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Evaluation of Quantitative Analysis of Cultures from Sonicated Retrieved Orthopedic Implants in Diagnosis of Orthopedic Infection▽

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To improve the microbiological diagnosis of device-related osteoarticular infections, we have developed a protocol based on the sonication of device samples, followed by concentration and inoculation of the sonicate in a broad variety of media in a quantitative manner. Sixty-six samples from 31 patients were included in the study (17 of them with clinical diagnosis of infection). The sonication procedure had a sensitivity of 94.1%, which is better than that of conventional cultures (88.2%). One case of contamination and six cases of unexpected positive cultures were detected (specificity of 42.8%): two of these were considered to represent true infection, while the other four were considered to be nonsignificant (corrected specificity of 50%), although the clinical importance of these isolates is questionable. When we analyzed the number of CFU, no breakpoint between significant and nonsignificant isolates could be established. Based on our results, the procedure of sonication of retrieved implants is better than conventional cultures for the diagnosis of device-related infections. The significance of some isolates in patients without clinical infection remains uncertain. However, they may become pathogens and cannot be routinely considered to be contamination.

Although prosthetic joint implantation has become an important medical procedure that improves quality of life for many patients, the majority of failures that lead to severe consequences remain unsolved. A significant proportion of these failures may be infectious, with the secondary risk of bone infection. These infections have severe consequences not only for the patient but also for society because of long hospital stays, long and expensive treatments, and multiple surgeries (4).

Septic implant analysis is especially interesting due to the fact that infected materials act as bacterium reservoirs, impairing implant function and propagating infection into the bone. Moreover, both the diagnosis and treatment of prosthetic osteoarticular infections are further complicated by the development of a bacterial biofilm, where the bacteria have changed their phenotypes to an extremely resistant sessile form of life (3, 5, 7). The surge of multiresistant microorganisms that easily adhere to inert surgical materials stresses the value of adequate diagnosis leading to proper therapy for these patients. Although periprosthetic tissue culture remains the standard microbiological diagnostic method, organisms adhered to the prosthesis are occasionally impossible to detect by common bacterial cultures. Different approaches to obtain data from the potentially infected prosthetic material (11, 14, 18, 21, 25) include sampling from the surface of the implant (direct swab), surrounding fluids, and the implant after sonication. False-negative and false-positive results are found with these methods, compared with the clinical diagnosis of infection. False-negative cultures frequently occur due to empirical antibiotic treatments, low numbers of circulating bacteria in the implant-surrounding tissues, or biofilm formation. On the other hand, false-positive cultures frequently come from contamination, as orthopedic infections are often caused by the same bacteria responsible for the contamination of cultures (4, 18), such as coagulase-negative *Staphylococcus* (CNS) or *Propionibacterium* spp. Highly sensitive techniques can lead to unacceptably high numbers of false-positive determinations. Another potential source of error is the contamination of the removed implant during transportation to the diagnostic unit, especially because of leakages in plastic transport bags (19). Besides, the basic media commonly used for bacterial isolation do not adequately allow the isolation of uncommon organisms such as mycobacteria or fungi, while the determination of bacterial DNA in the synovial fluid around the implant by means of PCR may increase the risk of false-positive determinations (15).

The ideal diagnostic approach would require high sensitivity and specificity to confirm orthopedic implant infection. We have designed a prospective study to evaluate the diagnostic value of quantitative cultures performed after orthopedic implant sonication, associated with the inoculation of a broad range of media, to discriminate between contamination and true infection according to the number of CFU detected in the cultures.

MATERIALS AND METHODS

Patients and samples. Patients with clinical diagnoses of osteoarticular infection directly related to an orthopedic device were included in the study from July 2006 to April 2007. Patients were selected among those attending two university

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hospitals in Madrid (Spain): Hospital Fundación Jiménez Díaz and Hospital La Princesa. A protocol was designed to evaluate clinical data for these patients, including demographics, underlying diseases, types of devices, and previous antibiotic treatments. Patients without clinical diagnosis of infection were also included as controls.

Clinical diagnosis of prosthetic infection was based on the standard preoperative and intraoperative signs associated with orthopedic implant infection: fistula, purulent discharge from the wound, intraoperative identification of periprosthetic purulence and/or a sinus tract communicating with the prosthesis, laboratory findings (maintained elevation of C-reactive protein and erythrocyte sedimentation rate), radiological signs, and/or gamma scan findings (2, 9, 17).

Removal of the prosthetic device was performed under aseptic conditions as a regular orthopedic surgery procedure. Multiple (three to five) periprosthetic tissue samples were sent to microbiology laboratories for routine cultures. Prosthetic devices were sent to the reference laboratory (Fundación Jiménez Díaz microbiology department) in sterile closed containers for specimen processing. Samples were stored at 4°C until processing (maximum delay of 24 h).

Sample processing. In the reference laboratory, samples were aseptically introduced and hermetically closed in 20- by 40-cm sterile plastic bags with 50 ml of sterile phosphate buffer (pH 6.8) (bioMérieux, Marcy-L'Étoile, France). Bags were previously steam sterilized and sealed until use. The samples were sonicated with an Ultrasons-H 3000840 low-power bath sonicator (J. P. Selecta, Abrera, Spain) during 5 min. The bags were visually inspected before and after sonication to detect leaks in the bag.

The sonicate was removed under an aseptical manipulation protocol and placed into 50-ml Falcon tubes. Samples were then centrifuged at 3,000 × *g* during 20 min, and the supernatant was discharged. The sediment was resuspended in 5 ml of sterile phosphate buffer, and 10 μl was inoculated onto the following culture media: tryptic soy–5% sheep blood agar, chocolate agar, Schaedler–5% sheep blood agar, MacConkey agar, Sabouraud-chloramphenicol agar, and Middlebrook 7H10 agar. Ten microliters was also prepared for Gram staining. Samples were streaked onto each medium for quantitative culture. The media were then incubated under different conditions: at 37°C in a 5% CO_2 atmosphere during 7 days (tryptic soy–5% sheep blood agar and chocolate agar) or 15 days (Middlebrook 7H10 agar), at 37°C under a normal atmosphere during 1 day (MacConkey agar), at 37°C under an anaerobic atmosphere during 7 days (Schaedler–5% sheep blood agar), and at room temperature and atmosphere during 30 days (Sabouraud-chloramphenicol agar). All media were inspected daily for microbial growth. Isolated organisms were identified according to commonly used commercial biochemical tests (API20NE, API Strep, and Rapid ID32A; bioMérieux, Marcy-L'Étoile, France) or commonly accepted protocols (6). Susceptibility testing of the organisms was performed using the Kirby-Bauer disc plate assay, and the results were interpreted according to Clinical and Laboratory Standards Institute (formerly NCCLS) standards (12).

When two phenotypically identical strains were isolated from different patients, the strains were analyzed by randomly amplified polymorphic DNA (RAPD) analysis using previously described protocols (24) with primers Akopyanz (CCG CAG CCA A), p3 (AGA CGT CCA C), and p15 (AAT GGC GCA G).

Statistical analysis. Fisher's exact test was used for evaluations of the presence of confluent bacteria related to clinical diagnosis. All calculations were performed with EPI-INFO 3.4.1 software (Centers for Disease Control and Prevention).

RESULTS

Sixty-six samples from 31 patients were included in the study (2.13 samples/patient). Seventeen of the patients (37 samples; 2.17 samples/patient) had a clinical diagnosis of prosthetic infection. In all these samples, the preoperative diagnosis of infection was clinically confirmed in the operation by macroscopic periprosthetic purulence. Additionally, in some cases, a sinus tract communicating with the prosthesis was observed. The other 14 patients (29 samples; 2.07 samples/patient) had no clinical diagnosis of infection (aseptic loosening in six cases, mechanical pain in three cases, pseudoarthrosis in two cases, and other conditions in three cases). Results for sonicates and conventional cultures are shown in Table 1.

The processed retrieved orthopedic materials were hip prostheses (15 cases; 38 samples), knee prostheses (3 cases; 10 samples), intramedullar nails (four cases; six samples), and other devices (9 cases; 12 samples).

Among the patients with clinical diagnosis of infection, all but one (with clinical diagnosis of total knee prosthetic infection) had positive results for the sonicate (sensitivity of 94.1%), and two had negative results by conventional culture methods (sensitivity of 88.2%). Specificity, however, was lower for the sonication (42.8%) than for the conventional culture (specificity of 100% for nine patients with conventional cultures). The positive predictive value was 66.7% for sonication and 100% for conventional cultures, and the negative predictive value was 85.7% for sonication and 81.8% for conventional cultures. One patient with total hip prosthetic infection had *S. aureus* isolated from the sonicate culture, but the conventional cultures were negative.

Of the cases with no clinical diagnosis of infection, but where conventional cultures were performed ($n = 9$), four had positive results for the sonicate. After reviewing the clinical chart, one of these cultures was considered to be a true-positive result (*Pseudomonas aeruginosa*), while the other three were considered to be without clinical significance (one *Stenotrophomonas maltophilia* and two *Burkholderia* sp. isolates). Of the cases where no conventional cultures were performed ($n = 5$), one was considered to be clinically significant after reevaluation (*Staphylococcus aureus*), and the true significance of another one was doubtful (one isolation of *Mycobacterium fortuitum*). The rest of these cases were considered to be of no clinical relevance (one isolate of *Burkholderia cepacia* and one isolate of *Sphingomonas paucimobilis*). The *S. maltophilia* isolate was considered to be contamination caused by visible leakage found in the plastic sonication bag. No other cases of bag leakage were detected.

After these late considerations, if we include all the cases with a final diagnosis of infection, the sensitivity of sonication remained at 94.7%, while conventional cultures showed a sensitivity of 84.2%. However, specificity remained lower for sonication (50%) than for conventional cultures (100% of eight cases). The positive predictive value rose to 75% for sonication (conventional cultures remained at 100%), but the negative predictive value was not affected.

In relation to the isolated organisms, all the samples from four patients grew two different organisms (*S. aureus* and *Streptococcus agalactiae*, *S. aureus* and *P. aeruginosa*, *S. aureus* and CNS, and CNS and *Aspergillus terreus*). All the other positive cultures grew only one organism: gram-positive cocci were the most common of them (*S. aureus* in nine cases and CNS in six cases); *P. aeruginosa*, *S. maltophilia*, and *Burkholderia* spp., were isolated in two cases each, and one isolate was detected for other organisms (Table 2). No identical strains were detected in two different patients according to RAPD results.

When we evaluated the data obtained from the Gram stain, 33.3% of all the positive samples gave a positive result. All but one of these samples showed a colony count higher than 100,000 CFU. Quantification of the isolates showed that no breakpoint could be established to evaluate if the organism was contamination, because in one case (diagnosed as contamination with *S. maltophilia*), the colony number was higher than 500,000 CFU; in other cases of possible contamination, the count was between 100,000 CFU (*B. cepacia* and both *Burk-*

TABLE 1. Results of conventional and sonicate cultures

Case	Implant[a]	Culture result of the sonicate	Conventional culture result	Clinical diagnosis of infection	Conventional cultures performed
1	Osteosynthesis material	*P. oralis*	*P. oralis*	Yes	Yes
2	Intramedullary nail	*S. aureus*		No	No
3	TKP			No	Yes
4	Osteosynthesis material	*S. aureus*	*S. aureus*	Yes	Yes
5	THP	*S. maltophilia*		No	Yes
6	Osteosynthesis material	*Burkholderia* sp.		No	Yes
7	THP	*Burkholderia* sp.		No	Yes
8	Intramedullary nail	*M. fortuitum*		No	No
9	Intramedullary nail	*S. paucimobilis*		No	No
10	Osteosynthesis material	*S. maltophilia*	*S. maltophilia*	Yes	Yes
11	THP	*S. aureus* + *S. agalactiae*	*S. aureus*	Yes	Yes
12	Osteosynthesis material	*S. aureus*	*S. aureus*	Yes	Yes
13	Osteosynthesis material	*P. aeruginosa*		No	Yes
14	TKP			Yes	Yes
15	Osteosynthesis material			No	No
16	Osteosynthesis material	*S. aureus*	*S. aureus*	Yes	Yes
17	THP	*S. aureus*	*S. aureus*	Yes	Yes
18	THP	-		No	Yes
19	THP	*B. cepacia*		No	No
20	THP	*B. fragilis*	*Escherichia coli* + *B. fragilis*	Yes	Yes
21	Intramedullary Nail			No	Yes
22	THP	CNS	SCN	Yes	Yes
23	Nail	*S. aureus* + CNS	*S. aureus*	Yes	Yes
24	THP	*P. aeruginosa* + *S. aureus*	*P. aeruginosa* + *S. aureus*	Yes	Yes
25	THP	CNS	CNS	Yes	Yes
26	THP	CNS	CNS	Yes	Yes
27	THP	CNS	CNS	Yes	Yes
28	THP			No	Yes
29	THP	*S. aureus*		Yes	Yes
30	TKP			No	Yes
31	THP	CNS + *A. terreus*	CNS	Yes	Yes

[a] TKP, total knee prosthesis; THP, total hip prosthesis.

holderia sp. isolates) and 250,000 CFU (*S. paucimobilis*), while in one case of a truly significant isolate (*Prevotella oralis*), the colony count was lower than 250 CFU. No statistical difference was found for the presence of confluent bacterial growth in the cultures related to the clinical diagnosis of infection ($P = 0.42$ by Fisher's exact test), even if the two cases of unsuspected infection were considered to be true positives ($P = 0.31$ by Fisher's exact test).

TABLE 2. Characteristics of the two groups of patients

Group	Clinical diagnosis of infection	No. of patients	No. of patients with device:				Avg delay in processing (h)	No. of positive cultures	Isolated species (no. of cases)	No. of cultures with >1 organism	No. (%) of cultures with confluent growth (%)[c]
			Hip prostheses	Knee prostheses	Intramedullar nail	Other					
1	No	14	5	2	3	4	5.79	8	*S. aureus* (1),[a] *P. aeruginosa* (1),[a] *Stenotrophomonas maltophilia* (1), *B. cepacia* (1), *Burkholderia* sp. (2),[b] *S. paucimobilis* (1), *Mycobacterium fortuitum* (1)[b]	0	2 (25)
2	Yes	17	10	1	1	5	9.76	38	*S. aureus* (8), CNS (6), *P. aeruginosa* (1), *Bacteroides fragilis* (1), *P. oralis* (1), *S. agalactiae* (1), *S. maltophilia* (1), *A. terreus* (1)[b]	4	14 (36.8)

[a] Reclassified as being true infections after culture results were known.
[b] Isolates of doubtful signification.
[c] The number of CFU isolated in 10 μl of sample was uncountable.

DISCUSSION

The diagnosis of infection related to biomaterials in orthopedic surgery remains a challenge for microbiology laboratories (1, 4, 14, 18, 25) because, despite all advances in microbiological techniques, there is no truly valid technique that could serve as a "gold standard" for this diagnosis.

Clinical evaluation, both perioperatively and intraoperatively, has many pitfalls, and several reports have shown that patients with clinically aseptic loosening may in fact have oligosymptomatic infection that cannot be diagnosed until microbiological evaluation is performed (13, 23).

The pathogenesis of these infections may explain the diagnostic problems. Biofilm is an extremely important form of bacterial life that seems to be a crucial part of the development of biomaterial-related infection (3, 5, 7, 8). Other potential factors related to infections are intracellular survival of bacteria and the ability of low-pathogenic-potential organisms to develop infection when foreign bodies are present through long time periods (16). All these factors lead to difficulties in diagnoses, as bacterial isolates that are part of a biofilm or are inside cells grow poorly in culture media. Moreover, there could be fastidious organisms involved, such as mycobacteria or fungi, that cannot be isolated unless special culture media or prolonged incubation times are used (10).

Sonication of the implanted material seems to be a valid approach for the diagnosis of device-related infections, as has been stated previously (19, 20, 22, 25). This technique dislodges the adhered organisms and allows their detection through conventional microbiological cultures. Reports describing approaches similar to the one used in the present work (20, 22) have shown better sensitivity results for sonication than for conventional cultures, as confirmed in our work. However, the specificity in our results was lower than that that reported previously by Trampuz et al. (20), while no specificity was reported previously by Tunney et al. (22). Several factors could explain this difference. First, the number of conventional cultures taken among patients without infection was very low in our report, so the specificity of conventional cultures could be lower than what was detected. Second, it is possible that sonication in bags could increase the number of positive cultures due to contamination, as previously stated (19), so the specificity reported previously by Trampuz et al. was lower than that of conventional cultures (88.5% versus 90.9%) (20); however, in those studies, the sonication bags were perforated, allowing the entrance of water from the sonicator with subsequent contamination of the sample; we detected only one case of a damaged bag in our work where *S. maltophilia* was subsequently isolated, while in all the other cases, no rupture could be detected despite careful inspection of the bags before and after sonication. Moreover, we changed the water of the sonicator after each sonication, and the sonicator remained empty so as to avoid bacterial overgrowth in the water and subsequent contaminations. Third, the number of culture media employed by us is higher than those used by Trampuz et al. (20), so there is a possibility that some isolates may not be detected if only conventional media are used; in fact, *Burkholderia* sp. isolates grew only on Middlebrook 7H10 agar plates, and *A. terreus* isolates grew both in Sabouraud-chloramphenicol agar tubes and on Middlebrook 7H10 agar plates; if we had used the culture media described previously (20), we would not have detected these isolates, and the specificity would have increased to 66.7%.

The fact that two cases of true infection caused by pathogenic organisms were detected without initial suspicion of infection raises the question of clinical evaluation as a gold standard (23).

On the opposite side, and even more interesting, is the fact that organisms isolated in our study as being false positives share a common characteristic: all of them are nonfermenter gram-negative rods or uncommon isolates (environmental mycobacteria and fungi) with a low pathogenic potential for humans. However, their true significance in these samples is questionable because no clinical symptoms were detected in the patients. These organisms could have been attached to the implant surface and may not have led to clinical infection but may have contributed to the loosening of the prosthesis in the long-term. The high number of colonies detected and the absence of these organisms in control cultures from the sonicator make this hypothesis reasonable. As the use of multiple culture media in our study was done to increase the detection possibilities of fastidious or uncommon organisms, the significance of these organisms as cause of contamination or a true pathogen remains doubtful. Although no clinical disease could be found, the patients with *Burkholderia* sp. isolates had symptoms leading to prosthesis removal (due to pseudoarthrosis and aseptic loosening), as was also the case for the patient with *M. fortuitum* (with motion problems). In the case of *A. terreus*, the fungus was isolated together with CNS, a more common pathogen, in a patient with clinical diagnosis of infection, so its role in the disease is difficult to establish.

In conclusion, the use of sonication, together with a broad spectrum of culture media, increases the possibilities for the diagnosis of device-related orthopedic infections. The significance of some isolates that appeared with high colony counts but without clinical symptoms or signs needs further evaluation to classify them properly as contaminants or pathogens.

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