C
in 5% CO2 or under anaerobic conditions. Identification
of bacteria was done with the MALDI-TOF mass spectrometer.
Susceptibility testing was done using the Vitek 2 microbial
identification system.

For intraoperative swabs, the eSwab system was used.
After arrival at the lab, 10 µL of liquid Amies was inoculated
onto Columbia 5% sheep-blood agar (BD), chocolate agar,
MacConkey agar, SCS agar, SNVS agar (all BioMérieux),
and thioglycolate broth (BD), and then Gram staining was
performed. Plates and broth were incubated for 2 days at 36°C
in 5% CO2 or under anaerobic conditions. Identification and
susceptibility testing was done as described earlier.

The incubation time was chosen according to microbiolo-
gical expertise (swabs for 2 days and tissue samples for
5 days). Swabs were transported in liquid Amies transport
media with flocked swabs. These swabs released their content
completely into the transport medium, which was used for
the inoculation of plates. On the other hand, the tissue had to
be ground before inoculation, and part of it was put into an
enrichment broth. Therefore, with tissue samples, sometimes
there were cases that were not culture-positive until day 5.

For histopathological diagnostics, the samples were fixed
in formalin and embedded in paraffin, and after the cutting
slices of 2 µm thickness, routine hematoxylin–eosin staining
was performed. The criterion for an implant-associated
Table 1 Clinical data

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Group NU</th>
<th>Group OM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>34</td>
<td>69.4</td>
<td>23</td>
</tr>
<tr>
<td>Female</td>
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<td>30.6</td>
<td>22</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (± standard deviation)</td>
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<td>45.2 (±16.68)</td>
<td></td>
</tr>
<tr>
<td>Median (range)</td>
<td>53 (22–81)</td>
<td>46 (18–80)</td>
<td></td>
</tr>
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</tr>
<tr>
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<td>17</td>
<td>34.7</td>
<td>9</td>
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<td>Former smokers/nonsmokers</td>
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</tr>
<tr>
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<td>79.6</td>
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<td>5</td>
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<tr>
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<td>6.1</td>
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<td>7</td>
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<td>40.8</td>
<td>16</td>
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<td>10.2</td>
<td>3</td>
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<td>Open 3°</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>16</td>
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<td>24.5</td>
<td>1</td>
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<td>8</td>
<td>16.3</td>
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<td>4</td>
<td>6</td>
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</tr>
<tr>
<td>6</td>
<td>2</td>
<td>4.1</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td>4.1</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
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<td>9</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>&gt;9</td>
<td>3</td>
<td>6.1</td>
<td>0</td>
</tr>
<tr>
<td>Mean (± standard deviation)</td>
<td>3.4 (±4.69)</td>
<td>1.0 (±1.15)</td>
<td></td>
</tr>
<tr>
<td>Median (range)</td>
<td>2 (1–31)</td>
<td>1 (1–2)</td>
<td></td>
</tr>
<tr>
<td>Time since fracture (years)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>&lt;1</td>
<td>15</td>
<td>30.6</td>
<td>15</td>
</tr>
<tr>
<td>1–2</td>
<td>16</td>
<td>32.7</td>
<td>24</td>
</tr>
<tr>
<td>2–5</td>
<td>9</td>
<td>18.4</td>
<td>6</td>
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<tr>
<td>5–10</td>
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<td>8.2</td>
<td>0</td>
</tr>
<tr>
<td>10–20</td>
<td>5</td>
<td>10.2</td>
<td>0</td>
</tr>
<tr>
<td>Mean (± standard deviation)</td>
<td>3.4 (±4.39)</td>
<td>1.5 (±0.98)</td>
<td></td>
</tr>
<tr>
<td>Median (range)</td>
<td>1.5 (0.5–15)</td>
<td>1.5 (0.5–3.5)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: NU, nonunion; OM, osteosynthetic material.

infection was at least 23 polymorphonuclear neutrophils per ten high-power fields.26

Statistical tests
The concordance of the results of the five diagnostic methods and of the clinical evaluation was calculated using Cohen's $\kappa$-test, and the interpretation of the test was as in Landis and Koch.27 The correlation between CRP concentration/white blood cell count and culture of either sonication fluid or tissue samples was calculated by the Spearman test. With regard to the clinical data (Table 1), differences between groups were calculated using the unpaired $t$-test (age), Mann–Whitney...
Bacterial infection and atrophic nonunions

U-test (number of previous surgeries at the same location), or Fisher’s exact test (all others).

Results

Evaluation of diagnostic methods in implant infections

Implants were removed from various locations (Table 1). Conventional microbial diagnostic of tissue samples and swabs was performed, as was histopathological examination of tissue samples. After sonication of the removed implant, the presence of bacteria in the fluid was assessed by conventional culture and eubacterial 16S rDNA PCR. As summarized in Table 2, more bacteria were detected following culture of sonication fluid (57.1% group NU, 40% group OM) compared to culture of tissue samples (10.2% group NU, 17.2% group OM), histology of tissue samples (9.3% group NU, 25% group OM), and PCR analysis of sonication fluid (11.9% group NU, 10.5% group OM). Culture of intraoperative swabs provided the least positive results (6.8% group NU, 0% group OM) (data summarized in Table 2).

Agreement or nonagreement of these five diagnostic methods was determined using Cohen’s $\kappa$-test. There was only one moderate agreement between culture of tissue samples and culture of intraoperative swabs; all other diagnostic tests revealed fair, slight, or no agreement (data summarized in Table 3).

Clinical evaluation

Patients with nonunions were evaluated according to the aforementioned clinical criteria for an infection (see Materials and methods section). In this group, 16 of 49 patients (32.7%) showed signs suspicious of an infection.

Table 2 Number of patient samples acquired from group NU and group OM, and number of positive results

<table>
<thead>
<tr>
<th>Group NU (n=49)</th>
<th>Number of patients</th>
<th>Positive</th>
<th>Group OM (n=45)</th>
<th>Number of patients</th>
<th>Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture of tissue samples</td>
<td>49</td>
<td>5/49 (10.2%)</td>
<td>Culture of tissue samples</td>
<td>29</td>
<td>5/29 (17.2%)</td>
</tr>
<tr>
<td>Culture of intraoperative swab</td>
<td>44</td>
<td>3/44 (6.8%)</td>
<td>Culture of intraoperative swab</td>
<td>29</td>
<td>0/29</td>
</tr>
<tr>
<td>Culture of sonication fluid</td>
<td>49</td>
<td>28/49 (57.1%)</td>
<td>Culture of sonication fluid</td>
<td>45</td>
<td>18/45 (40.0%)</td>
</tr>
<tr>
<td>16S rDNA PCR of sonication fluid</td>
<td>42</td>
<td>5/42 (11.9%)</td>
<td>16S rDNA PCR of sonication fluid</td>
<td>38</td>
<td>4/38 (10.5%)</td>
</tr>
<tr>
<td>Histopathology of tissue samples</td>
<td>32</td>
<td>3/32 (9.3%)</td>
<td>Histopathology of tissue samples</td>
<td>8</td>
<td>2/8 (25%)</td>
</tr>
</tbody>
</table>

Abbreviations: NU, nonunion; OM, osteosynthetic material; rDNA, ribosomal DNA; PCR, polymerase chain reaction.
Using the Cohen $\kappa$-score, we compared the clinical evaluation of an infection with culture of sonic fluid and with culture of tissue samples. There was no agreement between clinical evaluation and culture of sonic fluid ($\kappa<0$) or 16S rDNA PCR of sonic fluid ($\kappa<0$); there was slight agreement between clinical evaluation and culture of tissue samples ($\kappa=0.038$), as well as culture of intraoperative swab ($\kappa=0.006$), and fair agreement between clinical evaluation and histopathology of tissue samples ($\kappa=0.294$).

Standard laboratory tests (CRP concentration and white cell count) were correlated with positive results of culture of sonic fluid and of culture of tissue samples by means of the Spearman test. We found no statistically significant correlation between these parameters.

**Identification of bacterial species**

We compared the bacterial species that were detected following culture of sonic fluid or culture of tissue samples (Tables 4 and 5). Culture of sonic fluid revealed a broader spectrum of bacteria, and in particular *Propionibacterium* spp. were detected more reliably than by culture of tissue samples. When tissue samples were positive, only one bacterial species was detected by culture of tissue samples. Bacteria detected by culture of tissue sample and culture of sonic fluid in the same case were not always concurrent (Tables 4 and 5).

We also tried mapping bacteria found in certain locations, but due to the limited number of patients included in this study and the considerable variety of bacteria, no significant differences in distribution of bacteria could be determined (Table S1).

**Discussion**

Our results showed that 57.1% of atrophic nonunions of long-bone fractures were associated with bacteria on the implant surface if culture of implant sonication is performed. The strengths of the study were prospective study design, thoroughly documented clinical data, existence of a comparison group, and random testing of the sonication method. Limitations of the study were the number of patients...
included not being sufficient for mapping of bacterial species (Table S1), varying number of tissue samples retrieved, lack of a generally accepted definition for implant-associated infections, and negative PCR results, possibly due to a dilution effect.

Fracture repair is a complex multistep process, which when disturbed may result in nonunion of the bone. Bacterial infections are one possible reason for impaired fracture healing and diagnostics, and treatment of infectious nonunions still pose a challenge in the field of orthopedic surgery. Sonication of implants has led to an increased detection rate of bacteria in prosthetic joint infection. The aim of this study was to investigate whether more bacteria could be detected on implants of fracture nonunions by means of the sonication method.

In our study, culture of sonication fluid far outreached all other diagnostic methods, showing positive results in 57% of atrophic fracture nonunions. To test the sonication method for contamination, ten sterile screws were randomly chosen during different surgical procedures and processed according to the sonication protocol. Bacteria were not detected in any of these samples, making possible contamination during the sonication procedure unlikely. Culture of intraoperative swabs showed poor results, as previously described in the literature, and should therefore only be used if tissue samples or sonication are not available.

Using molecular methods to diagnose implant infections is controversial in the literature, though a number of conclusive reports exist that advocate the use of such methods. We therefore speculated that molecular diagnostics might significantly enhance the detection rate of bacteria also in atrophic nonunions. Surprisingly, eubacterial 16S rDNA PCR of sonication fluid showed poor results when compared to culture of sonication fluid. This might have been due to a dilution effect in the sonication fluid, which might have produced falsely negative results. When evaluating the five different diagnostic methods, we found that the agreement between all of them was mostly fair or slight only (Table 3), leading us to the conclusion that a discrepancy of diagnostic tests cannot safely rule out an infection.

We also compared the results of group NU with group OM. Interestingly, 40% of group OM were positive by culture of sonication fluid, even though the implants did not cause any clinical problems and fracture healing was not impaired in these patients. This finding has been previously described by Obst et al. According to their study, the majority of routinely removed implants were colonized, which raises the question whether bacterial biofilms on implants should be considered pathogenic at all and whether they are actually the cause of nonunions.

There are several possible explanations for this phenomenon. First, even though bacterial biofilms are not considered to be a highly aggressive form of life, there might be differences in virulence between various bacterial strains and hence in clinical significance. Furthermore, even though the immune system is capable of recognizing and attacking biofilms, there are possible reasons as to why the immune system fails to eliminate biofilm infection in some cases.

First, it could be a matter of time: how fast a biofilm develops (how many bacteria are present) and how fast the immune system responds. Once a biofilm has formed and the immune system fails to cope with the infection, a persistent inflammatory response ensues, which leads to osteoclast generation and tissue degradation.

Second, individual differences in the immune response have been described in association with nonunions. According to Szczęsny et al, genetic mutations, such as the TLR4 gene mutation 1/W, have been identified to be associated with ineffective recognition and elimination of bacteria, and thus predispose these patients to impaired fracture healing. Our data showing improved detection of bacteria on osteosynthetic materials by means of the sonication method are in line with results by others.

The question of whether bacteria detected by culture of sonication fluid are truly pathogenic or whether the

### Table 5 Bacterial species detected following culture of tissue samples and culture of sonication fluid of patients undergoing routine removal of osteosynthetic material (group OM)

<table>
<thead>
<tr>
<th>Species</th>
<th>Number of isolates</th>
<th>Culture of tissue samples</th>
<th>Culture of sonication fluid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coagulase-negative Staphylococcus spp.</td>
<td>2/5 (40.0%)</td>
<td></td>
<td>6/20 (30.0%)</td>
</tr>
<tr>
<td>Propionibacterium acnes</td>
<td>2/5 (40.0%)</td>
<td></td>
<td>8/20 (40.0%)</td>
</tr>
<tr>
<td>Enterococcus fecalis</td>
<td>1/5 (20.0%)</td>
<td></td>
<td>1/20 (5.0%)</td>
</tr>
<tr>
<td>Bacillus spp.</td>
<td></td>
<td></td>
<td>3/20 (15.0%)</td>
</tr>
<tr>
<td>Sporolactobacillus laevolacticus</td>
<td></td>
<td></td>
<td>1/20 (5.0%)</td>
</tr>
<tr>
<td>Delfia acidovorans</td>
<td></td>
<td></td>
<td>1/20 (5.0%)</td>
</tr>
</tbody>
</table>
sionization method should be considered too sensitive for clinical practice is controversial among orthopedic surgeons. It has been suggested that the sonication results be verified by a number – at least 50 colony-forming units per plate – and to discard fewer bacteria as insignificant. However, one should keep in mind that bacteria in biofilms adapt to altered environments (such as culture mediums) very slowly or not at all.40,41 There have also been reports of bacteria in a so-called viable but not culturable state, which could easily be misinterpreted as falsely negative if bacterial numbers are the sole criterion for an infection.42,43

Bacteria detected on implants of patients with atrophic nonunions should not be discarded as insignificant, because bacteria were also detected in 40% of patients without impaired fracture healing. As previously discussed, there are individual reasons why bacteria might add to the pathology of impaired fracture healing in some patients while the majority of fractures heal without complications, despite the presence of bacteria.

We were also interested in evaluating the type of bacteria that can be detected in fracture nonunions. Compared to other diagnostic tools, a wide spectrum of bacteria was found following sonication. Among those were Propionibacterium spp., which for a long time have been considered merely associated with skin-related diseases, but have been linked to implant infections as well.13,14 By means of the sonication procedure and by incubation for up to 14 days, we were able to detect propionibacteria that previously have gone unnoticed. In group OM, propionibacteria were even the most frequently detected bacteria (40%).

According to Grice et al,44 a specific combination of bacteria can be found at sebaceous, moist, and dry-skin areas. We wondered whether a mapping of bacteria detected by implant sonication might offer a clue concerning the origin of bacteria (patient’s skin, contamination from surroundings in open fractures, intraoperative contamination), and hence specify antibiotic prophylaxis more precisely. However, due to the large variety of bacteria and the limited number of patients, no significant differences in the distribution of bacteria were able to be detected.

It is widely accepted, that Staphylococcus spp. make up 50%–60% of implant-associated infections, and S. aureus is thought the major causative agent of bone infections.10–12 In our study, 55% of bacteria found in group NU and 30% found in group OM were in fact Staphylococcus spp.; however, S. aureus was not detected in a single case in either group. This highlights once more that new diagnostic methods have enabled us to detect a wider spectrum of bacteria that seem to be associated with implant infections.

Conclusion
Our data show that bacteria detected on implants by culture of sonication fluid might contribute to the pathology of a large number of atrophic fracture nonunions, and that a previously underestimated variety of bacterial species might be associated with impaired fracture healing.

The pathogenicity of detected bacteria should be discussed in an interdisciplinary setting for each individual case, and further studies on clinical outcome following different treatment strategies might support our finding that culture of sonication fluid is a valuable additional tool to determine a surgical course of action (eg, Masquelet two-step procedure) and to improve antibiotic treatment.

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Author contributions
All authors contributed toward data analysis, drafting and revising the paper and agree to be accountable for all aspects of the work.

Disclosure
The authors report no conflicts of interest in this work.

References


## Supplementary material

### Table S1 Distribution and number of bacterial species found by culture of sonication fluid: group NU and group OM

<table>
<thead>
<tr>
<th>Location</th>
<th>Species and number of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Humerus/olecranon (n=8)</td>
<td>Bacillus spp. (2), Staphylococcus spp. (3), Propionibacterium spp. (2)</td>
</tr>
<tr>
<td>Ulna/radius (n=13)</td>
<td>Propionibacterium sp. (1)</td>
</tr>
<tr>
<td>Clavicle (n=5)</td>
<td>Staphylococcus sp. (1), Delftia acidovorans (1), Bacillus sp. (1), Propionibacterium spp. (3)</td>
</tr>
<tr>
<td>Pelvis (n=1)</td>
<td>Staphylococcus sp. (1)</td>
</tr>
<tr>
<td>Femur (n=23)</td>
<td>Staphylococcus spp. (9), Micrococcus sp. (1), Lactobacillus sp. (1), Oceanobacterium sp. (1), Propionibacterium spp. (1)</td>
</tr>
<tr>
<td>Fibula/tibia (n=36)</td>
<td>Staphylococcus spp. (8), Propionibacterium spp. (2), Enterobacter sp. (1), Corynebacterium sp. (1), Brevibacterium sp. (1), Bacillus sp. (4), Sporolactobacillus sp. (1), Enterococcus sp. (1)</td>
</tr>
<tr>
<td>Foot (n=8)</td>
<td>Bacillus sp. (1), Staphylococcus sp. (1), Micrococcus sp. (1), Propionibacterium sp. (1)</td>
</tr>
</tbody>
</table>

Abbreviations: NU, nonunion; OM, osteosynthetic material.