Improved Diagnosis of Orthopedic Implant-Associated Infections by Inoculation of Sonication Fluid into Blood Culture Bottles

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Abstract

Sonication improved the diagnosis of orthopedic implant-associated infections (OIAI). We investigated the diagnostic performance of sonication fluid inoculated into blood culture bottles in comparison with intraoperative tissue and sonication fluid cultures. Consecutive patients with removed orthopedic hardware were prospectively included and classified as OIAI or aseptic failure (AF) according to standardized criteria. The diagnostic procedure included collection of five intraoperative tissue cultures and sonication of the removed device, followed by conventional culture of the sonication fluid. Cultures were incubated for 7 days (aerobic) or 14 days (anaerobic). In addition, 10 ml of sonication fluid were inoculated into each aerobic and anaerobic BacT/Alert FAN blood culture bottles and incubated in the automated blood culture system for 5 days. Of 75 included patients, 39 had OIAI and 36 AF. The sensitivity of sonication fluid was higher when inoculated into blood culture bottles (100%) compared to conventional sonication fluid (87%, p = 0.05) or intraoperative tissue cultures (59%, p <0.01). Previous antibiotic therapy reduced the culture sensitivity of conventional sonication fluid to 77% and of intraoperative tissue to 55%, while it remained 100% when blood culture-inoculated sonication fluid. The time to positivity was shorter in blood culture-inoculated sonication fluid, detecting 72% of microorganisms after one day of incubation, whereas intraoperative tissue and conventional sonication fluid cultures grew in 18% and 28%, respectively. In conclusion, sonication fluid inoculated into blood culture bottles improved the diagnosis of OIAI and considerably reduced the time to culture positivity compared to conventional sonication fluid and intraoperative tissue cultures.
Introduction

The pathogenesis of orthopedic implant-associated-infections (OIAI) is related to biofilms, rendering these infections difficult to diagnose. Microorganisms in biofilms are embedded in hydrated extracellular matrix, adhered to surface and transformed in a less metabolically active state (1). An accurate diagnosis of OIAI is crucial for the treatment success. A combination of various preoperative and intraoperative methods is usually needed for microbiological diagnosis of OIAI (2, 3). However, current diagnostic methods have limited sensitivity with 10-30% false-negative results and/or lack specificity (2).

Research and development of new diagnostic methods aim to improve the diagnostic accuracy and speed of microbial detection in foreign-body infections. Sonication of explanted implants, designed to remove attached biofilms, significantly improved the culture sensitivity compared to conventional microbiological methods using synovial fluid or intraoperative tissue samples. Sonication was evaluated in infections involving prosthetic joints, fracture-fixation devices, vascular grafts, neurosurgical shunts, breast implants and cardiac devices (4-7). Despite the use of sonication, however, cultures remain negative for several reasons, such as inappropriate culture media, short incubation time, loss of microbial viability during specimen transport or due to prior antimicrobial therapy (8).

Various methods have been investigated to further improve the diagnosis of OIAI. For example, the culture sensitivity was improved by inoculation of synovial fluid immediately after aspiration into aerobic and anaerobic blood culture bottles (9, 10). Similarly, authors have suggested inoculation of homogenized tissue suspension in blood culture bottles (11, 12). In a recent study of prosthetic joint infection (13), sonication fluid inoculated into blood culture bottles showed higher sensitivity compared to inoculated synovial fluid (88% versus 64%, p = 0.009), especially in cases with previous antibiotic treatment (81% vs. 52%, p = 0.031). This observation is not surprising as similar results have been demonstrated with conventional synovial and sonication fluid cultures on blood agar plates and enrichment growth media.

Other investigators routinely inoculated sonication fluid in blood culture bottles (14). However, it remains unclear whether inoculation into blood culture bottles increases the sensitivity of sonication fluid culture (especially in patients receiving antibiotics prior to sampling) and potentially reduce the time to culture positivity. This question has a high clinical relevance, which needs to be resolved before a general use of automated blood culture systems for sonication fluid can be routinely recommended.

In this study, we evaluated the diagnostic performance of sonication fluid inoculated into BacT/Alert FAN blood culture bottles and compared it with intraoperative tissue and conventional sonication fluid
cultures. Consecutive patients with removed orthopedic hardware were prospectively included. In all
patients - independent whether infection or aseptic reason was suspected - standardized comprehensive
preoperative and intraoperative diagnostic procedures were performed to minimize the probability of
misclassification of OIAI as aseptic failure (AF) or vice versa by interpreting contaminating
microorganisms as pathogens. To our knowledge, this is the first study, in which a direct diagnostic
performance comparison between conventional sonication fluid cultures and blood culture-inoculated
sonication fluid was performed.

Materials and methods

Study design. A cohort study was conducted in two tertiary medical care centers, Hospital del Mar (=400
beds) and Hospital de l’Esperança (=200 beds) in Barcelona, Spain. The study protocol was reviewed and
approved by the institutional review board before patient inclusion. A standardized comprehensive
diagnostic algorithm was applied to all patients to accurately determine the cause of prosthesis failure.
This algorithm included standardized sampling of five intraoperative tissue specimens, sonication of
removed orthopedic prosthetic components, prolonged incubation of synovial, intraoperative tissues, and
sonication fluid cultures and inoculation of sonication fluid into aerobic and anaerobic BacT/Alert FAN
Blood culture bottles with antimicrobial removal systems (BioMérieux, Marcy L’Etoile, France).

Study population. In the participating hospitals we prospectively included all consecutive patients aged
≥18 years hospitalized from June 2013 through December 2013, in whom a joint prosthesis or fracture
fixation device was removed for any reason. If obvious contaminations of the explanted components
occurred during surgery, transport or processing of the prosthesis in the laboratory, subjects were
excluded. The following information was recorded: demographic, clinical, radiological, laboratory and
microbiological data; information on type of surgical management and antimicrobial therapy.

Study definitions. OIAI was defined when at least one of the following criteria was present (15): (i)
visible purulence of the site aspirate or at the surgical site (as determined by the surgeon); (ii) the
presence of a sinus tract communicating with the implant and/or (iii) clinical signs of infection such as
warm, redness or wound drainage (as determined by the surgeon). Additionally, in prosthetic joint
infection were the following criteria also considered: (iv) acute inflammation in histopathology sections
of intraoperative tissue (as determined by the pathologist); (v) acute inflammation in preoperative joint
aspiration (leukocyte count >1.7 g/L or >65% granulocytes in a knee prosthesis (16) or leukocyte count
>4.2 g/L or >80% granulocytes in a hip prosthesis (17). AF was defined when the implant was removed in the absence of these criteria for OIAI. Previous antimicrobial therapy was defined as any antibiotic received for ≥24 hours within the 14 days prior to surgery.

**Synovial fluid.** Synovial fluid was aspirated preoperatively and transferred into two vials. One of the vials contained ethylenediaminetetraacetic acid (EDTA) for determination of leukocyte count and percentage of granulocytes. The other was a native vial for culture. In the microbiology laboratory, 0.1 ml was inoculated on each PoliVitex (BioMérieux, Marcy L'Etoile, France) agar plates (incubated 7 days aerobically at 37ºC with 5% CO₂) and Schaedler enriched with 5% of sheep blood (BioMérieux, Marcy L'Etoile, France) agar plates (incubated 14 days anaerobically at 37ºC). Additionally, 0.5 ml of synovial fluid was inoculated in thioglycolate broth (BBL™ Enriched Thioglycolate Medium with Vitamin K & Hemin, Becton Dickinson and Company, USA) and residual volume were inoculated into a BacT/Alert (BioMérieux, Marcy L’Etoile, France) anaerobic bottle and incubated for 5 days.

**Intraoperative tissue samples.** Tissue specimens were collected in native vials and were individually homogenized in 0.5 ml thioglycolate broth for 1 min using a mortar and pestle. Aliquots 0.5 ml of tissue homogenate samples were inoculated per plate in PoliVitex agar plates, Schaedler enriched with 5% of sheep blood agar plates and in thioglycolate broth. The aerobic cultures were incubated at 37ºC for 7 days and the anaerobic ones for 14 days. Each distinctive colony morphology was identified using standard microbiological techniques. Low-virulence microorganisms, such as coagulase-negative staphylococci, *Corynebacterium* spp, *Bacillus* spp, or *Propionibacterium* spp were considered pathogens when the same organism was isolated in at least two samples.

**Sonication of removed implants.** The removed orthopedic hardware were explanted aseptically in the operating room and transported to the microbiology laboratory in solid polyethylene containers, with screw tops and airtight inner seals. The containers were previously autoclaved at 121ºC for 15 min and double packed. Sonication of the hardware was performed in the microbiological laboratory, as previously described (18). The container with the hardware was vortexed for 30 seconds, followed by sonication for 1 min (at a frequency of 40 ± 5 kHz), then vortexed again for 30 seconds. For sonication, a Bransonic ultrasound bath (model SM25E-MT, Branson Ultrasonics Corporation, Geneva, Switzerland) was used. 0.5 ml aliquots of sonication fluid were plated onto PoliVitex chocolate agar plates, Schaedler agar plates enriched with 5% sheep blood, and inoculated into thioglycolate broth. The cultures were incubated at 37ºC for 7 days (aerobically) or 14 days (anaerobically). Sonication fluid cultures were
considered positive when ≥50 colony-forming units (CFU) of the same organism morphology grew per milliliter of sonication fluid, as previously defined (15). If the patient had previously received antibiotics, any growth in the sonication fluid culture was considered positive (19).

**Blood culture BacT/ALERT method.** Ten milliliters of sonication fluid were inoculated into aerobic and anaerobic BacT/Alert FAN blood culture bottles with antimicrobial removal systems. These bottles were incubated into the automated BacT/Alert system (BioMérieux, Marcy L’Etoile, France) for 5 days. For these bottles which flagged positive, a Gram stain was performed and an inoculum was plated onto PoliVitex chocolate agar plate, Blood agar plate (BioMérieux, Marcy L’Etoile, France) and Schaedler agar plates enriched with 5% sheep blood. If no organisms were seen on Gram stain, the BacT/Alert bottle was returned to the BacT/Alert instrument for further monitoring. The aerobic agar plates were incubated 2 more days, and the anaerobic ones at 37ºC to complete 14 days.

**Statistical Analysis.** Comparisons between categorical variables were performed using McNemar’s χ² or Fisher’s exact test, as appropriate. Continuous variables were compared using the Mann-Whitney U test. P values of less than 0.05 were considered statistically significant. Calculations and graphics were performed using Prism software (Version 6.05; GraphPad, La Jolla, CA).

**Results**

**Study population.** We included 75 patients in whom an orthopedic hardware was removed and submitted to the microbiology laboratory for sonication. No patients were excluded because of obvious contaminations of the implant during handling or in the microbiology laboratory. AF was diagnosed in 36 cases (48%) and OIAI in 39 cases (52%). Further characteristics of the 75 patients are summarized in Table 1. In 45 (60%) a joint prosthesis and in 30 (40%) a fracture fixation device was explanted. Patients with OIAI had most commonly visible pus surrounding the implant (72%) and acute inflammation in tissue histopathology (82%). About half of patients with OIAI (56%) received antibiotics within 14 days prior sampling.

**Performance of diagnostic methods.** Table 2 summarizes the culture accuracy of intraoperative tissue, conventional sonication fluid and sonication fluid inoculated into blood culture bottles from patients with OIAI and AF. The sensitivity of sonication fluid culture was significantly higher than the one of intraoperative tissue culture (87% vs 59%, p <0.01). The sensitivity was improved to 100% by inoculating the sonication fluid into blood culture bottles (p = 0.05).
Effect of previous antibiotic treatment on culture sensitivity. Figure 1 shows the culture sensitivity of intraoperative tissue cultures, conventional sonication fluid cultures and blood culture-inoculated sonication fluid. Previous antibiotic therapy reduced the culture sensitivity of intraoperative tissue from 65% to 55%, of conventional sonication fluid from 100% to 77%, while it remained 100% in blood culture-inoculated sonication fluid. Among the 36 cases with AF, none received antimicrobial treatment previously to surgery and all cases were negative by all three diagnostic methods.

Microbiological findings in patients with OIAI. Table 3 summarizes the microbiological findings of individual diagnostic methods in 39 patients with OIAI. Using conventional and blood culture-inoculated sonication fluid cultures more pathogens were detected (45 and 50 organisms, respectively) than by intraoperative tissue cultures (30 pathogens). In addition, mixed infections (i.e. isolation of ≥2 microorganisms) were detected more frequent in sonication fluid than in intraoperative tissue cultures (21% versus 13%). Negative cultures were observed less frequent in conventional sonication fluid than in intraoperative tissue (13% versus 41%, p <0.01). All patients with negative cultures received antibiotics prior to surgery. By inoculating sonication fluid into blood culture bottles, no false-negative cultures were observed. All 5 infections, which were detected only by blood-cultures inoculated sonication fluid, were acute infections caused by coagulase-negative staphylococcus (n = 1), S. aureus (n = 1), viridans group streptococcus (n = 1) and Gram-negative bacilli (n = 1).

Time to culture positivity in patients with OIAI. Figure 2 illustrates the time to culture positivity in 39 OIAI cases. After one day of incubation, 18% of intraoperative tissue and 28% of conventional sonication fluid cultures were positive, whereas blood culture-inoculated sonication fluid cultures were positive in 72% (p <0.01). All OIAI cases (100%) were detected by sonication fluid inoculated in blood culture bottles on day 5 of incubation. In contrast, sonication fluid culture detected 87% and intraoperative tissue culture 59% of OIAI cases on day 7 of incubation. In this series, prolonged anaerobic incubation of intraoperative tissue and conventional sonication cultures up to 14 days did not yield additional microorganisms.

Discussion

Inoculation of synovial fluid and intraoperative tissue homogenate into aerobic and anaerobic blood culture bottles was shown to improve the culture sensitivity in prosthetic joint infection (9-11). Later, several researchers demonstrated improved culture sensitivity of sonication fluid culture compared to
intraoperative tissue cultures in the diagnosis of OIAI (18, 20-22). A recent publication, the inoculation of sonication fluid into blood culture bottles has been compared with inoculation of synovial fluid (13). However, the same sample (in this case sonication fluid) was not compared in this study. Therefore it remained unclear whether inoculation of sonication fluid into blood culture bottles increases the sensitivity culture compared to conventional sonication fluid cultures. Especially it remained unclear whether inoculation may reduce the time to culture positivity or patients receiving antibiotics prior to sampling may benefit from this method.

In this study, we compared the performance of sonication fluid inoculated into blood culture bottles with the one of intraoperative tissue and sonication fluid cultures. When sonication fluid was inoculated into blood culture bottles, the cultures sensitivity was higher (100%) compared to conventional sonication fluid (87%) and intraoperative tissue samples (59%). Despite sonication may be harmful to microorganisms, especially to gram-negative bacilli and anaerobic bacteria (23), three additional OIAI cases were detected by sonication. caused by *P. acnes* and 2 by Gram-negative bacilli, corroborating the efficacy of sonication procedure.

Inoculation of sonication fluid in blood culture bottles has several advantages over conventional solid media cultures. First, previous studies suggested that microorganisms are present in the sonication fluid in culture-negative cases of prosthetic joint infection (or at least their DNA), as detected by broad-range PCR (24) or multiplex PCR (15, 25). However, it remains unclear whether culture negativity is caused by non-viability of the microorganisms or just low microbial quantity, below the detection limit of conventional sonication fluid cultures. Second, by use of blood culture system, a 20-fold increased volume of sonication fluid is investigated than on agar plate cultures (10 ml versus 0.5 ml). This study also suggests that larger sample volume does not compromise the culture specificity since no false-positive cultures in AF cases were observed. Third, growth media in blood culture bottles contain antimicrobial removal systems and allow growth of microorganisms immediate after inoculation. The main disadvantage of inoculation of sonication fluid in blood culture bottles is a potential decrease in specificity by losing the ability of set colony count thresholds to define a positive culture. Therefore, a combination of independent microbiologic diagnostic tests should be performed, including periprosthetic tissue cultures and conventional sonication fluid cultures.

In the study by Shen et al. (13) researchers detected six additional infections by inoculated sonication fluid compared to synovial fluid in patients who had previously received antibiotics. In our study,
previous antibiotic therapy reduced the culture sensitivity of conventional sonication fluid culture (from 100% to 73%) and intraoperative tissue culture (from 65% to 55%). However, the sensitivity of inoculated sonication fluid into blood culture bottles was not affected (100%). Therefore, inoculation of sonication fluid culture into blood culture bottles with antimicrobial removal systems may be particularly useful in patients who previously received antimicrobials.

Interestingly, five additional cases of OIAI were detected in our study by inoculation of sonication fluid into blood culture bottles (and not by conventional sonication fluid culture). All were acute infections, receiving previous antibiotics. This can be explained by better antibiotic activity on early biofilms in acute infections, as compared to mature biofilms in chronic infections. This explanation is in line with previous observation that sonication increased the cultures sensitivity in chronic, but not in acute prosthetic joint infections (19). We hypothesize that biofilms in chronic infections involve more layers and are more firmly attached to the surface, therefore, sonication can fully exhibits its detachment effect and improved detection characteristics.

Despite the use of sonication improves the diagnosis of OIAI, a considerable number of these infections are culture-negative (8, 19, 25, 26). Culture-negative OIAI may be due to the use of prior antimicrobial therapy, inadequate diagnostic methods, or because of the inability of a pathogen to grow with the current available culture methods (18, 27, 28). In our study, sonication succeeded in significantly reducing the rate of culture-negative OIAI from 41% (by intraoperative tissue culture) to 13%. These results are in agreement with the prior study, which demonstrated that sonication fluid provided faster microbial detection than intraoperative tissue samples (8). In our study, we also observed that the time to microbial detection was further reduced by inoculating the sonication fluid culture into blood culture bottles, detecting 72% of infections already after one day of culture incubation.

Blood culture bottles are widely used in microbiology laboratories for the diagnosis of sepsis or different type of infections such as arthritis when inoculated with sterile fluids (10). However, contamination risk may be increased. Careful manipulation should be applied especially when inoculation of sonication fluid into blood culture bottles is performed. In our study, we applied an enhanced diagnostic algorithm to all included patients independent of microbiological findings and did not observe false-positive culture in AF. By inoculating sonication fluid into blood culture bottles, no false-positive results in this method were observed, therefore increasing the sensitivity without compromising specificity. By counting single-positive tissue samples, the specificity was compromised, as shown by false-positive tissue cultures in...
aseptic failure (29). In contrast, in the study by Shen et al. (13), coagulase-negative staphylococci were isolated from some blood culture bottles inoculated with sonication fluid from patients with AF, which may reflect contamination or misclassification of patients.

In conclusion, sonication fluid inoculated into blood culture bottles with antimicrobial removal systems improved the diagnosis of OIAI and considerably reduced the time to culture positivity compared to conventional sonication fluid and intraoperative tissue cultures. This method demonstrated high sensitivity and specificity, especially in patients receiving antibiotics previously to surgery. By inoculation of sonication fluid in blood culture bottles, 72% of microorganisms were detected after one day of incubation, whereas intraoperative tissue and conventional sonication fluid cultures grew after one day in only 18% and 28% of OIAI. This simple and readily available diagnostic method may significantly improve the diagnosis of implant associated infections in future.

Acknowledgements

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Table 1. Characteristics of 75 study patients with aseptic failure and implant-associated infection.

<table>
<thead>
<tr>
<th></th>
<th>Aseptic failure (n = 36)</th>
<th>Orthopedic implant-associated infection (n = 39)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient age, years, median (range)</td>
<td>73 (27 - 89)</td>
<td>73 (48 - 87)</td>
<td>0.968</td>
</tr>
<tr>
<td>Male sex</td>
<td>12 (33%)</td>
<td>15 (43%)</td>
<td>0.554</td>
</tr>
<tr>
<td>Type of prosthesis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Joint prosthesis (n = 45)</td>
<td>27 (75%)</td>
<td>18 (46%)</td>
<td>0.091</td>
</tr>
<tr>
<td>Fracture fixation device (n = 30)</td>
<td>9 (25%)</td>
<td>21 (54%)</td>
<td><strong>0.004</strong></td>
</tr>
<tr>
<td>Clinical signs of infection</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sinus tract</td>
<td>0</td>
<td>8 (21%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Other local signs of infection$^a$</td>
<td>0</td>
<td>19 (49%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Visible pus surrounding the implant</td>
<td>0</td>
<td>28 (72%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Acute inflammation in tissue histopathology</td>
<td>0</td>
<td>32 (82%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Synovial fluid cell count, mean (range)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leukocyte count, G/l</td>
<td>0.4 (0.05-0.5)</td>
<td>52 (1.2-78)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Granulocytes, %</td>
<td>17 (2-40)</td>
<td>95 (55-98)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>No. of patients who received antibiotics prior to sampling</td>
<td>0</td>
<td>22 (56%)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

NOTE. Values represent numbers (%), if not indicated otherwise.

$^a$ Local signs of infection include warmth, redness or wound drainage.
Table 2. Diagnostic performance of three diagnostic methods in 75 patients with removed orthopedic hardware (39 with orthopedic implant-associated infection and 36 with aseptic failure).

<table>
<thead>
<tr>
<th>Diagnostic method</th>
<th>Sensitivity, % (95% CI)</th>
<th>Specificity, % (95% CI)</th>
<th>PPV, % (95% CI)</th>
<th>NPV, % (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intraoperative tissue samples</td>
<td>59 (42-74)</td>
<td>100 (90-100)</td>
<td>100 (85-100)</td>
<td>69 (55-81)</td>
</tr>
<tr>
<td>Conventional sonication fluid</td>
<td>87 (73-96)</td>
<td>100 (90-100)</td>
<td>100 (90-100)</td>
<td>88 (74-96)</td>
</tr>
<tr>
<td>Inoculation of sonication fluid in blood culture bottles</td>
<td>100 (91-100)</td>
<td>100 (90-100)</td>
<td>100 (91-100)</td>
<td>100 (90-100)</td>
</tr>
</tbody>
</table>

Note. 95% CI, 95% confidence interval; PPV, positive predictive value; NPV, negative predictive value.
Table 3. Microbiological findings in 39 orthopedic implant-associated infection cases according to type of diagnostic method

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Intraoperative tissue culture</th>
<th>Conventional sonication fluid culture</th>
<th>Sonication fluid inoculated in blood bottles</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. microorganisms detected</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>18 (46%)</td>
<td>26 (67%)</td>
<td>31 (79%)</td>
</tr>
<tr>
<td>≥2</td>
<td>5 (13%)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8 (21%)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8 (21%)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>0</td>
<td>16 (41%)</td>
<td>5 (13%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Total No. of microorganisms isolated</td>
<td>30</td>
<td>45</td>
<td>50</td>
</tr>
<tr>
<td>Gram-positive cocci</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coagulase-negative staphylococci</td>
<td>8</td>
<td>16</td>
<td>17</td>
</tr>
<tr>
<td>S. aureus</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Viridans group streptococci</td>
<td>1</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Enterococcus spp.</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Gram-negative bacilli</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. coli</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Enterobacter spp.</td>
<td>2</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Klebsiella spp.</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Proteus spp.</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Anaerobes</td>
<td>2 (7%)</td>
<td>5 (11%)</td>
<td>5 (10%)</td>
</tr>
<tr>
<td>P. acnes</td>
<td>2</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Other microorganisms</td>
<td>2 (7%)</td>
<td>3 (7%)</td>
<td>3 (6%)</td>
</tr>
<tr>
<td>Candida spp.</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Corynebacterium spp.</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

Note:

<sup>a</sup>Klebsiella spp + Proteus spp; Enterococcus spp + Proteus spp + Pseudomonas spp; S. epidermidis + S. warneri; Corynebacterium spp + Enterococcus spp + Proteus spp; S. epidermidis + S. simulans.
Klebsiella spp + Proteus spp; Enterococcus spp + Proteus spp + Pseudomonas spp; S. epidermidis + S.

warneri; Corynebacterium spp + Enterococcus spp + Proteus spp; S. epidermidis + S. simulans; S.

epidermidis + Streptococcus spp; Corynebacterium spp + S. epidermidis + Enterococcus spp; S.

epidermidis + P. acnes.
Figure 1. Culture sensitivity of intraoperative tissue, conventional sonication fluid and sonication fluid inoculated into BacT/Alert bottles in 39 orthopedic implant-associated infection cases (stratified to whether or not receiving antibiotics prior to sampling).
Figure 2. Time to culture positivity of 39 orthopedic implant-associated infection cases.