Concentration of Sonication Fluid through Centrifugation Is Superior to Membrane Filtration for Microbial Diagnosis of Orthopedic Implant-Associated Infection

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Microbial identification of orthopedic implant-associated infections using sonication fluid (SF) submitted to a concentration step by membrane filtration (SMF) was compared with the standard centrifugation (SC) method. Among 33 retrieved infected implants, sonication identified microorganisms in 26 (78.8%). The sensitivity of SC was higher than that of SMF (78.8% versus 30.3%; \( P < 0.001 \)).

Several authors have applied a sonication technique as an adjunctive diagnostic tool to increase the identification of causative agents of different orthopedic implant-associated infections (OIAIs) (1–3). However, sonication may also give false-negative results in at least 10% to 20% of cases when applying only phenotypic methods (4). We hypothesized that submitting the sonication fluid (SF) to a validated method of sample concentration, such as membrane filtration, in which the total volume of SF is submitted to filtration through a membrane filter with a pore size smaller than the bacterial size, would improve the microbial diagnosis of orthopedic implant-associated infection.

We prospectively included 35 patients who underwent surgical removal of infected prosthetic joints (knee and hip) and internal fracture-fixation devices, including different sizes of plates and screws, between March 2013 and July 2014 at the orthopedic department of the Santa Casa de São Paulo School of Medical Sciences (Brazil). Subjects were excluded when clear contamination occurred during implant removal, transportation, or processing in the microbiology laboratory or if membrane obstruction was observed. Subject demographics, type of orthopedic implants, and indication for surgical removal were recorded. The study protocol was reviewed and approved by the Institutional Review Board. OIAI was diagnosed if at least one of the following criteria was present: open wound exposing fractured bone and/or osteosynthesis and prosthetic joint devices with gross evidence of purulence, intraoperative tissue with visible purulence, presence of a draining fistula communicating with the internal implant, and/or acute inflammation in intraoperative osteosynthesis/prosthetic joint tissue detected by histopathology (3).

In the operating room, surgically removed orthopedic implants were placed in sterile polyethylene containers to which 650 ml of Ringer solution was added and then sealed with an airtight cover for transportation. The time limit for processing samples was 6 h. In the microbiology laboratory, containers with the retrieved implants were vortexed for 30 s at maximum power using the Vortex Genie 2 (Scientific Industries, Inc., Bohemia, NY, USA) and then sonicated (BactoSonic ultrasound bath; Bandelin GmbH, Berlin, Germany) for 5 min at a frequency of 40 ± 2 kHz and power density of 0.22 ± 0.04 W/cm², followed by an additional 30 s of vortexing.

In order to concentrate the SF, centrifugation was performed with 50-ml aliquots at 2,500 × g for 5 min. The supernatant was aspirated, leaving 0.5 ml (100-fold concentration). Aliquots of 0.1 ml of concentrated SF were then plated onto aerobic blood sheep agar and chocolate agar and incubated aerobically at 35°C ± 2°C for 7 days and at 5% CO₂ for 14 days, respectively, and inspected daily for bacterial growth. Additionally, 4 ml of the remaining concentrated SF was inoculated in 10 ml of thioglycolate broth (BD Diagnostic Systems, Sparks, MD), plated as described above, and if turbid, incubated aerobically and anaerobically for 7 and 14 days, respectively. The remaining 600 ml of SF was separated into four equal parts of 150 ml and individually submitted to four filtrations through a 0.30- to 0.45-µm-pore-size, 25-mm-diameter, 150-µm-thick cellulose sterile membrane filter (MF-Millipore). These four membranes were then placed directly onto blood agar, chocolate agar, and aerosol plates but also into thioglycolate broth. As described above, aerobic and anaerobic plates were incubated aerobically and anaerobically for 7 and 14 days, respectively. Additionally, thioglycolate broth was incubated for 14 days, and the turbid thioglycolate broth was subcultured on blood agar plates when cloudy. Colonies of isolated microorganisms growing on plates were quantified (number of CFU/ml SF) and identified, and their susceptibility to antibiotics was tested according to standard microbiological techniques. Due to the addition of centrifugation and membrane filtration concentration steps to the SF culture, a cutoff of 50 CFU/plate was considered positive and used for ideal sensitivity analysis (3, 6). Likewise, patients who received antibiotics for at least 24 h in the 14 days prior to surgery were considered positive regardless of their CFU.


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values (1, 3). Characteristics of the subjects were summarized as frequencies and percentages or means (range) and standard deviations. Comparison of categorical data was performed using the chi-squared or Fisher’s exact test. Continuous data were analyzed using the t test. Differences were considered significant when the P value was <0.05 (two tailed). Data were analyzed using the SPSS statistical software package for Windows, version 19.0 (IBM Corporation, Chicago, IL).

Thirty-five infected implants from 35 subjects were consecutively retrieved and submitted to sonication; 2 subjects were excluded after culture plate contamination and membrane obstruction (0.30 μm porosity). In total, 33 implants from 33 patients with a diagnosis of OIAI were analyzed, 25 (75.8%) osteotomies and 8 (24.2%) arthroplasties (5 hip and 3 knee). No statistically significant differences were observed regarding demographic parameters, implant types, or surgical indications for implants in the study population (Table 1). Overall, SF submitted to concentration steps by centrifugation (SC) and membrane filtration (SMF) identified pathogens in 78.8% (26/33) and 30.3% (10/33) of cases, respectively (P < 0.001). Solid media cultures of SC yielded bacteria in 30.3% (10/33) of patients, whereas SC inoculated in thioglycolate broth yielded bacteria in 78.8% (26/33) of patients (P < 0.001). Additionally, SMF plated on solid media cultures yielded bacteria in 24.2% (8/33) of patients, whereas SMF inoculated in thioglycolate yielded bacteria in 30.3% (10/33) of patients (P = 0.58).

Table 2 summarizes the microorganisms identified by the SC and SMF. A higher number of microorganisms were identified by SC than by SMF of explanted devices (33 versus 11 pathogens; P < 0.001). Gram-positive bacteria were the most common and detected almost equally by SC and SMF (60.6% and 72.7%, respectively; P = 0.46). Although not reaching statistical significance, a larger proportion of Staphylococcus aureus isolates were identified with SMF (45.4%) than with SC (21.2%), and a larger proportion of coagulase-negative staphylococcus isolates were identified with SC (24.2%) than with SMF (9.1%).

To our knowledge, this is the first study applying the membrane filtration technique on SF resulting from infected orthopedic implants. The membrane filtration technique has been used successfully in the microbiology laboratory as a pressure- or vacuum-driven separation process that allows the passage of water but removes contaminants, primarily through a size exclusion mechanism using membranes of different pore sizes (5). Depending on the size of the retrieved implant and the container used, the total volume of Ringer’s solution used to fill the container to partially cover the device may vary from 50 ml (sonication of screws and small plates) to 500 ml (large prosthetic joints). Therefore, many researchers have added an SF concentration step using sample centrifugation (3, 6–9). Applying filtration to the total volume of SF through known-pore-size membranes and plating these membranes onto solid and in liquid culture media would increase the number of microorganisms identified. Nevertheless, compared to SMF, SC showed statistically significant greater efficiency in concentrating and detecting bacteria, with SC and SMF sensitivities of 78.8% and 30.3%, respectively. We argue that some

### Table 1: Characteristics of 33 patients who underwent elective orthopedic implant removal due to infection

| Characteristic          | Total patients (no. [%]) (n = 33) | No. (%) positive with SC (n = 26) | No. (%) positive with SMF (n = 10) | P value*
|-------------------------|-----------------------------------|----------------------------------|----------------------------------|-----------
| **Demographic**         |                                   |                                  |                                  |           
| Male                    | 17 (51.5)                         | 17 (65.3)                        | 9 (90)                           | 0.13      
| Age (yr, mean [range])  | 42.7 (8–88)                      | 41.7                             |                                  |           
| **Implant type**        |                                   |                                  |                                  |           
| Osteosynthesis          | 25 (75.8)                        | 20 (76.9)                       | 8 (80)                           | 0.84      
| Hip arthroplasty        | 5 (15.2)                          | 3 (11.5)                         | 1 (10)                           | 0.89      
| Knee arthroplasty       | 3 (9.0)                           | 3 (11.5)                         | 1 (10)                           | 0.89      
| **Implant indication**  |                                   |                                  |                                  |           
| Fracture                | 20 (60.6)                        | 15 (57.7)                        | 7 (70)                           | 0.49      
| Arthrosis               | 5 (15.1)                          | 3 (11.5)                         | 0 (0)                            | 0.26      
| Neoplastic lesion       | 3 (9.1)                           | 3 (11.5)                         | 2 (20)                           | 0.51      
| Osteomyelitis           | 3 (9.1)                           | 2 (7.6)                          | 1 (10)                           | 0.82      
| Osteonecrosis           | 2 (6.1)                           | 2 (7.6)                          | 0 (0)                            | 0.36      

*SC, sonication and centrifugation.

### Table 2: Pathogen isolated in SC and SMF of retrieved infected implants.

| Microorganism                  | SC (n = 33) | SMF (n = 11) | P value*
|-------------------------------|------------|-------------|-----------
| CoNS                          | 8          | 1           | 9.1       | 0.28      
| Staphylococcus aureus         | 7          | 2           | 5         | 45.4      | 0.11      
| Acinetobacter baumannii       | 4          | 12.1        | 2         | 18.2      | 0.61      
| Pseudomonas aeruginosa        | 3          | 9           | 0         | 0         | 0.3       
| Enterococcus sp.              | 3          | 9           | 1         | 9.1       | 1         
| Escherichia coli              | 2          | 6           | 0         | 0         | 0.4       
| S. maltophilia                | 2          | 6           | 0         | 0         | 0.4       
| Streptococcus viridans        | 2          | 6           | 1         | 9.1       | 0.72      
| Enterobacter sp.              | 1          | 3           | 1         | 9.1       | 0.4       
| Bacillus sp.                  | 1          | 3           | 0         | 0         | 0.55      
| Gram-positive bacteria        | 20         | 60.6        | 8         | 72.7      | 0.46      
| Gram-negative bacteria        | 13         | 39.4        | 3         | 27.3      | 0.46      

*a Microorganism descriptions using frequencies, percentages, and chi-squared test. SC, sonication and centrifugation; SMF, sonication and membrane filtration.

*b P values of <0.05 were considered statistically significant.

*c CoNS: coagulase-negative staphylococci.
bacteria may have passed through a 0.45-μm-pore-size membrane (5). Regarding the performance of the culture media applied, bacterial growth was significantly higher on thioglycolate than on agar medium from SC. Previous studies have shown that liquid cultures seem to provide better conditions to sessile bacterial growth than solid media (9, 10). As already shown in previous studies addressing OIAI and prosthetic joint infection, Gram-positive bacteria were the most common sessile pathogens isolated in SC (60.6%) and SMF (72.7%), followed by Gram-negative bacilli, which have been increasingly identified among subjects presenting OIAIs (3, 11). Our study had limitations, including the small sample size analyzed and the lack of a universally accepted clinical diagnosis of OIAI. We concluded that as a concentration step, SC is superior to SMF, identifying a higher number of microorganisms. Thioglycolate broth provided a better environment than solid media to sessile bacterial growth.

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