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Sonication technique improves microbiological diagnosis in patients treated with antibiotics before surgery for prosthetic joint infections

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SUMMARY

Microbiological diagnosis is crucial for the appropriate management of implant-associated orthopedic infections (IAOIs). Sonication of biomaterials for microbiological diagnosis has not yet been introduced in routine clinical practice. Aim of this study was to describe the advantages and feasibility of this procedure in the clinical setting. We prospectively studied 56 consecutive patients undergoing revision because of IAOI and compared the sensitivity of sonication of explanted orthopedic implants with standard cultures. Patients were divided into two groups: those with foreign body infection (FBI, 15 patients) and those with prosthetic joint infection (PJI, 41 patients). Clinical, radiological and microbiological features were recorded. In the PJI group the sensitivity of sonication in detecting bacterial growth was higher than conventional culture (77% *vs* 34.1% respectively, $p<0.002$), while no difference was observed in the FBI group (85.7% *vs* 86% respectively, $p>0.05$). Coagulase-negative Staphylococci accounted for 90% of the bacteria detected by sonication. Moreover, we found that in the PJI group the sensitivity of sonication was not affected by the timing of antibiotic interruption before surgery. Sonication remains an important tool to improve microbiological diagnosis in PJIs, especially in patients who received previous antimicrobial treatment.

KEY WORDS: Implant-associated orthopedic infections (IAOIs), Sonication, Biofilm infection, Prosthetic joint infection.

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INTRODUCTION

In the last two decades, the use of orthopedic implants for the treatment of degenerative bone diseases has significantly improved quality of life, especially in the elderly population. However, the development of implant-associated orthopedic infections (IAOIs) remains one of the major complications in the setting of joint replacement surgery (Kurtz *et al*., 2005). These infections have severe consequences because of prolonged hospitalization, long-term antimicrobial treatments, additional surgery and increased costs (Piper *et al*., 2009; Kurtz *et al*., 2012). A major challenge in the management of IAOIs is a correct microbiological diagnosis in order to select the appropriate antimicrobial agents and to eradicate the infection. Common bacteria in IAOIs are typically organized in a biofilm, which is a microbial network enclosed within an extracellular matrix where microorganisms are in a stationary growth phase (Donlan *et al*., 2002). Due to the location of bacteria, cultures obtained from the tissues around the joint are often negative (Bereza *et al*., 2013). To

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date, the gold standard of microbiological diagnosis is based on culture of preoperative aspirated joint fluid and intraoperative periprosthetic tissue samples (at least 3 and optimally 5 or 6 tissue specimens) followed by inoculation on both aerobic and anaerobic culture media (Osmon *et al.*, 2012).

However, the sensitivity of conventional cultures is not satisfactory (35% of false negatives despite a histological result specific for infection) (Atkins *et al.*, 1998), especially in patients who received previous antimicrobial agents (Trampuz *et al.*, 2007; Berbari *et al.*, 2012). Sonication before culture of the explanted prostheses has been used to disrupt biofilm and to enhance bacterial growth by releasing sessile organisms (Holinka *et al.*, 2011). Although recent data suggest that sonication could be a useful tool to improve the microbiological diagnosis of IAOIs (Donlan, 2002; Trampuz *et al.*, 2003; Trampuz *et al.*, 2007; Piper *et al.*, 2009), this method has not yet been introduced into routine clinical practice.

Aim of this study was to evaluate the usefulness of the implant sonication method to improve microbiological diagnosis of IAOIs in clinical practice. We studied a cohort of patients with IAOI and compared pathogen detection in the sonication culture with standard culture of the explanted orthopedic implant. The effect of antibiotic treatment on the sensitivity of pathogen detection was also assessed.

METHODS

Study population

The study was conducted in three clinics from two referral centers (Sapienza University of Rome and Molise University, Italy). A total of 56 consecutive patients (35 females, 21 females) who underwent revision or resection of orthopaedic implant for IAOIs between January 2008 to January 2010 were enrolled. All study participants gave informed written consent. Patients were excluded if less than three periprosthetic tissue specimens were sent to the laboratory for culture. Criteria for classification of IAOIs were in accordance with the standard criteria of prosthetic or implant infection (Berbari *et al.*, 1998) and fulfilled at least one of the following:

1) visible purulence in the synovial fluid or surrounding the orthopedic implant;
2) growth of the same organism in two or more cultures of synovial fluid or intraoperative periprosthetic tissue;
3) acute inflammation on histopathologic examination of permanent tissue section;
4) sinus tract communicating with the orthopedic implant.

Patients were included in the study even if they did not meet previous criteria but the same organism was cultured from at least two periprosthetic tissue samples and from the sonication fluid (with acceptable quantity over the cut-off). Antimicrobial therapy was defined as administration of antibiotic treatment within 15 days before the removal of the orthopedic implant. Criteria for exclusion were contamination of any kind of samples (periprosthetic tissues and explanted devices) during explant, transport and/or microbiological analysis. Fifty-six patients with IAOIs (21 prosthetic hip infections, 20 prosthetic knee infections, 15 internal device infections) were observed. Subjects were divided into two groups: those with foreign body infection including intramedullary nails, plates, screws, Kirschner wires (FBI group) and those with prosthetic joint infection (PJI group). Demographic, clinical, microbiological and laboratory data were prospectively recorded for each patient. The study was approved by the institutional review board of the Infectious Diseases Unit, Department of Public Health and Infectious Disease, Sapienza University of Rome.

Sample collection

All surgical procedures were performed in a clean-air laminar-flow environment. When possible aspirated joint fluid was sent to the laboratory for leukocyte count and microbiological identification. The explanted implants, aseptically removed, were put in sterile polypropylene containers with screw caps depending on the size of implants and sterile Ringer's solution was added to fill-up the container covering the device. All closed tight sample containers were sent to the laboratory within 4 hours after revision. For each removed component, standard culture and sonication technique, when possible, were performed in parallel.

Conventional culture

In the operating room, a mean of 5.2 periprosthetic tissue specimens were taken from the main inflammatory area involved and sent in sterile conditions to the laboratory for histopathology and standard microbiological culture. Synovial fluid was inoculated in both liquid media (brain-heart-infusion broth or, when possible, into a BACTEC Ped Plus/F bottle) and solid culture plates (aerobic blood agar, chocolate agar and anaerobic blood agar). All samples were incubated for 7 days. The devices explanted from patients with IAOI were inoculated in Trypticase Soy Broth (TSB), incubated for 24 h at 37°C and then cultured on aerobic and anaerobic blood agar plates for 5 days. TSB (500 µl) was used to do the serial dilutions for bacterial count.

The cut-off to detect the pathogen microorganism was 5 CFU/ml. The periprosthetic tissues were put in specific tubes (Tube-drive IKA Ultraturrax IKA WERKE GMBH & CO.KG) and homogenized in 3 ml of brain-heart infusion broth for 1 minute. The homogenate was inoculated in aliquots of 0.5 ml on aerobic and anaerobic sheep blood agar plates, which were further incubated at 35-37°C in 5-7% carbon dioxide aerobically and anaerobically for up to 4 days and 7 days, respectively. Strain identification and analysis of antibiotic susceptibility patterns were performed using a Vitek-2 system (bioMérieux, Marcy l'Etoile, France). The samples were considered positive when the same microorganism with an identical antibiotic susceptibility pattern grew from two or more tissue specimens.

Sonication culture of explanted implants

The explanted orthopedic devices of patients with IAOI were inoculated in TSB and incubated for 24 h at 37°C.

After the removal of TSB, each device was further covered with Ringer's solution, vortexed and sonicated as previously described (Trampuz *et al.*, 2007; Holinka *et al.*, 2011). Briefly, the container was vortexed for 30 seconds using a vortex mixer (VELP Scientifica), sonicated at a frequency of 40kHz in 400 ml Ringers solution at 22°C for 5-7 minutes and vortexed again for 30 seconds. For sonication the Bactosonic ultrasound bath (BANDELIN Elettronic GmbH & Co. KG, Berlin, Germany) was used. Sonication fluid was subsequently centrifuged (3200 rpm for 15 minutes) and the supernatant was aspirated.

The sediment was used for direct microscopy after Gram staining and placed onto aerobic Columbia sheep blood agar plates and onto anaerobic Schaedler sheep blood agar for 5 days. (Trampuz *et al.*, 2006 a); Monsen *et al.*, 2009). In order to compare the number of bacteria grown in sonication fluid with that grown in traditional culture, 500 µl of sonication fluid was inoculated onto aerobic and anaerobic sheep blood agar and the numbers of CFU/ml were counted after 24h of incubation. The minimum detection level was 5 CFU/ml.

Microorganisms were identified by standard automatic methods (Vitek-2 system; bio Mérieux, Marcy L'Etoile, France). Even if this method was not standardized for a quantitative approach, we performed an overnight incubation before counting the number of bacteria in order to compare the results from the traditional culture with those from sonication fluid culture (Oliva *et al.*, 2013).

Statistical analysis

Statistical analysis was performed using the two-tailed Fisher exact test or Chi-squared test with Yates' correction for continuity, as appropriate. Statistical significance was defined as a p-value of less than 0.05. 95% confidence intervals for proportion were obtained using the modified Wald method. The aim was to examine differences between the FBI group and the PJI group and the different microbiological methods used.

RESULTS

Characteristics of study population

Fifty-six patients with IAOIs were included in the study: 15 patients with infections of orthopedic devices (FBI group) which consisted in 5 intramedullary nail, 4 plates, 4 screw, 2 Kirschner wires and 41 patients with prosthetic joint infections (PJI group).

In the FBI group 80% of cases (12/15 [95% confidence interval (CI): 54.05% to 93.72%]) were localized in the inferior limb. In the PJI group,

TABLE 1 - *Demographic and clinical characteristics of study population*

Characteristics	*Foreign Body Infections (n=15)*	*Prosthetic Joint Infection (n=41)*	*p*
Age Mean (±S.D.)	44.9 (±13.8)	70.9 (±6.31)	<0.0001
Sex n° (%) Male Female	 10 (67) 5 (33)	 11 (27) 30 (73)	 0.016
Underlying disorder n° (%) Diabetes mellitus Previous joint surgery Congenital abnormalities	 1 (7) 0 (0) 2 (13)	 12 (29) 8 (19) 0 (0)	 0.15 0.09 0.07
Timing of infection n° (%) Early Delayed Late	 7 (46) 6 (40) 2 (13)	 14 (34) 11 (27) 16 (39)	 0.5 0.5 0.1
Fever n°(%)	8 (53)	9 (22)	0.05
Presence of sinus tract n° (%)	8 (53)	3 (7)	0.0005
Clinical signs of acute inflammation 3/5 n° (%)*	13 (86)	19 (46)	0.01
Preoperative laboratory findings (%) Blood leukocyte count >10x10^3/ml Erythrocyte sedimentation rate >30 mm/hr Serum C-reactive protein>1.0 mg/dl	 7 (46) 12 (80) 12 (80)	 9 (22) 17 (41) 20 (49)	 0.1 0.01 0.06

*Clinical signs of acute inflammation are considered at least 3 of five: swelling, redness, heat, local pain, loss of function.

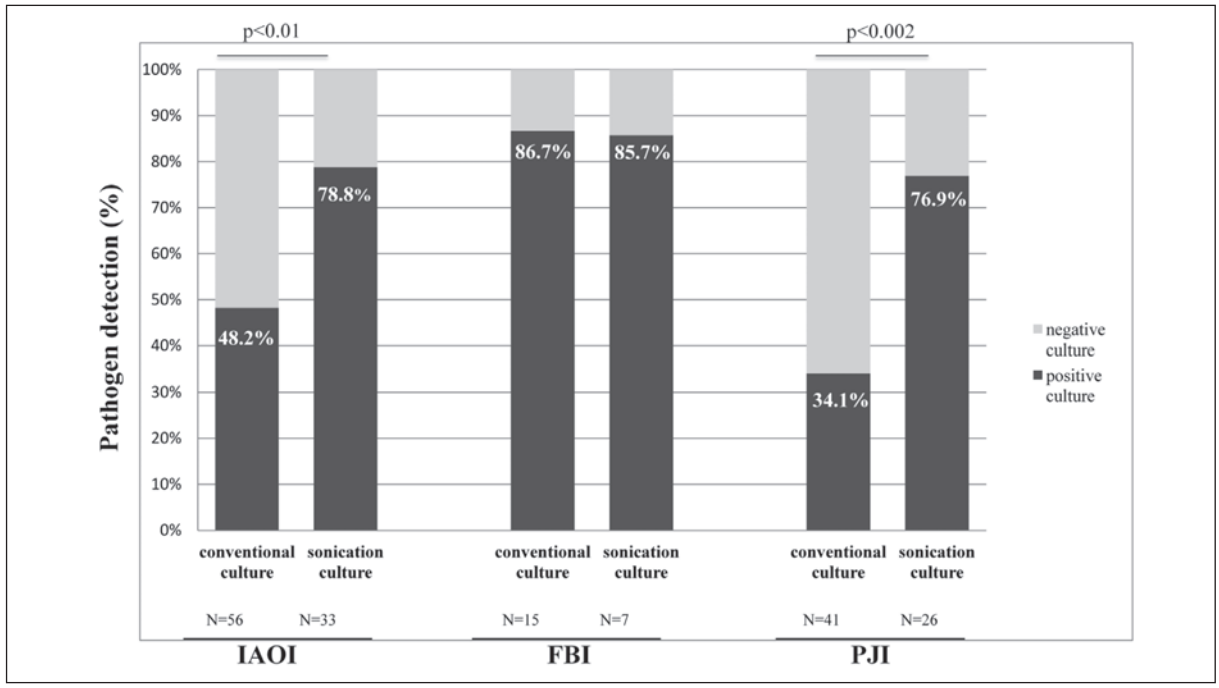

FIGURE 1 - *Rate of pathogen detection in conventional culture and sonication fluid culture. In all IAOI the rate of pathogen detection was higher for sonication culture (p<0.01). For FBI no differences were found, whereas in the PJI group culture fluid sonication of explanted devices was more sensitive than conventional culture (p<0,002). IAOI: implant-associated orthopedic infections, FBI: foreign body infection, PJI: prosthetic joint infections.*

51% of cases (21/4 [95% CI: 36.48% to 65.75%]) were hip-prosthesis infections, whereas 49% (20/4 [95% CI: 34.25% to 63.52%]) were knee-prosthesis infections.
The demographic and clinical features of FBI and PJI groups are shown in Table 1. In the PJI group there was a higher percentage of females than in the FBI group; in addition, patients with PJIs were older than those with FBIs. Fever, presence of sinus tract and clinical signs of acute inflammation were more common in the FBI group.

Rate of pathogen detection in conventional culture and sonication fluid culture

Conventional culture of specimens was done in all 56 cases; moreover, sonication was also performed in a subset of 33 patients (7 FBI and 26 PJI).
Overall, the rate of pathogen detection was significantly higher in sonication culture than in conventional culture (78.8% [95% CI: 61.95% to 89.62%] *vs* 48.2% [95% CI: 35.67% to 60.99%], p<0.01).
In FBI patients, sonication detected bacteria in 85.7% [95% CI: 46.65% to 99.47%] of cases and conventional culture in 86.7% of cases [95% CI: 60.86% to 97.52%], (p>0.05). On the other hand, in PJI patients sonication detected bacteria in 76.9% [95% CI: 57.61% to 89.30%] of removed implants (20/26), whereas conventional culture was positive in only 34.1% [95% CI: 21.50% to 49.51%] of cases (14/41) (p=0.0016) (Figure 1). The agreement between two tests was 60% (K=0.29) in all explanted devices: 71% (K=0.25) in FBI group, 57% (K=0.27) in PJI group.

Microorganism identification

Overall, 30 microorganisms were detected from 33 explanted prosthetic implants by sonication and 28 microorganisms were detected from 56 periprosthetic tissues using the conventional method. Most organisms were Coagulase-negative Staphylococci (CoNS). Four patients in the PJI group had polymicrobial infections detected only by sonication (Table 2).
All microbiological findings belonging to the FBI group were concordant between the different diagnostic methods used. In the PJI group, 9 cases were concordant in term of microorganism growth between standard and sonication methods, whereas in 11 cases bacterial growth was detected only in sonication fluid culture. In 4 cases we found discordant microbiological results. Only in 8 cases collect synovial fluid before surgery.
All but one patient had concordant results comparing the synovial fluid culture with samples collected during surgery. In the discordant patient, the culture of synovial fluid was negative whereas *Staphylococcus xylosus* and *Sphingomonas paucimobilis* grew sonication fluid culture of an intra-operative sample.
In another patient, *Staphylococcus warnerii* was found both in synovial fluid and in sonication fluid culture whereas standard culture of intraoperative sample was negative.

TABLE 2 - *Distribution of microorganism detected by both conventional culture and sonication culture in 33 devices explanted.*

Microorganism detected	*Conventional culture*	*Sonication culture*
Staphylococcus spp	1	2
S. epidermidis	3	7
S. lentus	2	3
S. haemolyticus	1	2
S. warneri	0	2
S. xylosus	1	1
S. hominis	3	3+1[a]
Staphylococcus aureus	2	2
Corynebacterium spp	0	2[a]
Rhizobium radiobacter	1	1
P. aeruginosa	1	1
Sphingomonas paucimobilis	0	1[a]
Stenotrophomonas maltophilia	0	1
Candida albicans	0	1[a]

[a]The marked microorganism was detected as secondary positive pathogen (polymicrobial infection).

Clinical features and bacterial growth

We also investigated if there was any correlation between bacterial growth and different

clinical features such as fistula, fever, clinical signs (swelling, redness, heat, loss of function), inflammatory markers, site of infection (hip or knee joint prosthesis) and timing of infection (early and not early).

We did not find a significant association between microbiological growth and clinical features in the FBI group, whereas we observed that the site of infection could affect the microbiological isolation in the PJI group.

In hip infection using conventional culture a low detection rate was obtained in comparison to sonication culture (20% [95% CI: 2.03% to 64.04%] *vs* 94% [95% CI: 69.69% to >99.99%]; p<0.04). Sonication method increased the rate of microbiological detection in both hip and knee infection, with better evidence for hip prosthetic infection (hip 94% [95% CI: 69.69% to >99.99%] *vs* knee 50% [95% CI: 51.09% to 100%], p=0.02).

Impact of antibiotic treatment on pathogen detection

Regarding interruption of antimicrobial treatment before foreign body explantation we observed that 13 patients (87% [95% CI: 60.86% to 97.52%]) in the FBI group stopped antibiotic therapy at least 15 days before surgery, while only 23 patients (56% [95% CI: 41.03% to 70.12%]) in the PJI group stopped antibiotic therapy at least 15 days before surgery. In the FBI group the sensitivity of cultures was similar regardless of the microbiological diagnostic method used.

Concerning the PJI group, the rate of microbiological isolation by conventional culture was very low in patients who stopped antibiotic therapy for less than 15 days before surgery in comparison to the patients who stopped therapy for a longer period of time (16.6% [95% CI: 5.01% to 40.05%] *vs* 56.5% [95% CI:

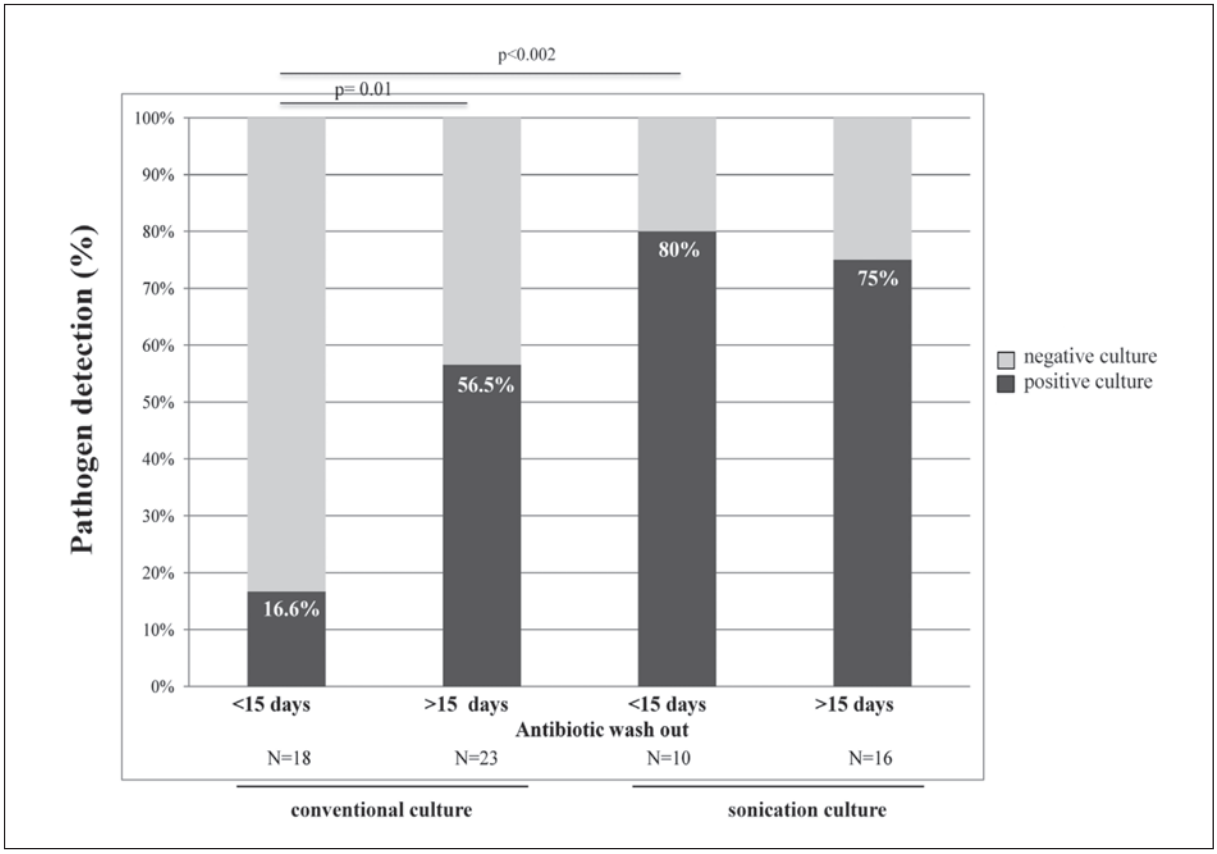

FIGURE 2 - *Rate of pathogen detection in the PJI group according to the timing for stopping antibiotic treatment. Conventional culture showed a very low rate in patients who stopped therapy for less than 15 days before explant, whereas sonication maintains a high rate of detection independently of antibiotic wash-out timing.*

36.79% to 74.39%] respectively, p=0.01). On the other hand, when a sonication technique was performed, the rate of pathogen detection was higher than conventional culture regardless of timing of antibiotic interruption before surgery (80% [95% CI: 47.94% to 95.41%] *vs* 16.6% [95% CI: 5.01% to 40.05%] for antibiotic interruption less than 15 days and 75% [95% CI: 50.03% to 90.29%] *vs* 56.5% [95% CI: 36.79% to 74.39%] for an antibiotic interruption longer than 15 days respectively). The most significant differences were observed in patients who stopped therapy for less than 15 days before surgery (Figure 2).

DISCUSSION

The microbiological diagnosis of IAOIs is crucial to manage optimal antimicrobial treatment. Nowadays the gold standard is the culture of synovial fluid and multiple intraoperative-periprosthetic tissues, but standard cultures still have limited sensitivity. Standard procedures do not include sonication method even though promising results have already been obtained in several clinical investigations (Trampuz *et al.*, 2007).

Identifying the causative agent of IAOIs is difficult for several reasons. First of all, the causative organisms tend to have low virulence with a low replicative rate; moreover, subjects with clinical infection often receive antibiotic therapy before surgery.

Sonication of explanted prosthetic material has proved more sensitive than conventional microbiological culture in the diagnosis of foreign body infection, especially for orthopedic prosthesis and breast implants (Padgett *et al.*, 1995; Atkins *et al.*, 1998; Donlan 2002; Trampuz *et al.*, 2003; Sia *et al.*, 2005; Trampuz *et al.*, 2006 a); Trampuz *et al.*, 2006 b); Berbari *et al.*, 2007; Trampuz *et al.*, 2007; Monsen *et al.*, 2009; Piper *et al.*, 2009; Rieger *et al.*, 2009; Westrich *et al.*, 2010; Holinka *et al.*, 2011). Recently, our colleagues demonstrated that sonication of explanted cardiac implants improves microbial detection in cardiac device infections (Oliva *et al.*, 2013). In summary, sonication represents a reproducible and simple technique to dislodge bacteria from infected devices.

In the present study the sonication method showed the potential reliability and feasibility to improve diagnosis of IAOIs in case of PJI. In particular, sonication was able to increase the microbiological detection rate in hip infections from 20% of conventional culture to 94%, indicating a useful role of sonication culture in this kind of infections. On the other hand, sonication methods do not seem to add benefits in case of internal device infections, even if the population studied was small (only 15 patients). These findings could be explained by the timing of infection which was usually acute or subacute in the FBI group (not enough time for biofilm formation) and by the fact that the 87% of patients were free of antimicrobial pressure (more than 15 days of antibiotic interruption) compared to 56% of the PJI group.

Our study showed a lower sensitivity of conventional culture compared to literature data in which standard culture reaches values of 60.8% (Trampuz *et al.*, 2007).

This could be explained by the small numbers of patients free of antimicrobial treatment for more than 14 days and by the small number of synovial fluid samples obtained. The 11 cases of microbiological growth detected only by sonication methods were almost all (91%) Coagulase-negative Staphylococci. Our study has some limitations, such as the lack of negative controls to assess the specificity of sonication and no molecular study carried out to confirm the pathogen identification.

Several data showed that the previous use of antimicrobial treatment before surgery could impair the rate of microbiological isolation (Stewart *et al.*, 2001; Trampuz *et al.*, 2005; Trampuz *et al.*, 2008; Esposito *et al.*, 2008; Moran *et al.*, 2010; Achermann *et al.*, 2010). However, our study found that in the PJI group the sensitivity of pathogen detection by sonication methods was not affected by the timing of antibiotic interruption before surgery. In particular, sonication also allowed us to obtain the microbiological diagnosis in patients who could not stop therapy 15 days before implant removal. We can assume that sonication could make a difference with the standard culture, in terms of the pathogen detection rate, in patients undergoing biomaterial removal while on antimicrobial therapy.

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