Original Article

Exploring the Role of Sonication Fluid Culture in Periprosthetic Joint Infection: A Comparative Study with Conventional Methods

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Abstract

Objective: The aim of this study is to evaluate and compare the diagnostic effectiveness of sonication fluid culture (SFC) compared to conventional methods in identifying the causative microorganisms in periprosthetic joint infections. **Methods:** In this study, three cultures were evaluated for diagnosing periprosthetic joint infection intraoperative periprosthetic tissue culture, implant culture, and SFC. The sensitivity, specificity, and predictive values were calculated for each method, using the 2018 definition of periprosthetic hip and knee infection and clinical evaluation as references. Of the 92 patients who had implants removed, 49 were for mechanical reasons and 43 for infection. **Results:** Positive cultures were obtained in 13 out of 49 patients with mechanical issues and 31 out of 43 with infections. The sensitivity of periprosthetic tissue cultures (53.5%) is slightly higher than SFC (48.8%), suggesting better detection of positive cases. However, SFC's specificity (83.7%) is higher, indicating more accurate identification of negative cases compared to periprosthetic cultures (73.5%). However, SFC identified additional pathogens in patients with negative periprosthetic tissue and implant cultures. Examination of the infected knee and hip prostheses showed that SFC enhanced pathogen detection, particularly in patients with negative implant cultures. Despite this, SFC was not statistically superior to other methods. **Conclusion:** This study supports the combined use of periprosthetic tissue culture and SFC for identifying causative microorganisms in implant infections. Despite not being statistically superior, SFC provides additional pathogen detection, especially when other methods fail, thereby enhancing overall diagnostic accuracy.

Keywords: *Conventional culture, diagnostic tool, periprosthetic joint infection, sensitivity, sonication fluid culture, specificity*

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Introduction

The use of orthopedic implants has increased with the development of operative techniques and the exponential growth in the volume of arthroplasties. However, this increase in implant usage has also brought complications, the most important of which is implant-associated infection. These infections can lead to long-term antibiotic use, multiple surgeries, and prolonged hospitalizations, which can create a heavy financial and psychological burden.[1]

The treatment process for implant-associated infections is lengthy and demanding, and the pathogen may not be detected using conventional periprosthetic tissue culture methods. The sensitivity of current methods is limited, and false-negative rates as high as 10%–30% or false-positive results can be observed.[2-5] The most accurate diagnosis of implant-related infection is made by the combination of laboratory, histopathology, microbiology, and imaging methods.[6]

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In prosthetic joint infections, microorganisms form a biofilm on the prosthesis surface. Biofilms provide protection against phagocytosis and antibiotics for microorganisms enclosed within a glycocalyx matrix, which complicates pathogen detection and elimination. The formation of biofilms often necessitates the removal of implants for effective treatment. To ensure successful surgical treatment, it is crucial to perform meticulous debridement, meticulously removing all devitalized material and foreign bodies that harbor mature biofilm.[7]

Numerous methods have been developed for the detection of causative agents. One such method is sonication.[8] Sonication is based on the high- and low-pressure areas created when low-frequency ultrasound waves pass through the fluid surrounding the removed implant. As a result, bacteria within the biofilm and on the implant surface are released, facilitating the detection of microorganisms.[3,9] Due to its low cost and ease of application, this method is considered useful and promising.[9] Many studies have demonstrated higher sensitivity and specificity compared to periprosthetic tissue culture.[9-14] However, an inhibitory effect on some bacterial species, particularly gram-negative bacteria, has been revealed at different temperatures, durations, and compositions.[9] However, Van Diek *et al*. have explored implant sonication's low sensitivity when infection screening.[15]

In this study, the hypothesis is sonication provides more accurate results and allows for microorganism identification in more patients compared to the conventional methods of periprosthetic tissue culture and implant culture.

Methods

This prospective study was conducted at Trakya University Hospital between the years 2019 and 2021. Patient informed consent was obtained for the study and ethical approval was granted by the ethics committee under protocol code 2018/356. All patients aged 18 and above who underwent orthopedic implant removal for any reason at our hospital during these years were included in the study. The implants of 92 patients were removed, 49 (53.3%) had mechanical reasons (aseptic), and 43 (46,7%) had an infection. Demographic, clinical, laboratory, and surgical data for the patients, along with comorbidities and complications, were noted [Table 1].

The diagnosis of orthopedic implant-associated infection was established when at least one of the following elements was present: a purulent appearance in the surgical field or puncture fluid; an implant-associated sinus tract; clinical redness, swelling, increased temperature, and wound discharge. And additionally, the criteria from the 2018 definition of periprosthetic hip and knee infection were utilized.[16]

Samples were collected from suspicious areas during surgery for each patient. The removed implant was transported to the laboratory under sterile conditions. In this study, three different cultures were examined: intraoperative periprosthetic tissue culture, implant culture, and sonication fluid cultures (SFCs).

Intraoperative periprosthetic tissue culture

The tissue specimens were aseptically disrupted in a sterile mortar and pestle with brain–heart infusion (BHI) broth. Aliquots of tissue specimens were inoculated onto sheep blood agar (SBA), chocolate agar, BHI broth, and thioglycolate broth. The SBA plates, BHI broth, and thioglycollate broth were incubated aerobically at 35–37°C for 5 days. The chocolate agar plates were incubated at 35–37°C in 5–10% carbon dioxide for 5 days. For anaerobic culture, the homogenized tissue was inoculated onto SBA. The inoculated plates were incubated in an anaerobic jar at 35–37°C for 14 days.

The prosthetic device, implant culture

Before the sonication process, the Ringer's lactate solution (15–50 ml) in the container in which the prosthesis or implant was placed was transferred to a conical centrifuge tube. The fluid was centrifuged at 3200 × g for 20 min. Around 0.1 ml of sediment was processed in the same manner as tissue samples.

Sonication fluid culture

The sonication protocol was adapted from Monsen *et al*. Briefly, an appropriate volume of Ringer lactate solution (30–700 ml) was added to polypropylene containers until the prostheses were covered.[9] The container was vortexed for 30 s, followed by sonication (at a frequency of 40 kHz) in an ultrasound bath (Wisd Ultrasonic Cleaner, Model WUC-D06H, Daihan Scientific, Korea) for 5 min and then vortexed again for 30 s. Sonication fluid (15–50 ml) was centrifuged at 3200 × g for 20 min in sterile conical centrifuge tubes. Around 0.1 ml of sediment was inoculated into each medium, and procedures were performed in the same manner as in periprosthetic tissue samples.[9]

Organisms were identified utilizing an automated microbial identification system (Vitek 2 Compact; BioMérieux, France), while standard phenotypic methods were employed for further organism identification.[17,18]

Demographic characteristics, the duration between the initial surgery and material extraction, microorganisms, and comorbidities were assessed using descriptive

statistics. Sensitivity, specificity, positive predictive value, and negative predictive value were computed for intraoperative periprosthetic tissue culture, implant culture, and SFC, with consideration given to the criteria from the 2018 definition of periprosthetic hip and knee infection and the clinical evaluation.[16] McNemar' and Cochren *Q* tests were employed for categorical variables, while the Mann–Whitney *U* tests were used for continuous variables. Chi-square or Wilcoxon rank-sum tests were implemented for baseline comparisons between groups. All calculations were executed using IBM statistical package for the social sciences (SPSS) version 15.0 software (IBM Corp., Armonk, NY, USA).

Results

Thirteen of 49 patients with mechanical reasons had a positive culture. Thirty-one of 43 patients with implant infection had a positive culture. Thirteen of 49 patients who underwent implant removal due to mechanical reasons and were not considered infectious based on the criteria had positive cultures. These 49 patients were further evaluated as the control group for assessing sensitivity and specificity. Table 2 presents the microorganisms detected in patients who underwent implant removal due to mechanical reasons.

Table 1: Demographic and clinical characteristics of the patients

	Mechanical reasons (*n*=49)	Infection (*n*=43)
Age (mean)	39	61
Sex		
• Male	30	15
• Female	19	28
Material		
• Knee prosthesis	0	13
• Hip prosthesis	2	10
• Plate screw, etc.	47	20
Day after surgery (mean)	815	1884
Comorbidities		
• Hypertension	9	29
• Diabetes mellitus	5	16
• Hypothyroidism	0	3
• Malignancy	2	2
CRP elevation	*6*	32
ESR elevation	5	30
Sinus tract	0	18
Positive cultures	13	31
• Periprosthetic tissue culture	11	23
• Implant culture	7	21
• Sonication fluid cultures	8	21

CRP=C-reactive protein, ESR=Erythrocyte sedimentation rate

Out of the 43 patients who underwent material extraction due to infection, cultures were positive in 31 of them. No statistically significant difference was observed between periprosthetic tissue culture, implant culture, and *SFC* ($\chi^2(2) = 4.71$, $P = 0.79$). Table 3 presents the sensitivity and specificity of the various cultures.

In this study, positive cultures were obtained in at least one sample from 31 out of 43 patients with implant infections. In 23 cases, periprosthetic tissue cultures showed positive results, with the isolation of gram-positive bacteria in 19 samples (70.3%) and gram-negative bacteria in 8 samples (29.6%). Both gram-positive and gram-negative bacteria were isolated in four cases. In 21 cases, implant cultures were positive, with the isolation of gram-positive bacteria in 15 cultures (60%) and gram-negative bacteria in 10 cultures (40%). Among the 21 SFCs that showed positive results, gram-positive bacteria were isolated in 17 (68%), and gram-negative bacteria were isolated in 8 (32%). The most commonly isolated gram-positive bacteria were coagulase-negative staphylococci and *Staphylococcus aureus*. Table 4 presents the pathogens that were identified in the 31 patients who required material extraction due to infection.

Twenty-three infected knee and hip prostheses were removed. Seventeen patients had positive cultures. In one patient, 2 pathogens (*S. aureus* + Citrobacter) were detected. Ten patients had positive periprosthetic tissue cultures. Ten patients had positive implant cultures. Thirteen patients had positive SFC. There was no significant difference between periprosthetic tissue culture, implant culture, and SFC ($\chi^2(2) = 1.63$, $P = 0.441$). But extra pathogens were detected with SFC. SFC was positive in 6 of 13 patients with negative periprosthetic tissue cultures. SFC was positive in 4 of 13 patients with negative implant cultures. Table 5 shows the comparative tables of periprosthetic tissue culture and SFC.

Table 2: Pathogens in 13 patients with implant extraction for mechanical reasons (aseptic)

Pathogens	Periprosthetic tissue cultures (*n*=11)	Implant cultures (*n*=7)	Sonication fluid culture (*n*=8)
Coagulase-negative staphylococci	8	5	6
Gram + difteroid	1	–	–
Pseudomonas aeruginosa	1	1	1
Serratia mercendes	1	1	1

Table 3: Sensitivity and specificity of cultures

Culture type	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
Periprosthetic tissue cultures	53.5	73.5	65.7	65.5
Implant cultures	48.8	85.7	75	65.6
Sonication fluid cultures	48.8	83.7	72	65

Table 4: Pathogens in 31 patients who had material extraction due to infection.

Pathogens	Periprosthetic tissue cultures (+) *n*=23 (4 patients had 2 different pathogens)	Implant cultures (+) *n*=21 (4 patients had 2 different pathogens)	Sonication fluid cultures (+) *n*=21 (5 patients had 2 different pathogens)
Coagulase-negative staphylococci	10	6	8
Staphylococcus aureus	6	8	5
Citrobacter	1	1	1
Morganella morganii	1	1	1
Escherichia coli	1	1	1
Gram + difteroid	1		1
Candida albicans	–	–	1
Pseudomonas aeruginosa	1	2	2
Serratia mercendes	–	1	–
Enterobacter cloacae	2	2	2
Proteus mirabilis	1	1	1
Klebsiella pneumoniae	1	1	1
Enterococcus faecalis	2	1	1
Grup D streptococcus	–	–	1

Table 5: The comparative table of implant culture and sonication fluid culture of knee and hip prosthesis with infection

	Sonication fluid cultures		Total
	Negative	Positive	
Periprosthetic tissue cultures			
Negative	7	6	13
Positive	3	7	10
Total	10	13	23

Table 6: The comparative table of implant culture and sonication fluid culture of knee and hip prosthesis with infection

	Sonication fluid cultures		Total
	Negative	Positive	
Implant cultures			
Negative	9	4	13
Positive	1	9	10
Total	10	13	23

Infected knee and hip prostheses were examined separately and are shown in Table 6. In 13 infected knee prostheses, positive results were found in 6 periprosthetic tissue cultures, 4 implant cultures, and 5 SFC. Among the seven patients with negative periprosthetic tissue cultures, pathogens were detected by SFC in two cases. Similarly, in nine patients with negative implant cultures, pathogens were detected by SFC in two cases. Although pathogen detection was enhanced by SFC for knee prostheses, it was not found to be statistically superior to other methods ($\chi^2(2) = 0.85$, $P = 0.89$).

With regard to the 10 infected hip prostheses, positive results were found in 4 periprosthetic tissue cultures, 6 implant cultures, and 8 SFC. In cases with negative implant cultures (six patients), pathogens were successfully detected by SFC in four patients. Despite the improvement in pathogen detection, SFC was not found to be statistically superior to other methods ($\chi^2(2) = 6.00$, $P = 0.074$). Table 7 shows the types of infection and culture results.

Discussion

Ninety-two patients who had implant removal due to infection or mechanical reasons were included in this study. In addition to clinical findings, this study utilized the 2018 definition of periprosthetic hip and knee infection criteria, which is more up-to-date compared to the studies cited in the literature.[16]

Many studies in the literature indicate that the sensitivity of SFC is better than periprosthetic tissue culture. A meta-analysis revealed that the polled sensitivity of sonication fluid was 79%.[10] In this study, the sensitivity of SFC was 48.8% and the sensitivity of periprosthetic tissue culture is 53.5%. Contrary to the literature, SFC's sensitivity was less than periprosthetic tissue and implant

Table 7: Types of infection and culture results of knee and hip prosthesis with infection

Culture	Pathogen	Patient (*n*=23)
Positive intraoperative periprosthetic tissue culture and SFC	*Staphylococcus aureus* + *Citrobacter (1)*	7
	Coagulase-negative staphylococci (4)	
	Escherichia coli (1)	
	Morganella morganii (1)	
Negative intraoperative periprosthetic tissue culture, positive SFC	*S. aureus* (1)	6
	Coagulase-negative staphylococci (3)	
	Candida albicans (1)	
	Gram + difteroid (1)	
Positive intraoperative periprosthetic tissue culture, negative SFC	Coagulase-negative staphylococci (2)	3
	Gram + difteroid (1)	
Negative intraoperative periprosthetic tissue culture and SFC, positive prosthetic device, implant culture	*S. aureus* (1)	1
Negative cultures		6

SFC=Sonication fluid culture

culture. In the literature, there is a study by Van Diek *et al*., which is parallel to these results.[15] In addition, Puig and Verdi showed that the sensitivity of SFC in early orthopedic implant-associated infections was not statistically superior.[13]

In this study, a control group was included, which comprised patients who had material removal performed due to irritation, patient request, mechanical reasons, or aseptic loosening and who exhibited no clinical complaints concerning infection. From this perspective, this study did not solely encompass aseptic loosening cases for sensitivity and specificity comparisons, and a more reliable group was established relative to studies in the literature.[8,15,19,20] This aspect has constituted a strength of this research.

In the literature, the source of infection is mostly gram-positive bacteria.[3,20] However, there are also publications in the literature in which gram-positive and gram-negative bacteria were detected at equal rates.[14] In this study, more gram-positive bacteria were detected as the source of infection. Gram-positive bacteria were detected 70.3% in periprosthetic tissue culture, 60% in implant fluid, and 32% in sonication fluid.

The most commonly detected bacteria are coagulase-negative staphylococci and *S. aureus*, which is similar to the present results.[12,14,15,19,21]

Sonication was found to be beneficial, especially when evaluating patients who had knee and hip prostheses removed due to infection, despite the low sensitivity of SFC. Microorganisms were detected in the SFC of 6 (26%) patients who had no growth in periprosthetic tissue culture, out of 23 patients who had prosthesis removal due to infection. Detected microorganisms in knee and hip prostheses included coagulase-negative staphylococci in three patients, *S. aureus* in one patient, gram-positive diphtheroid in one patient, and Candida albicans in one patient. In a study by Trampuz *et al*., the prosthesis was removed from 79 patients due to infection.[19] Although there was no growth in periprosthetic tissue culture in 14 of these patients, 14 (17.7%) microorganisms were detected in the SFC. Similarly to this study, the most frequently detected microorganisms were coagulase-negative staphylococci in five patients and *S. aureus* in three patients. Furthermore, a *Candida*-type fungus was detected in one patient.

When separately analyzing infected knee and hip prostheses, similar outcomes were noted. In certain cases, microorganisms were identified through sonication despite negative periprosthetic tissue and implant cultures. However, these results did not yield statistical significance. The constrained patient sample size in this study could be a contributing factor to the absence of statistical significance. Nevertheless, sonication was determined to be advantageous, particularly in the identification of novel organisms in knee and hip prostheses.

The main reasons for false results in culture samples are defined as the patient having used antibiotics prior to the culture sampling and sample contamination.[22] It is thought that especially stopping antibiotic therapy <2 weeks before the operation is ideal for culture sensitivity.[19] None of the patients included in this study, who were clinically suspected of having an infection, were started on antibiotic therapy before implant removal. Antibiotic therapy targeted to the causative agent was initiated intravenously after implant removal for patients with positive culture results.

There are various factors that can affect the sensitivity and specificity of SFC, including sample contamination, antibiotic use, and transportation time. For example, the incubation period is still a topic of debate in current literature, with similar studies using incubation periods ranging from 0 to 7 days. There are also studies advocating for an extension of the incubation period to 14 days that is necessary to detect low-virulent and difficult-to-detect microorganisms.[7] The limitation of this study is the variation in the incubation period, which ranges from 3 to 7 days. This may have prevented us from detecting microorganisms with lower virulence and longer incubation periods.

Another factor that may have led to a decrease in sensitivity and specificity in this study is the lack of clear boundaries for the time elapsed between the collection of the material in the operating room and its cultivation in the laboratory, which may have been prolonged due to the COVID-19 pandemic.

All isolated bacteria were considered infectious agents, and no cutoff value was used, which is a weakness of this study.[7,10] Despite this, the sensitivity and specificity of SFC were lower than those of periprosthetic tissue culture. Some studies suggest adding sonication fluid to blood culture bottles or performing polymerase chain reaction to improve SFC's sensitivity and specificity.[21] In this study, the method of adding sonication fluid to blood culture bottles was not utilized, and unlike the literature, the sonication fluid was also cultured.

Conclusion

In conclusion, after a comprehensive evaluation of both the literature and this study, the combined use of periprosthetic tissue culture and sonication is regarded as advantageous for identifying the causative microorganisms, even though it is not deemed the gold standard. This approach proves to be particularly beneficial when the infectious agent remains undetected, as it aids in the identification of microorganisms.

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Conflicts of interest

There are no conflicts of interest.

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