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Improved Diagnosis of Infection Associated with Osteosynthesis by Use of Sonication of Fracture Fixation Implants

Maysa Harumi Yano,[a] Giselle Burlamaqui Klautau,[b] Cely Barreto da Silva,[c] Stanley Nigro,[c] Osmar Avanzi,[d] Marcelo Tomanink Mercadante,[d] Mauro Jose Costa Salles[b]

Infection Control Unit, Santa Isabel Hospital, São Paulo, Brazil[a]; Division of Infectious Diseases, Department of Internal Medicine,[b] Department of Laboratory Medicine and Pathology,[c] and Department of Orthopedic Surgery,[d] Santa Casa de São Paulo School of Medicine, São Paulo, Brazil

Previous studies have shown that sonication fluid cultures from removed orthopedic devices improved the microbiological diagnosis of orthopedic implant-associated infections; however, few of these investigations have applied sonication to the removed fracture fixation devices to evaluate its utility for the diagnosis of osteosynthesis-associated infection (OAI). We compared sonication fluid to conventional tissue cultures from 180 subjects with different sizes of plates and screws ($n = 156$), spinal implants ($n = 26$), and intramedullary nails ($n = 3$), of whom 125 and 55 subjects had OAI and noninfected osteosynthesis (NIO), respectively. The sensitivity for detecting OAI was 90.4% for sonication fluid culture and 56.8% for periprosthetic tissue cultures ($P < 0.05$), and the specificities were 90.9% and 96.4%, respectively. Sonication fluid culture detected more pathogens than peri-implant tissue culture (113 versus 71; $P < 0.001$), while polymicrobial infections were diagnosed by sonication fluid cultures and tissue cultures in 20.8% and 8% ($P < 0.001$), respectively. Microbiological diagnosis was achieved exclusively by sonication fluid cultures for 47 (90.4%) subjects, and among them, 18 (38.3%) had previously received antibiotics, whereas in five (9.6%) infected subjects, tissue culture was positive and the sonication fluid culture was negative. Among 39 (31.2%) OAI cases receiving antibiotics, the identification of the organisms occurred in 38.5% and 82.1% of the tissue and sonication fluid cultures, respectively ($P < 0.049$). We demonstrated that sonication fluid culture from removed osteosyntheses has the potential for improving the microbiological diagnosis of OAI.

Due to the increasing occurrence of trauma and injuries, particularly those associated with road traffic accidents (1), surgical implantation of orthopedic devices for fracture fixations (or osteosynthesis), including intramedullary nails, different size plates, screws, and external fixation pins, have increased. Indeed, the acute management of bone fractures in trauma patients has evolved steadily, and there is an expanded indication for osteosynthesis, with the aim of early stabilization of the fractures. Depending upon the physiological condition of the patient, especially those with unstable polytrauma and significant lower extremity injuries, surgeons must choose the less aggressive external fixation first step for fracture stabilization (2). Once the adequate local wound debridement is ensured, which includes the removal of all dead and infected tissues and the stabilization of the systemic clinical condition of the patient, secondary major osteosynthesis is then performed with either plates and screws or intramedullary nails (3).

Following orthopedic surgery, secondary soft tissue and implant-associated bone infections are still the most important negative aspects and limiting factors of success, leading to significant morbidity, including delayed bone union or nonunion, multiple additional debridement, or even amputation. Wound contamination with microorganisms occurs in up to 65% of open fractures, and depending upon the severity of injury, especially for third-degree open fractures, the infection rate after osteosynthesis is up to 30% (4, 5). Diagnosis is challenging and often requires a combination of clinical, laboratory, histopathology, imaging, and isolation of microorganism approaches from several samples of periprosthetic tissues (6). Wound cultures in open fractures do not seem to play a role in predicting deep infection, as some authors demonstrated that infections following open fractures were not caused by the organism identified at the predebridement stage, whereas open fractures with negative cultures collected at the predebridement phase ended up in osteomyelitis (7–9). Moreover, conventional periprosthetic tissue cultures can be false negative in up to 30% of cases (10).

Aiming to improve the microbiological diagnosis of orthopedic implant-associated infections, Trampuz et al. (11) applied vortexing and low-intensity ultrasound (sonication) on retrieved implants to dislodge bacterial cells from the biofilm, followed by culture of the resultant sonicate fluid; as a result, they identified the etiology of infection in 78.5% of the cultures, compared to 60.8% of periprosthetic tissue cultures (11). Indeed, different clinical experiences using sonication fluid cultures from prosthetic joints removed during surgery have demonstrated good results for the microbiological diagnosis of orthopedic implant-associated infections. In a recently published meta-analysis of sonication fluid cultures from prosthetic components for the diagnosis of prosthetic joint infections, which included 12 studies, the pooled sensitivity and specificity were 0.80 and 0.95, respectively (12). Nevertheless, the clinical utility of sonication as an adjunctive diagnostic tool for osteosynthesis-associated infection (OAI) has

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Address correspondence to Mauro Jose Costa Salles, mcsalles@osite.com.br.
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not been well studied. Since validated guidelines for the proper diagnosis of osteosynthesis-associated infections are lacking, we hypothesized that applying sonication to the removed fracture fixation devices in sterile containers would improve the diagnosis of infection compared to that with conventional tissue cultures. Furthermore, we evaluated the influence of the previous use of antibiotics on the sensitivities of both tissue and sonication fluid cultures.

MATERIALS AND METHODS

Study population. We prospectively included 180 subjects who, for any reason, underwent complete or partial removal of internal fracture fixation devices, including different size plates, screws, spinal implants, and intramedullary nails, between September 2010 and October 2013 at the orthopedic department of Santa Casa de São Paulo School of Medicine (Brazil). Subjects were excluded when fewer than two periprosthetic tissues were cultured, the osteosynthesis did not fit within a specified plastic container, or contamination occurred during implant removal, transportation, or processing in the microbiology laboratory. Subject demographics, location and type of osteosynthesis, comorbidities, previous orthopedic surgeries, number of tissue samples collected per patient, length of time between implantation and retrieved osteosynthesis, and the previous use of antibiotics up to 14 days before the removal of osteosynthesis were recorded. The study protocol was reviewed and approved by the Santa Casa de São Paulo Institutional Review Board.

Diagnosis of osteosynthesis infection. Osteosynthesis infection was defined if at least one of the following criteria was present: open wound exposing fractured bone and/or osteosynthesis devices with gross evidence of purulence; intraoperative tissue with visible purulence, as determined by the surgeon; presence of a draining fistula communicating with the internal implant; and/or acute inflammation in intraoperative osteosynthesis tissue detected by histopathology (5, 6).

Specimen collection and microbiological methods. In the surgical ward, more than one periprosthetic tissue sample was collected and processed for microbiology and histopathology. Tissue was homogenized in 3 ml of brain heart infusion (BHI) broth for 1 min and inoculated onto aerobic sheep blood agar, chocolate agar, and anaerobic blood agar, as well as into thioglycolate broth (BD Diagnostic Systems, Sparks, MD). The time limit for processing samples was 6 h. Sheep blood agar and chocolate agar were incubated aerobically at 35 to 37°C in 5 to 7% CO_2 for 7 days, and anaerobic blood agar was incubated anaerobically at 37°C for 14 days. Additionally, 0.5 ml of tissue homogenate was inoculated in thioglycolate broth and incubated for 14 days, and the turbid thioglycolate broth was subcultured on blood agar plates when cloudy. Colonies of microorganisms growing on plates were identified, and their susceptibilities to antibiotics were tested according to standard microbiological techniques. Low-virulence microorganisms (coagulase-negative staphylococci, *Corynebacterium* spp., *Chryseobacterium* spp., and *Bacillus* spp.) were considered pathogens when the same organism was identified in at least two different tissue samples or when at least one additional (culture-independent) criterion for OAI was also fulfilled.

Osteosynthesis sonication. In the operating room, the explanted internal fracture fixation device was aseptically removed, placed in sterilized solid polyethylene containers, to which 50 to 250 ml of Ringer solution was added (depending upon the osteosynthesis width), and sealed with an air-tight cover. In the microbiology laboratory, the containers with the retrieved implants were vortexed for 30 s using a Vortex-Genie 2 (Scientific Industries, Inc., Bohemia, NY, USA) and then sonicated (in the ultrasound bath BactoSonic; Bandelin GmbH, Berlin, Germany) for 5 min at a frequency of 40 ± 2 kHz and power density of 0.22 ± 0.04 W/cm_2, followed by an additional 30 s of vortexing, according to the technique of Trampuz et al. (11). To concentrate the resulting sonication fluid, centrifugation was performed in 50-ml aliquots at 2,500 rpm for 5 min (13). The supernatant was aspirated, leaving 0.5 ml (100-fold concentration), and aliquots of 0.1 ml of concentrated sonicate fluid were then plated onto aerobic sheep blood, chocolate, and anaerobic sheep blood agar, and they were incubated aerobically at 37°C for 7 days and anaerobically at 37°C for 14 days and inspected daily for bacterial growth. Additionally, 4 ml of the remaining concentrated sonication fluid was also inoculated in 10 ml of thioglycolate broth, plated as described above, and incubated aerobically at 35° to 37°C in 5% CO_2 for 2 days, and anaerobically at 37°C for 14 days. The colonies of isolated microorganisms growing on plates were quantitated (number of CFU/ml of sonication fluid) and identified, and their antimicrobial susceptibilities were tested according to standard microbiological techniques. Due to the addition of a concentrating step to the sonication fluid culture, a cutoff of 50 CFU/plate was considered positive and used for ideal sensitivity and specificity analyses (14). Furthermore, for those subjects undergoing antimicrobial therapy or who had previously received antibiotics for ≥24 h in the 14 days prior to surgery, any growth of organism in the sonication fluid culture was considered positive (15). Explanted osteosynthesis cases due to aseptic loosening were used for negative controls and equally processed as described for the retrieved infected osteosynthesis implants.

Statistical analysis. The characteristics of subjects with infected and noninfected osteosyntheses were summarized as frequencies and percentages or means (range) and standard deviations (SD). Descriptive comparisons between the categorical variables were performed using a chi-square or Fisher's exact test, as appropriate. Continuous variables were compared with Student's *t* test (normally distributed) or a Mann-Whitney U test (nonnormally distributed). The sensitivities, specificities, positive predictive values, and negative predictive values were compared between tissue culture and sonication fluid culture using the McNemar test of paired proportions. Ninety-five percent confidence intervals (CIs) were calculated as exact binominal confidence intervals. Differences were considered significant when the *P* value was <0.05 (two tailed). The data were analyzed using the SPSS statistical software package for Windows, version 19.0 (IBM Corporation, Chicago, IL).

RESULTS

Study population and devices. Two hundred twenty internal fracture fixation devices were consecutively retrieved from 215 subjects. Fifteen subjects were excluded from further analysis because the implants did not fit within the specified plastic container, 11 were excluded due to a submission of fewer than two tissue samples for culture, and nine were excluded due to clear contamination detected during removal, transportation, or laboratory work. One-hundred eighty-five osteosyntheses from 180 subjects were analyzed (five subjects had two different devices explanted from distinct anatomical sites), of which 152 (84.4%) were different size plates and screws retrieved mainly from the tibia and fibula (29.4%), 25 (13.8%) were spinal implants, and only three (1.7%) were intramedullary nails. Osteosynthesis-associated infections (OAI) and noninfected osteosyntheses (NIO) were diagnosed in 125 (69.4%) and 55 (30.6%) subjects, respectively. Demographic parameters, the median age of the implants at the time of resection surgery, the mean number of tissue samples collected for cultures from each patient, and the clinical characteristics of the study population are shown on Table 1.

Microbiology. Table 2 summarizes the microorganisms identified in 180 subjects included in the study. More pathogens were detected with the sonication fluid cultures than with the periimplant tissue cultures (113 versus 71; $P < 0.001$). The most frequent organisms isolated in both tissue and sonication fluid cultures were *Staphylococcus aureus*, followed by Gram-negative bacilli and coagulase-negative staphylococci (CoNS). *S. aureus* was more likely to be isolated in tissue culture than in sonication fluid culture (41.8 versus 28%; $P = 0.03$). On the other hand, CoNS was identified in 17.7% and 22.7% of the tissue and sonication fluid cultures, respectively ($P = 0.38$). Gram-negative

TABLE 1 Characteristics of 180 subjects with OAI and NIO[a]

Demographics[b]	Subjects with OAI ($n = 125$)	Subjects with NIO ($n = 55$)	P value[c]
Male sex (no. [%])	78 (62.4)	27 (49.0)	0.09
Age (median [range]) (yr)	41 (21–60)	37 (12–62)	0.002
Age of implants (median [range]) (mo)	20.9 (0–53.0)	30.21 (1.4–60.18)	0.006
Clinical characteristics (no. [%])			
Diabetes mellitus	17 (9)	1 (1)	0.012
Coronary diseases	5 (3)	3 (2)	0.708
Liver cirrhosis	0 (0)	1 (1)	0.315
Solid malignancy	3 (2)	3 (2)	0.381
Corticosteroids	4 (2)	0 (0)	0.309
Rheumatoid arthritis	4 (2)	0 (0)	0.309
Alcohol abuse	11 (6)	0 (0)	0.018
Smoking	30 (17)	3 (2)	0.02
No. (%) of revisions (>1)	22 (12)	3 (2)	0.023
No. (median range) of peri-implant tissue samples taken	2.9 (2–12)	2.64 (2–8)	0.54
Prior use of antimicrobials (no. [%])[d]	39 (31.2)	1 (1.81)	<0.001

[a] OAI, osteosynthesis-associated infection; NIO, noninfected osteosynthesis.
[b] All percentages are in relation to the number of subjects with OAI or NIO, unless otherwise indicated.
[c] The patient characteristics were summarized as frequencies and percentages or median values and compared using the Pearson chi-square test or Fisher's exact test, as appropriate, with nominal variables and the Mann-Whitney test or *t* test, as appropriate, with continuous variables (SPSS version 19.0). All tests were two sided, and P values of <0.05 were considered statistically significant.
[d] Patient who received a minimum of 1 day of antibiotic therapy within 14 days prior to the removal of the implants.

TABLE 2 Distribution of microorganisms detected by sonication fluid culture and tissue culture[a]

Microorganism(s)	Sonication fluid culture ($n = 150$)		Tissue culture ($n = 79$)		P value[b]
	No.	%	No.	%	
Staphylococcus aureus	42	28	33	41.8	0.034
CoNS[c]	34	22.7	14	17.7	0.38
Pseudomonas aeruginosa	15	10	11	13.9	0.28
Enterobacter sp.	12	8	3	3.8	0.19
Klebsiella pneumoniae	8	5.3	5	6.3	0.09
Serratia marcescens	7	4.7	5	6.3	0.59
Enterococcus sp.	5	3.3	2	2.5	0.73
Streptococcus sp.	4	2.7	0	0	0.14
Corynebacterium sp.	4	2.7	1	1.3	0.49
Bacillus sp.	4	2.7	3	3.8	0.21
Escherichia coli	3	2	0	0	0.21
Acinetobacter baumannii	3	2	1	1.3	0.21
Chryseobacterium indologenes	2	1.3	0	0	0.3
Proteuss sp.	2	1.3	0	0	0.3
Candida sp.	2	1.3	0	0	0.3
Citrobacter sp.	1	0.7	0	0	0.46
Providencia sp.	1	0.7	1	1.3	0.64
Burkholderia cepacia	1	0.7	0	0	0.46
Stenotrophomonas maltophilia	1	0.7	0	0	0.46
Gram-positive species	85	65	49	62	0.43
Gram-negative species	52	34	26	32	0.78
Polymicrobial flora	27	15	10	5.5	0.001

[a] Description of agents identified among 180 subjects in the study.
[b] The microorganisms were described as frequencies and percentages and were compared using the Pearson chi-square test or Fisher's exact test, as appropriate. All tests were two sided, and P values of <0.05 were considered statistically significant.
[c] CoNS, coagulase-negative staphylococci.

bacilli were almost equally detected in tissue and sonication fluid culture (34% and 32%, respectively; $P = 0.78$). Polymicrobial infections were diagnosed by sonication fluid and tissue cultures in 20.8% (26/125) and 8% (10/125), respectively ($P < 0.001$), and all subjects presenting with polymicrobial infections had OAI.

Comparison of microbiological tests and discordant results between sonication fluid and tissue cultures. One hundred eighteen subjects (65.5%) had positive sonication fluid cultures (113 OAI and 5 NIO), and 73 (40.5%) had positive tissue cultures (71 OAI and 2 NIO) ($P < 0.001$). The sensitivity for the microbiological diagnosis in 125 subjects with OAI in the sonication fluid culture was higher than that with peri-implant tissue culture (90.4% versus 56.8%, respectively; $P < 0.05$). The specificities and positive predictive values of sonication fluid and peri-implant tissue cultures showed no statistically significant difference, at 90.9%, and 96.4%, respectively, and 95.8%, and 97.3%, respectively. However, the negative predictive value was higher for sonication fluid culture than that for tissue culture (80.6% versus 49.5%, respectively; $P < 0.05$) (Table 3). The global concordance (positive and negative results) between the tissue and sonication fluid cultures was 65.5% (118/180). Among the subjects with OAI, the tissue and sonication fluid cultures were concordant in 58.7% (73/125). There were 52 discordant results between the sonication fluid and tissue cultures. Table 4 summarizes the discordant microbiological results between the sonication fluid and tissue cultures. Microbiological diagnosis was achieved exclusively by sonication fluid cultures for 47 subjects with OAI (10 subjects with polymicrobial infections), of which only 18 (38.3%) had previously received antibiotics. Twenty-nine out of 47 subjects (61.7%) with OAI detected only by sonication fluid cultures had no previous use of antibiotics. Among them, a polymicrobial infection was identified in six (20.7%) subjects, whereas for 23 subjects, sonication fluid culture identified one pathogen. In six subjects with OAI, the tissue culture was positive and the sonication fluid cul-

TABLE 3 Comparison between sonication fluid culture and periprosthetic tissue culture[a]

Culture type	Sensitivity[b]		Specificity[c]		Positive predictive value		Negative predictive value	
	% (no. detected/ total no.)	95% CI (%)	% (no. detected/ total no.)	95% CI (%)	% (no. detected/ total no.)	95% CI (%)	% (no. detected/ total no.)	95% CI (%)
Sonication fluid	90.4 (113/125)	83.9–94.4	90.9 (50/55)	80.4–96	95.8 (113/118)	90.6–98.2	80.6 (50/62)	68.2–87.7
Tissue	56.8 (71/125)	47.6–65.3	96.4 (53/55)	87.7–99.6	97.3 (71/73)	90.7–99.2	49.7 (53/107)	41.7–60.1

[a] Comparison is among all subjects of study ($n = 180$).
[b] The sensitivities of different culture methods were compared by McNemar's test of paired proportions ($P < 0.001$).
[c] The specificity of different culture methods were compared by McNemar's test of paired proportions ($P = 0.45$).

ture was negative. Among them, only one patient previously received antibiotics. A polymicrobial infection was detected solely by tissue culture in one case. There were seven discordant results between the sonication fluid and tissue cultures among subjects with NIO. Five subjects had sonication fluid culture-positive and tissue culture-negative results. Among 20 subjects with OAI, there were microbiological discrepancies when both the sonication fluid and tissue cultures were positive (see Table S4.1 in the supplemental material). Regarding antimicrobial resistance, we identified methicillin-resistant *S. aureus* (MRSA) in 34 of 75 (45.3%) of the isolates; among them, there were 13 strains cultured from sonication fluid, showing an MIC of ≥1.5 μg/liter for vancomycin, as obtained by Etest. Six subjects with OAI due to MRSA presenting with an MIC of 2.0 μg/liter for vancomycin received a long course of glycopeptide therapy without removal of the infected implant. The antimicrobial resistance rates for *Pseudomonas* spp., *Enterobacter* spp., and *Klebsiella* spp. isolated from sonication fluid culture were higher than those of the tissue culture isolates, of which the rates of carbapenem-resistant *Pseudomonas* spp. and *Klebsiella* spp. isolated from sonication fluid and tissue cultures were 35.7% and 0.9%, and 42.9% and 20%, respectively. *Enterobacter* spp. showing 45% resistance to third-generation cephalosporins was identified only in cultures from sonication fluid.

Previous antimicrobial therapy. Thirty-nine out of 125 (31.2%) subjects with OAI received a minimum of 1 day of antibiotic therapy within 14 days prior to the surgical debridement and removal of the implants, and among them, identification of organisms occurred in 38.5% (15/39) and 82.1% (32/39) of the tissue and sonication fluid cultures, respectively ($P < 0.001$). In 86 subjects presenting with OAI and no previous use of antibiotics (68.8%), tissue and sonication fluid cultures were positive in 65.1% (56/86) and 94.2% (81/86) ($P < 0.001$), respectively. Both tissue and sonication fluid cultures showed higher rates of sensitivity for subjects with no previous use of antibiotic than subjects for whom antibiotics were prescribed within 14 days prior to surgery (Fig. 1).

DISCUSSION

Surgical implantation of plates, screws, intramedullary nails, and many other orthopedic devices for fracture fixations has increased, and infections associated with osteosynthesis have become more common (4, 5). The identification of the causative microorganisms solely by tissue cultures has been unhelpful in a large proportion of cases, particularly in those with previous use of antibiotics (10). For this reason, several researchers have applied a sonication (low-intensity ultrasound) technique to displace microorganisms from the surface of removed prosthetic devices, and they eventually were able to demonstrate an increase in the rate of etiological diagnosis compared to that with periprosthetic tissue cultures (11, 13–28). Since skin-contaminating bacteria produce many osteosynthesis infections, comparing the sonication results of prosthetic joint infection to those of fracture fixation infections is not an easy task. Few of these previous studies evaluated the role of sonication in the microbiological diagnosis of osteosynthesis infections (17, 20, 22, 26, 27). Holinka et al. (20) sonicated only six osteosynthesis devices and therefore could not draw any conclusions regarding the advantage of sonication compared to conventional techniques (20). Esteban et al. (22) used sonication and molecular techniques on 73 osteosynthesis infections (41 intramedullary nails and 32 screws, dynamic screws, and plates), but no improvements in microbiological diagnosis were made (22). Also, they had to deal with contamination, possibly associated with sonication occurring in plastic bags. Borens et al. (26) recently combined sonication and microcalorimetry to speed up the diagnosis of orthopedic device-related infection, but only six screws, three plates, and one cement nail were included in the analysis. In another study in which 31 intramedullary nails were sonicated in plastic bags nine polymicrobial infections were detected out of 15 culture-positive results. Again, in at least four of these positive cases, it is possible that contamination with bacteria typically associated with water was detected due to sonication occurring in plastic bags (27).

In our study, 180 subjects (125 fitting the criteria for OAI) were consecutively included, mainly those with different size plates and screws retrieved from the tibia and fibula; to avoid contamination, sonication was performed exclusively in sterilized solid polyethylene containers. We demonstrated that sonication fluid culture from a removed osteosynthesis has the potential for improving the microbiological diagnosis of OAI. The sensitivity and negative predictive value of sonication fluid culture were significantly higher than those with peri-implant tissue culture (90.4% versus 56.8%, and 80.6% versus 49.5%, respectively). Our results emphasize the concept that OAI is typically a biofilm-associated infection, as bacteria preferentially adhere to rough and porous biomaterials, such as long stainless steel plaques, and to the irregularities that conform to the shapes of screws, increasing the bacterium-surface contact area and leading to the formation of a large amount of biofilms (29). Another possible explanation for the increased detection of pathogens using sonication was that for each patient, the explanted components were placed all together (plates and screws) into the container, thus detaching large amounts of bacteria from the biofilm. In fact, we were able to identify 113 and 71 pathogens through sonication fluid and tissue cultures, respectively. Conversely, the low sensitivity of tissue cultures, collecting up to 12 samples per patient (median of 2.9 samples), might be partially explained by the fact that in more than one-third of the infected osteosyntheses, patients had received antibiotics within 14 days prior to the surgery.

TABLE 4 OAI subjects with discordant results between sonication fluid culture and tissue culture[a]

Case	OAI	Tissue culture result	Polymicrobial	Sonication fluid culture result	Polymicrobial	Antibiotic prior to surgery[b]
SS28	Yes	Negative		*S. aureus*	No	No
SS31	Yes	Negative		CoNS	No	No
SS32	Yes	Negative		*S. aureus*	No	Yes
SS36	Yes	Negative		CoNS and *Streptococcus* sp.	Yes	Yes
SS40	Yes	Negative		*Enterococcus* sp. and CoNS	Yes	Yes
SS42	Yes	CoNS and *Enterococcus* sp.[c]	Yes	Negative		No
SS48	Yes	Negative		CoNS	No	Yes
SS49	Yes	Negative		*S. aureus* and *Bacillus* sp.	Yes	Yes
SS50	Yes	Negative		CoNS	No	No
SS59	Yes	Negative		CoNS	No	No
SS60	Yes	Negative		*S. aureus*	No	No
SS65	Yes	Negative		*P. aeruginosa*	No	Yes
SS69	Yes	Negative		*S. aureus*	No	No
SS83	Yes	Negative		*Enterococcus* sp.	No	No
SS93	Yes	Negative		CoNS	No	Yes
SS113	Yes	Negative		CoNS	No	No
SS118	Yes	Negative		*Corynebacterium* sp.	No	No
SS123	Yes	Negative		*S. aureus*	No	No
SS128	Yes	Negative		CoNS and *Streptococcus* sp.	Yes	No
SS141	Yes	Negative		CoNS	Yes	No
SS144	Yes	Negative		*Proteuss* sp.	No	Yes
SS151	Yes	Negative		*S. aureus*	No	No
SS157	Yes	Negative		*Corynebacterium* sp.	No	Yes
SS183	Yes	Negative		*Corynebacterium* sp.	No	Yes
SS191	Yes	*S. aureus* and *A. baumannii*	Yes	Negative		Yes
SS198	Yes	Negative		CoNS	No	No
SS206	Yes	Negative		*Enterobacter* sp.	No	Yes
SS208	Yes	Negative		CoNS	No	No
SS217	Yes	Negative		CoNS	No	No
SS219	Yes	Negative		CoNS	No	No
SS224	Yes	Negative		CoNS	No	Yes
SS228	Yes	Negative		*P. aeruginosa* and *Citrobacter* sp.	Yes	Yes
SS233A	Yes	Negative		*P. aeruginosa* and *Serratia* sp.	Yes	No
SS233B	Yes	Negative		*Serratia* sp.		No
SS237	Yes	Negative		*A. baumannii*	No	No
SS242	Yes	Negative		*Enterobacter* sp.	No	Yes
SS253	Yes	Negative		CoNS	No	Yes
SS254	Yes	Negative		*Enterobacter* sp.	No	No
SS255	Yes	Negative		*S. aureus* and *C. indologenes*	Yes	Yes
SS257	Yes	Negative		*K. pneumoniae*	No	Yes
SS260	Yes	Negative		*S. aureus* and CoNS	Yes	No
SS288	Yes	*S. aureus*	No	Negative		No
SS293	Yes	Negative		*K. pneumoniae*	No	No
SS294	Yes	Negative		*K. pneumoniae*	No	Yes
SS300	Yes	Negative		*S. maltophilia*	No	No
SS301	Yes	CoNS	No	Negative		No
SS313	Yes	Negative		*K. pneumoniae* and *P. aeruginosa*	Yes	No
SS317	Yes	Negative		*A. baumannii*	No	No
SS325	Yes	Negative		*E. coli*	No	No
SS348	Yes	*P. aeruginosa*	No	Negative		No
SS352	Yes	Negative		CoNS	No	No
SS360	Yes	Negative		*S. aureus* and CoNS	Yes	No

[a] OAI, osteosynthesis-associated infection.
[b] Patient received a minimum of 1 day of antibiotic therapy within 14 days prior to the removal of the implants.
[c] CoNS, coagulase-negative staphylococci.

Classically, OAI affecting trauma patients is associated with skin microorganisms, mainly Gram-positive cocci, such as *S. aureus*; however, multiple pathogens are usually recovered from the infected bone, including Gram-negative bacilli and, less frequently, anaerobic bacteria (6–9). Bacteriological studies focusing on internal fracture fixation devices have been a critical step for adequate treatment of OAI, because even if staphylococcal (*S. aureus* and CoNS) bone infections are still the number one cause of OAI,

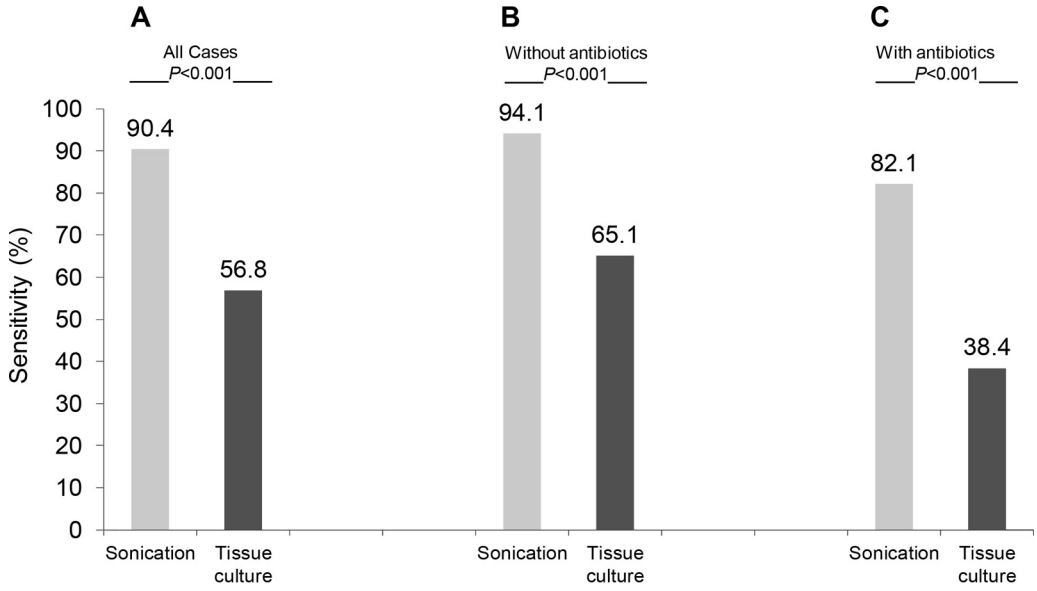

FIG 1 Sensitivities of sonication fluid culture and tissue culture. (A) Overall sensitivity of sonication fluid culture (white bars) and periprosthetic tissue culture (black bars) from 125 subjects with ($n = 39$) and without ($n = 86$) previous antimicrobial therapy. Also shown are the sensitivities of sonication fluid and tissue culture among subjects without (B) and with (C) previous use of antibiotics. The differences in sensitivities were compared by McNemar's test of paired proportions.

the growing frequency of MRSA and the increase in Gram-negative bacterial infections have been highlighted in some case series (7–9). In our results, as expected, *S. aureus* and CoNS were the commonest planktonic and sessile pathogens retrieved from tissues and implants, respectively. Interestingly, MRSA represented 45.3% of all *S. aureus* isolates, and among them, more than one-third of the sessile strains presented MICs of >1.5 μg/liter for vancomycin. Not surprisingly, many of these patients were on a prolonged course of intravenous vancomycin without removal of the infected implant. Gram-negative bacilli (GNB) were frequently isolated in both tissue and sonication cultures, but strains expressing resistances to different classes of antibiotics seemed to be much more common when GNB were embedded into the biofilms, as carbapenem-resistant *Pseudomonas* and *Klebsiella* spp. were frequently isolated from sonication fluid. Currently, due to the widespread use of antibiotics in the general population, the epidemiology of bacterial contamination of bone fractures has changed (30). Chen et al. (9) reported higher rates of MRSA and Gram-negative infections among trauma patients with open fractures, suggesting that current antibiotic regimens for open fractures need to be revised. In fact, recently published guidelines emphasize the importance of additional Gram-negative coverage for type III open fractures (31).

In the present study, 47 out of 125 (37.6%) subjects with OAI had positive microbial identification in sonication fluid but not on tissue cultures, in which sonication identified a single pathogen in 14 out of 47 (29.7%) subjects, including skin-contaminating bacteria, such as CoNS, and *Corynebacterium* species. Such a discordant result between the tests may be due to larger extension of biofilms attached to osteosyntheses and therefore releasing a higher number of bacteria after sonication. Nevertheless, the classification of some results as either infection or contamination from surgery or laboratory processes remains under debate. Although sonication techniques have provided new microbiological information for the management of orthopedic implant-associated infections, a formal consensus regarding its application to OAI is lacking (12, 13).

Recently published studies observed that antibiotic intake prior to prosthetic removal negatively affected the microbial detection of tissue and sonication cultures (11, 13, 15), while in other series, the use of antibiotics had no influence (14, 20, 24). In our results, both tissue and sonication fluid cultures showed higher rates of sensitivity in those for whom no antibiotic was prescribed than for those on antimicrobial therapy (65.1% and 38.5%, and 94.2% and 82.1%, respectively). However, the diagnostic accuracy of tissue culture seemed to have been more affected by the common practice of prescribing empirical antibiotics for OAI. As already speculated by Trampuz et al. (11), we also argue that free-living planktonic bacteria in peri-implant tissues may be much more affected by antibiotics than sessile microorganisms living in biofilms, which have the intrinsic ability to resist to commonly used antimicrobial agents.

Our study has some limitations, including the important lack of a universally accepted definition of OAI. In fact, the diagnosis of implant-associated bone and soft tissue infections in trauma patients is not easy to perform, as clinical examination may not be helpful, inflammatory blood markers are usually unhelpful, and imaging tests are nonspecific (6, 7). Therefore, we applied to our data a definition from previous studies that analyzed the role of sonication in orthopedic implant-associated infections, including in osteosyntheses (14, 15, 20, 22, 26). Also, some technical aspects for sonication still need to be standardized, including the number of microorganisms required to be defined as an infection, the total volume of Ringer's solution for containers, especially for small implants, such as screws and small plates, and concentration steps (15).

In summary, our results demonstrate that sonication fluid culture is superior to conventional tissue culture for osteosynthesis-associated infection diagnosis, especially in patients receiving antibiotics prior to surgery. Furthermore, MRSA and multidrug-resistant Gram-negative bacilli were recovered significantly more

often from sonication fluid cultures, which would prompt clinicians to choose this as the most appropriate antimicrobial therapy. Further studies using sonication for OAI diagnosis should be encouraged in order to confirm our results.

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