Assessment of sonication-based culture in diagnosing orthopedic implant infections: a comparative analysis with microbiological diagnostic approaches

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ABSTRACT

Aims: This study aimed to investigate the diagnostic advantages of microbiological culture, histopathological examination, and the management of sonication in the diagnosis of infections related to orthopedic implants and prostheses.

Methods: The study included 21 patients suspected of orthopedic implant or prosthesis-related infections. The classification of implant and prosthesis-related infections and the choice of treatment were based on the Infectious Diseases Society of America diagnostic and treatment guidelines. During the operations, samples were taken from the implant and inflamed tissue around the implant for each patient, and these samples were evaluated using standard culture, histopathological examination, and sonication methods.

Results: The sonication method exhibited a higher sensitivity in comparison to both tissue cultures and cultures acquired from implants and prostheses without the application of sonication (61.1% vs. 38.8% vs. 27.7%, P < 0.05, respectively). The count of isolated microorganisms was greater in the sonication method when compared to both tissue cultures and conventional cultures taken from implants and prostheses (16 vs. 10 vs. 6, P < 0.05, respectively). The sensitivity of the sonication method was found to be higher compared to conventional cultures, even among patients who had been administered preoperative antibiotics (p<0.05).

Conclusion: In the diagnosis of orthopedic implant and prosthesis infections, the sonication method was more effective as a diagnostic approach compared to conventional methods. A greater number of agents can be identified using the sonication method in infected tissues.

Keywords: Prosthesis, implant, infection, sonication

INTRODUCTION

The frequency of orthopedic prosthesis and implant applications has increased over the past two decades. It has been reported that in the United States, approximately one million people undergo total hip and knee arthroplasty surgery each year, and this number is projected to reach four million annually by 2030.[1,2] While complications are infrequent after orthopedic implant and prosthesis procedures, the growing number of patients undergoing these surgeries leads to a higher overall incidence of complications. One of the most serious complications after surgery are prosthetic and implant-related infections. These infections result in increased morbidity and mortality rates, as well as longer hospitalization, long-term antibiotic use and more surgical interventions.[3-5]

In diagnosing implant-induced infections, the best approach is to culture the tissues near the implant during an operation and isolate the responsible microorganism. However, in patients with suspected orthopedic implant infections (SOII), isolating the causative microorganism from the surrounding tissue cultures is not always feasible. The reason for this is that the bacteria hide under the biofilm layer they form on the implant. On the other hand, most of the patients take antibiotics before and during surgery and the likelihood of isolating the causative microorganism in culture decreases.[6] In addition, the likelihood of isolating the causative microorganism in culture may vary among diagnostic methods.

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One of the diagnostic methods for prosthesis and implant infections is the sonication of the extracted implant or prosthesis. Sonication is the decomposition of the biofilm layer on the implant with ultrasonographic sound waves. It is suggested that the sonication method increases the likelihood of isolating the the infection-causing microorganism relative to the tissue culture near the implant, and that this also applies to patients who undergo antibiotic treatment prior to the surgery.[7] Therefore, this study aimed to compare the microbiological culture, histopathological, and sonication methods to identify the causative agents in patients who had their implants or prostheses removed due to SOII or suspected joint prosthesis infection (SJPI).

METHODS

This prospective cross-sectional study was conducted at the Gülhane Training and Research Hospital Orthopedics and Traumatology Clinic from July 2015 to December 2015, in accordance with the Helsinki Declaration and the Good Clinical Practice Guidelines. The study received approval from the Gülhane Training and Research Hospital Clinical Researches Ethics Committee (Date: 22.06.2015, Decision No: 1491-88-14/1648.4-467). Informed consent was obtained from all cases included in the study.

The study included 21 patients who admitted with SOII or SJPI and subsequently had an implant or prosthesis removal procedure. The Infectious Diseases Society of America (IDSA) guidelines were employed for classifying and determining treatment options for these infections.[1] Eligibility criteria for the study inclusion encompass patients meeting at least one of the following: the identification of the same microorganism from cultures taken during two or more surgeries or from both preoperative aspiration and intraoperative cultures; the observation of purulent fluid in the prosthesis area without any other known cause; detection of acute inflammation in the peri-prosthetic tissue or on the prosthesis during histopathological examination after surgical debridement or prosthesis removal; and the presence of an externally opening sinus tract associated with the prosthesis. Additionally, patients who did not meet any of the aforementioned criteria but exhibited at least two of the following symptoms in the implant or prosthesis area-pain, limited motion, increased temperature, swelling, or necrosis at the incision site-were also included in the study, considering they might have a prosthesis infection. Patients with ongoing pain following prosthesis placement, who reported restricted movement and, irrespective of elevated erythrocyte sedimentation rate (ESR) or C-reactive protein (CRP) values, exhibited indications of non-union, pseudoarthrosis, or implant

loosening in radiographic examinations, were also included in the study due to suspected implant or prosthesis infection. Patients with contamination in any extracted sample such as peri-prosthetic tissue or implant or an infection originating from another source were excluded from the study.

Antimicrobial therapy was described as the use of antibiotics within the 14 days before the extraction of the implant or prosthesis. Demographic, clinical, microbiological, pathological, and laboratory findings were recorded for each patient. The implant age was defined as follows: the median time elapsed from the initial surgery to the point when infection was suspected. The reference ranges for laboratory parameters were as follows: For leukocytes, $4\text{-}10\times10^9$/L; for ESR, 0-20 mm/h; and for CRP, 0-5 mg/L.

Sample Collection

All surgical procedures were conducted in an environment with clean laminar airflow. In appropriate cases, joint fluid was aspirated preoperatively, and leukocyte count and microbiological examination were performed. The extracted implants were taken aseptically and stored in sterile polypropylene containers with screw caps. Every sample was transported to the microbiology lab within a two-hour window after surgery. Tissue cultures surrounding the implant or prosthesis were taken from all patients. Removed implants and prostheses were evaluated both by conventional culture and through sonication. Additionally, tissue samples from around the implant or prosthesis, as well as the sonication fluids of the removed implants or prostheses, were sent to the pathology laboratory for histopathological and cytological examination.

Conventional Culture

During the operation, for each patient, four tissue samples were taken from the implant and the inflamed tissue surrounding the implant. The samples were dispatched to laboratories under sterile conditions for both histopathological and microbiological analyses.

Procedures on the Implant

Fifty ml of sterile distilled water was added to the implants inside the sterile polypropylene tubes, followed by 30 seconds of vortexing. At this stage, 100 microliters from the obtained liquid was inoculated onto 5% sheep blood agar, chocolate agar, and eosin methylene blue (EMB) agar (Salubris, Istanbul, Turkey), both in aerobic and anaerobic environments. After the inoculation, the implant sample was sonicated at 50 kilohertz for five minutes (Elma D-78224 Singen/Htw, Germany), and then vortexing was repeated for 30 seconds. The sample was centrifuged at 13,000 G force

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for 15 minutes. The supernatant was discarded, and 100 microliters from the remaining liquid at the bottom was inoculated onto 5% sheep blood agar, chocolate agar, and EMB agar, in both aerobic and anaerobic environments. Aerobic cultures were incubated at 37°C for 48 hours in an incubator containing 5% CO_2. Anaerobic cultures were incubated for 7 days in an anaerobic chamber incubator (Bactron, Sheldon Manufacturing Inc. OR, ABD), with conditions being checked every other day.

Tissue Cultures

Tissue specimens, harvested under sterile conditions and shipped to the lab in sterile vessels, were placed into sterile mortars. They were then mixed with 1 ml of tryptic soy broth and crushed. From the crushed specimen-broth blend, 100 microliters were sampled and cultured on 5% sheep blood agar, chocolate agar, and EMB agar under both aerobic and anaerobic conditions. Aerobic cultures were incubated for 48 hours in an incubator containing 5% CO_2, while anaerobic cultures were incubated for 7 days in an anaerobic chamber incubator, with daily checks.

Colony counts of the microorganisms grown on the plates were determined and noted as colony forming units (cfu)/ml. The proliferating microorganisms were identified using conventional methods and the Phoenix 100 automated phenotypic identification device (BD, Maryland, USA). Antibiotic susceptibility tests were conducted using the Phoenix 100 automated phenotypic identification device (BD, Maryland, USA) and the Kirby-Bauer disk diffusion test, in accordance with the Clinical and Laboratory Standards Institute (CLSI) criteria. Results were evaluated quantitatively as cfu/ml in categories of pre-sonication and post-sonication of the implant, as well as in the tissue category. Bacterial typing was conducted at the genus and species level, together with the results of antibiotic susceptibility tests.

Statistical Analysis

All data were analyzed with IBM SPSS Statistics for Windows 20 (IBM Corp., Armonk, NY, USA). Numerical data determined to be normally distributed based on the results of Kolmogorov-Smirnov tests are given as mean±standard deviation values, while non-normally distributed variables are given as median (min-max) values. Accordingly, Student t-test and Mann-Whitney U test were used for comparisons between two groups. Categorical variables were presented as numbers and percentages, and comparisons between groups were performed using Chi-square and Fisher exact tests. The sensitivity values was determined using the formula: True positives / (True positives + False negatives).8 Significance was accepted at $p<0.05$ (*) for all statistical analyses.

RESULTS

The study population consisted of 21 cases, including 11 cases with SJPI (mean age: 60.9±16.0 years) and 10 cases with SOII (mean age: 38.6±12.1 years). The median implant age was higher in the SJPI group compared to the SOII group (19 months vs. 7 months, $p < 0.001$). The rate of preoperative antibiotic use was higher in the SOII group compared to the SJPI group (100% vs. 45.5%, p=0.007). In the SJPI and SOII groups, at least two of the symptoms such as pain, limited mobility, increased temperature, discharge, swelling, and necrosis in the incision area were present. In both groups, the ratio of patients with leukocyte values within normal limits was similar, while the ratio of patients with elevated CRP was higher in the SJPI group compared to the SOII group (72.7% vs. 10%, p=0.005). Demographic and clinical characteristics of patients with SOII and SJPI are presented in **Table 1**.

Table 1. Demographic and clinical findings of the patients suspected of prosthesis and implant infection

Variables	SJPI n=10	SOII n =11	p value
Age, years	61 (21-83)	39 (20-83)	<0.001*
Median implant age, months	19 (1-60)	7 (1-18)	<0.001*
Preoperative antibiotic use, n (%)	10 (100)	5 (45.5)	0.007*
Female gender, n (%)	8 (80.0)	5 (45.5)	0.113
Symptom duration, month	2 (1-3)	1.5 (0.5-2.0)	0.035*
Pain-Limited mobility	7 (70.0)	10 (90.9)	0.235
Drainage	0	5 (45.5)	0.017*
Increased temperature	9 (90.0)	8 (72.7)	0.325
Necrosis	0	5 (45.5)	0.017*
Leukocytosis	2 (20.0)	1 (9.0)	0.482
Elevated ESR	8 (80.0)	6 (54.5)	0.227
Elevated CRP	10 (10.0)	8 (72.7)	0.005*
Time between infection suspicion and surgery, days	22 (16-32)	14 (13-27)	0.015*
Follow-up time, months	11 (1-12)	10 (1-12)	0.863

Data are shown as mean ±SD or median (min-max) or number and percentage (%). * p <0.05 shows statistical significance. Abbreviations: CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; SJPI, patients who suspected of joint prosthesis infection; SOII, patients who suspected orthopedic implant infection.

In the histopathological examination, active or chronic inflammation findings were detected in the tissues surrounding the implants and prostheses and in sonication fluids of the implants and prostheses in 18 out of 21 patients. In the remaining three patients, no signs of inflammation were found in the histopathological examination, and no pathogens were isolated in the microbiological cultures. In these three patients, there were complaints of pain in the implantation area prior to surgery. Additionally, they had elevated ESH or CRP levels and showed signs of loosening in direct radiography. Consequently, these patients underwent surgical procedure based on suspicions of infection. Given that no pathogens were detected in the microbiological

cultures and there were no indications of inflammation in the histopathological analysis for these patients, the possibility of implant infection was ruled out, and they were evaluated as cases of aseptic loosening.

In the 18 patients with positive histopathological results, the sonication method exhibited higher sensitivity in detecting pathogens compared to the tissue and conventional culture methods (61.1% vs. 38.8% vs. 27.7%, p<0.05, respectively). Similar findings were also detected in the SJPI and SOII subgroups (Table 2).

Table 2. Comparison of sensitivity between conventional, tissue, and sonication fluid cultures.

Variables	Culture Identified / Total	Sensitivity (%)
All population		
Tissue culture	7 / 18	38.8
Conventional culture	5 / 18	27.7
Sonication fluid culture	11 / 18	61.1*
SJPI group		
Tissue culture	3 / 10	30.0
Conventional culture	2 / 10	20.0
Sonication fluid culture	5 / 10	50.0*
SOII group		
Tissue culture	4 / 8	50.0
Conventional culture	3 / 8	37.5
Sonication fluid culture	6 / 8	75.0*

Data are shown as number for cultures. * p< 0.05 for sonication fluid culture method vs. tissue and conventional culture methods. Abbreviations: SJPI, patients who suspected of joint prosthesis infection; SOII, patients who suspected orthopedic implant infection.

Identified Microorganisms

A total of 32 microorganisms were identified from all the cultures. The microorganisms most commonly identified were Staphylococcus species and *Ralstonia pickettii*. In the three separate cultures taken from the patients, similar microorganisms were identified, excluding *Acinetobacter baumannii*, *Peptoniphilus assaccharolyticus*, and *Pseudomonas aeruginosa*. The number of detected microorganisms was higher in the sonication method compared to other cultures (Table 3).

Table 3. Distribution of the isolated bacteria according to tissue, conventional, and sonication culture methods.

Microorganisms	Cultures		
	Tissue	Conventional	Sonication fluid
Acinetobacter baumannii	-	-	1
Enterococcus species	1	-	1
Escherichia coli	-	1	1
Enterobacter cloacae	1	-	1
Peptoniphilus assaccharolyticus	1	-	-
MRCNS	5	1	4
Corynebacterium striatum	1	1	1
Ralstonia pickettii	-	1	4
MSSA	1	1	1
Pseudomonas aeruginosa	-	-	1
Serratia marcescens	-	1	1
Total	10	6	16

Data are shown as number for cultures. Abbreviations: MSSA, Methicillin-sensitive *Staphylococcus aureus*; MRCNS, Methicillin-resistant coagulase-negative staphylococci

Inconsistencies between tissue culture results around the implant or prosthesis and sonication culture results were detected in seven patients. In four patients, while the tissue culture around the implant or prosthesis was negative, growth was detected in the sonication. In one patient, discrepancies were detected between the tissue culture results from the implant or prosthesis area and the results obtained through the sonication method. In two cases, in addition to the microorganisms obtained from the tissue culture around the implant or prosthesis, polymicrobial agents were isolated using the sonication method (Table 4). In two cases within the SOII group, a greater number of bacteria were detected using the sonication method in addition to the bacteria or bacteria isolated from tissue cultures.

In three out of 15 patients who continued antibiotic treatment before surgery, no signs of inflammation were found in the histopathological examination, and no agents were detected in microbiological cultures. In three patients, pathogens were detected using the sonication method despite the tissue cultures around

Table 4. Patients with discrepancy between tissue culture and implant-prosthesis sonication fluid culture.

No	Tissue culture	Polymicrobial	Sonication fluid cultre	Polymicrobial	Preoperative antibiotic use
1	MRCNS *Enterococcus* species	Yes	MRCNS *Enterococcus* species *Acinetobacter baumannii*	Yes	Yes
2	Negative	No	Esherichia coli	No	Yes
3	MRCNS	No	*Ralstonia pickettii*	No	Yes
4	Negative	No	*Ralstonia pickettii*	No	No
5	MSSA	No	MSSA *Ralstonia pickettii*	Yes	Yes
6	Negative	No	*Ralstonia pickettii* *Pseudomonas aeruginosa*	No	Yes
7	Negative	No	*Serratia marcescens*	No	Yes

Abbreviations: MSSA, Methicillin-sensitive *Staphylococcus aureus*; MRCNS, Methicillin-resistant coagulase-negative staphylococci

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the implant and prosthesis being negative. In two cases, more microorganisms were detected with the sonication method compared to tissue cultures. For the other patients, results from conventional cultures matched those from the sonication method. The sonication technique identified pathogens at a higher rate irrespective of antibiotic utilization. In patients who received antibiotic treatment, the sonication method exhibited a sensitivity of 66.6%. In contrast, the conventional or prosthesis culture technique had a 33% sensitivity, and the tissue culture method recorded a sensitivity of 46%.

DISCUSSION

The results of this study indicate that the sonication method is a more effective diagnostic approach compared to conventional methods in patients with orthopedic implant and prosthesis infections. The sonication method allowed for the detection of a greater number of pathogens in infected tissues.

In orthopedic implant and prosthesis infections, making a definitive diagnosis is of great importance for initiating appropriate antimicrobial treatment.[9,10] Although the examination of preoperative synovial fluid is considered the gold standard for diagnosis, standard cultures exhibit limited sensitivity.[11] This may be related to the microorganisms generally being of low virulence, the use of antibiotics prior to surgery, and the biofilm layer that forms on the implant. Therefore, several methods, including the sonication technique which targets the separation of the biofilm layer, have been identified for more effective diagnosis.[12,13]

Symptoms such as necrosis and discharge, as well as signs of acute inflammation, were higher in the SOII group compared to the SJPI group, while the age of the implant was lower. These findings suggest that bacterial agents in early-onset prosthesis/implant infections have higher virulence and this might lead to more dominant clinical symptoms in these patients.[14,15] However, the age of the SJPI group was higher. This difference may be associated with primary osteoarthritis and, consequently, joint replacement surgeries being performed at older ages.[16] Additionally, the fact that implantations for bone fixation, typically due to fractures, are frequently performed in the physically active younger age group might explain the age difference.[17]

In previous limited studies, the sonication technique has been demonstrated to be more effective than conventional methods for identifying infections caused by prosthetic or orthopedic implants.[7,17] It is known that pre-operative antibiotic use adversely affects the isolation of the pathogen.[18,19] Despite this, it has been reported that the sonication method displayed a more

effective diagnostic performance even in the patient group receiving antibiotic treatment.[7,17] Besides, in a study where the sonication fluid was evaluated with the PCR method, the sonication method displayed higher sensitivity in antibiotic-treated patients than in those without antibiotic treatment.[20] In the present study, even though all of the SJPI patients and approximately 50% of the SOII patients were receiving antibiotic treatment, the sonication method detected microorganisms with higher sensitivity compared to other methods in both the SOII and SJPI groups. Additionally, in three out of the 15 patients who used antibiotics, while conventional culture results were negative, bacteria were only identifiable via the sonication method. Moreover, the detection of polymicrobial etiology with sonication in two cases who had received antibiotic treatment, compared to standard tissue cultures, further underscores the effectiveness of the sonication method even in the presence of antibiotic use.

Staphylococcus was the most frequently isolated microorganism via the sonication method, consistent with existing literature.[7] This was followed by *Ralstonia pickettii*, a bacterium that can cause serious infections, especially in immunosuppressed patients. Out of the four patients in whom *Ralstonia pickettii* was detected, two had osteosarcoma, while the other two were elderly and diagnosed with diabetes. These findings suggest that the types of proliferating microorganisms might vary in immunosuppressed patients

This study had some significant limitations. First, it was a single-center study. Second, the sample size was small. Third, a majority of the patients had used antibiotics before surgery. These factors might affect the sensitivity of the culture methods.

CONCLUSION

Orthopedic implant and prosthetic infections are commonly encountered nowadays and can pose challenges in achieving a definitive diagnosis. The sonication method has a high sensitivity in these infections. Its diagnostic efficacy remains superior to conventional microbiological diagnostic methods, even in patient groups using antibiotics. Therefore, the sonication method can be a significant screening tool in determining the causative agents in SOII and SJPI patients.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Gülhane Training and Research Hospital Clinical Researches Ethics Committee (Date: 22.06.2015, Decision No: 1491-88-14/1648.4-467).

Informed Consent: Written consent was obtained from the patient participating in this study.

Referee Evaluation Process: Externally peer reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

Author Contributions: All the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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