ORIGINAL PAPER/PŮVODNÍ PRÁCE

The Role of Sonication in Improving the Detection of Periprosthetic Joint Infections: a Prospective Analysis

Využití sonikační metody v diagnostice periprotetické kloubní infekce: prospektivní studie

JAKUB RAPI[1], VASILEIOS APOSTOLOPOULOS[1,4], FILIP RŮŽIČKA[2], MARTINA VANĚRKOVÁ[3], PAVEL BRANČÍK[1], TOMÁŠ TOMÁŠ[1,4]

[1] First Department of Orthopaedic Surgery, St. Anne's University Hospital and Faculty of Medicine, Masaryk University, Brno

[2] Department of Microbiology, St. Anne's University Hospital and Faculty of Medicine, Masaryk University, Brno

[3] Centre for Cardiovascular Surgery and Transplantation, Brno

[4] International Clinical Research Center, St. Anne's University Hospital, Brno

Corresponding author:

Assoc. Prof. MUDr. Tomáš Tomáš, Ph.D.
First Department of Orthopaedic Surgery, St. Anne's University Hospital and Faculty of Medicine, Masaryk University, Brno
Pekařská 53
602 00 Brno, Czech Republic

tomas.tomas@fnusa.cz

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ABSTRACT

Purpose of the study

The diagnosis of periprosthetic joint infection (PJI) can be particularly challenging in cases of low-grade chronic infection. The suspicion of infection is typically confirmed through cultures of synovial fluid and periprosthetic tissue. However, these methods may not always detect low-grade infections, which can lead to persistent infection and early failure of the prosthesis. The purpose of this study was to evaluate the effectiveness of sonication in enhancing the detection of PJI using polymerase chain reaction (PCR) analysis.

Material and methods

A prospective cohort of 26 patients, suspected of having mitigated PJI, underwent surgery at the First Department of Orthopaedic Surgery, St. Anne's University Hospital in Brno between 2019 and 2024. The cohort included 16 women and 10 men, aged 56 to 82 years, with infections involving hip (11 cases) or knee prostheses (15 cases). Standard PCR and sonication followed by PCR were used to confirm PJI.

Results

In 20 out of 26 cases, both standard PCR and sonication-assisted PCR detected the infection(p= 0.014). However, in 6 cases, standard PCR failed to identify the pathogen, whereas sonication followed by PCR confirmed the infection. Among these, 4 cases had significantly positive results, and 2 showed weak positivity. The most common pathogens detected were coagulase-negative *Staphylococcus* (12 cases), followed by *Staphylococcus aureus*, *Pseudomonas aeruginosa,* and others.

Conclusions

The findings of this study indicate that the integration of sonication with PCR markedly enhances the detection of PJI, especially in instances where standard PCR techniques may be insufficient, such as in low-grade chronic infections.

Key words: periprosthetic joint infection, polymerase chain reaction, sonification, sonication-assisted PCR, arthroplasty.

INTRODUCTION

Periprosthetic joint infection (PJI) is a serious complication that can occur following total joint arthroplasty, such as hip or knee replacement surgeries. It is characterized by the presence of infection in the area around the prosthetic joint. PJI is a challenging and costly complication that can lead to significant morbidity and impose a financial burden on the healthcare systém (2, 4, 12). The diagnosis and management of PJI are complex, with no universally accepted set of diagnostic criteria (6, 12, 23). Various definitions and criteria have been proposed to aid in the diagnosis of PJI, but there is ongoing debate and variation in the management of this condition. PJI can be acute or chronic, and distinguishing between

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septic and aseptic failures can be difficult (11). Effective diagnosis and treatment of PJI are crucial to prevent further complications and improve patient outcomes (4, 13, 24, 25).

The diagnosis of PJI can be particularly challenging in cases of low-grade chronic infection. The suspicion of infection is typically confirmed through cultures of synovial fluid and periprosthetic tissue. However, these methods may not always detect low-grade infections, which can lead to persistent infection and early failure of the prosthesis (3). There is a lack of a universal tool for the diagnosis of PJI, and various methods have been examined to increase the sensitivity of pathogen detection (7, 15).

The use of sonication in the diagnosis of periprosthetic infections of hip and knee joints has gained attention in recent years due to its potential to detect bacteria that are difficult to culture with standard tissue cultures. Sonication involves the use of ultrasound waves to dislodge bacteria from the surface of the implant, allowing for their detection in the resulting fluid. This technique has been shown to lead to clinically relevant changes in treatment, as it can detect infections that might otherwise go undetected (18).

Sonication has been found to improve the sensitivity of PJI diagnosis, particularly in cases of low-grade infection. In a study of 226 patients who underwent revision surgery of a hip or knee prosthesis, sonication was found to have a sensitivity of 80.5% and a specificity of 97.8%, compared to a sensitivity of 94.3% and a specificity of 99.3% for perioperatively taken tissue cultures (8). Although the sensitivity and specificity of sonication were lower than those of tissue cultures, sonication led to clinically relevant changes in treatment for 9% of patients suspected of infection due to a positive sonication fluid culture. This indicates that sonication may be a useful diagnostic tool in clinical practice (8, 14, 19).

However, there is limited clinical validation of sonication in the diagnosis of PJI, and the implementation of sonication in standard clinical practice remains uncertain. The purpose of the present study is to evaluate a cohort of patients with diagnosed PJI utilizing sonication and to analyze whether sonication improves the detection of PJI.

MATERIAL AND METHODS

The study prospectively monitored a cohort of 26 patients who underwent surgery at the First Department of Orthopaedic Surgery, St. Anne's University Hospital in Brno between 2019 and 2024. These patients were highly suspected of having a mitigated periprosthetic joint infection. The cohort included 16 women and 10 men, ranging in age from 56 to 82 years. Among them, 11 patients had infections involving a hip prosthesis, while 15 had infections involving a knee prosthesis (Table 1).

Table 1. **Sample characteristics**

FEATURE		VALUE
Overal (n)		26
Age at inclusion (years)		71.3 (± 7.4)
Sex		
	Male	10 (38.5%)
	Female	16 (61.5%)
Type of procedure		
	TKA	15 (57.7%)
	THA	11 (42.3%)
Comorbidities (n)		
	Yes	13 (50%)
	No	13 (50%)
Symptom duration (months)		2.93 (± 1.80)

All patients underwent a standard clinical examination, X-ray imaging, and basic laboratory tests focusing on blood count, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and D-dimer levels. Subsequently, under strict aseptic conditions, joint aspiration was performed – typically without navigation for knees and under ultrasound (USG) guidance for hips. The aspirated material was sent for both standard and extended cultures, as well as for PCR diagnostics.

After careful consideration, the medical team proceeded with revision surgery for the affected prostheses. In all cases, a two-stage approach was adopted: first, the prosthesis was explanted, followed by the temporary or, in some cases, permanent implantation of an articulating spacer, which was always supplemented with a local antibiotic carrier. No antiseptic irrigation was performed perioperatively until the prosthesis was removed.

During surgery, at least three tissue samples were taken – one from the bed of each explanted component and from the synovial lining of the joint. These samples were sent for microbiological culture examination under both standard and extended conditions. In the operating room, the explanted prosthesis components were placed into pre-prepared sterile, hermetically sealed containers filled with sterile Ringer's solution. The containers were then placed into a sterile bag and immediately transported to the Department of Microbiology, St. Anne's University Hospital for sonication.

The containers were specifically selected to ensure they fit the size of the prosthesis components, minimizing the amount of fluid in the container and reducing the dilution of the agents obtained through sonication. Immediately after sonication, the container was opened under aseptic conditions, and the sonicate was collected for further analysis. The sonicate underwent both standard and extended culture

examinations. Additionally, one sample was sent for PCR diagnostics to the same laboratory that performed the preoperative PCR test.

Antibiotics were administered in the operating room just before the start of surgery and continued until the PCR and culture results were available. After consultation with the antibiotic center at the Department of Microbiology, St. Anne's University Hospital, the treatment was adjusted based on the sensitivity results. Antiseptics and local antibiotic carriers were strictly used only after the prosthesis was removed.

Patients with clear signs of florid periprosthetic infection, such as a joint fistula, pus in the joint aspirate, significant local signs of infection (severe redness, high local joint temperature), body temperature above 38 °C, CRP levels above 50 mg/l, or leukocytosis above 20,000/ml, were not included in the cohort. These patients required immediate initiation of broad-spectrum antibiotic therapy and, therefore, did not meet the definition of a mitigated infection.

Sonification process

A modified sonication technique was used to assess microbial colonization of implants as previously described by Trampusz et al. (21). In brief, all implants were sonicated for 10 minutes in a sterilized 1L polypropylene container containing 400 mL of Ringer's solution and sealed with an airtight lid. Sonication was performed using a BANDELIN Sonorex Digiplus DK 102 P ultrasonic bath (Bandelin, Germany) at a frequency of 35 kHz. Sonication was followed by additional vortexing for 2 minutes.

Bacterial cultivation

The resulting sonication fluid was inoculated in 500 µL aliquots onto Columbia Blood Agar (Oxoid) and Chocolate Agar (Hi Media Laboratories) for 7 days at 37 °C to detect aerobic bacteria. The same volume of the sonication fluid was inoculated onto Wilkins Chalgren Anaerobic Agar with 7% sheep blood and vitamin K (Hi Media Laboratories) and incubated anaerobically for 14 days at 37 °C in an Anaerobic Work Station Concept 400 (Ruskinn Technology) with an atmosphere of 80% N_2, 10% CO_2, and 10% H_2. After incubation, the bacterial colonies growing on plates were assessed.

Identification of the isolated microorganisms was performed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). This analysis was performed on a common diagnostic platform, the MALDI Biotyper with FlexControl 3.4 software (Bruker Daltonik), according to the manufacturer's instructions. Briefly, isolated colonies were applied as a thin film onto a section of a MALDI 96-target plate, overlaid with 1 µL of 70% formic acid, and dried at room temperature. Dried bacteria were overlaid with 1 µL of matrix solution, saturated α-cyano-4-hydroxycinnamic acid solution in acetonitrile±water±trifluoroacetic acid (50:47.5:2.5, v/v) and allowed to dry before spectral acquisition. Mass spectra were processed using BioTyper 3.1 software, and the manufacturer´s recommended cut-off scores were used for identification (_2 species-level identification; 1.7±1.999 genus-level identification, and <1.7 no identification). Isolates with an initial score <1.7 were retested, and the highest score was used for identification.

Genetic analysis

DNA isolation

For genetic analysis, 4 ml of the sonication fluid was collected in a sterile DNA/RNA-free tube. Sonicate fluid underwent DNA enzymatic pre-treatment following by DNA extraction using QIAamp DNA Blood Mini kit (Qiagen, Germany) according to the manufacturer´s instruction.

PCR

PCR amplification of the 16S rRNA gene was carried out using one pair of universal primers (forward primer UNB1 and reverse primer UNB2b) covering the variable region V8-V9. PCR mixes were decontaminated with 8-methoxypsoralen and UV light cross-linking before template DNA was added and controls for potential PCR inhibitors and contamination were performed (22).

An internal standard was added into decontaminated PCR vials for confirmation, that reaction was inhibition free, followed by 5 µl of DNA template. Positive control for bacteria broad range PCR detection system (UNB), as well as negative control (PCR-grade water), and DNA isolation process control (water instead of tissue sample) were added into each PCR run.

Electrophoresis

The length of the amplified product was 371 bp that allowed discrimination of most of the clinical important bacteria. Internal standard was amplified as a 519 bp long band. Products were loaded into ethidium bromide-stained agarose gel and separated by electrophoresis.

Sanger sequencing and sequence alignment

Bands of 371 bp were cut out, the result amplicons were sequenced using Applied Biosystems BigDye Terminator v3.1 Cycle Sequencing Kit (ThermoFisher Scientific,USA), purification of DNA was performed using BigDye XTerminator Purification Kit (ThermoFisher Scientific,USA) to eliminate unincorporated dNTPs or other impurities and the product was sequenced on the ABI PRISM 3130 Genetic Analyzer (ThermoFisher Scientific,USA). We reported species identification only when sequences produced significant alignment with reference sequence higher than 99%. The highest 16S rRNA gene homology was shown using the BLAST tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and SepsiTest BLAST database (https://sepsitest-blast.com/en/index.php).

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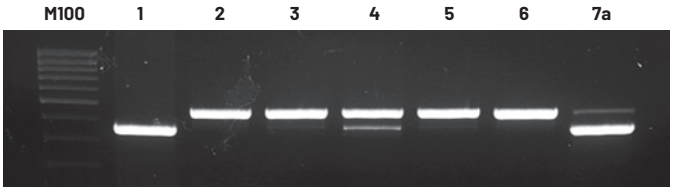

Fig. 1. **Photograph of gel electrophoresis of UNB product. Lane 1: positive ; lane 2 and 3: negative, only a band of 477 bp corresponding to internal standard is seen; lane 4: slightly positive; lane 5: negative DNA isolation process control;. lane 6: negative control; lane 7: positive control. M100: Refers to the molecular size markers, which are used as a reference to determine the size of the DNA fragments in the other lanes.**

Statistical evaluation

Statistical analysis was done using R software (version 4.0.5) in the RStudio development environment. A McNemar's test was used analyze the difference between paired proportions, the same subjects are assessed with two different methods. A p-value below the 0.05 was considered to be the threshold for statistical significance

RESULTS

In the cohort of 26 patients, periprosthetic joint infection was confirmed using the available methods. In 20 cases, the infection was detected by both the standard PCR method and PCR supplemented with sonication of the explanted material. However, in 6 samples, discrepancies were observed in the PCR results: the standard method of sampling periprosthetic fluid did not detect the infectious agent, but when sonication was added followed by PCR analysis, the infection was confirmed ($p = 0.014$). Of these 6 samples, 2 showed a quantitatively weak positive result, while 4 had a significantly positive result.

The mean CRP level of patients prior to implant removal was 26.2 ± 11.3 mg/L. The mean white blood cell (WBC) count was 8.4 ± 4.1 × 10^9/L. Elevated D-dimer levels (above 500 ng/mL) were observed in 11 patients, indicating a positive result. Radiographic signs of infection, such as radiolucent lines or implant loosening, were present in 12 patients.

The most commonly detected pathogen was *coagulase-negative Staphylococcus*, found in 12 cases. This was followed by *Staphylococcus aureus* in 4 cases, *Pseudomonas aeruginosa* in 5 cases, *Streptococcus pyogenes* in 3 cases, *Salmonella enterica* in 1 patient, and *Escherichia coli* in 1 sample (Table 2).

In the samples that showed positive microbial agents only after the additional sonication process, *coagulase-negative Staphylococcus* was identified in 3 cases (Fig. 2), *Pseudomonas aeruginosa* in 2 cases, and *Streptococcus pyogenes* in 1 case. No instances were found where culture examination of either the standardly collected fluid or the sonicate culture revealed an infection without concurrent confirmation by the PCR method.

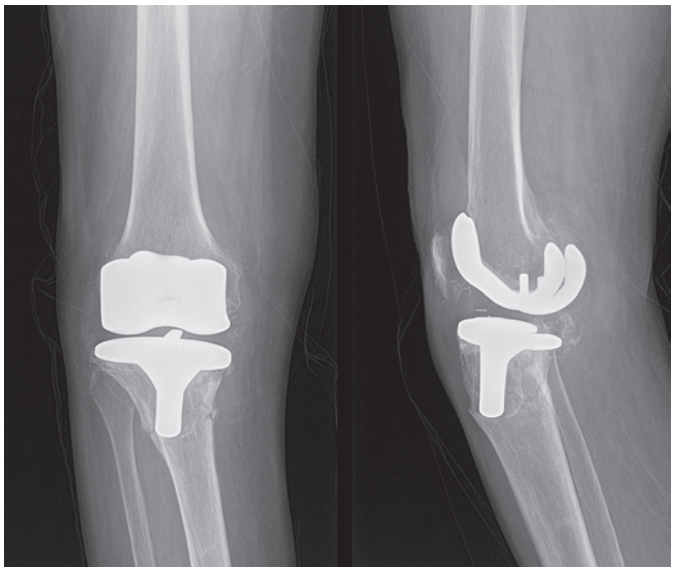

Fig. 2. **Radiographic imaging of a loose TKA in anteroposterior and lateral views (patient No. 6). Although preoperative cultures were negative, implant removal was necessary due to prosthetic loosening. *Coagulase-negative Staphylococcus* was detected exclusively through PCR analysis following sonication.**

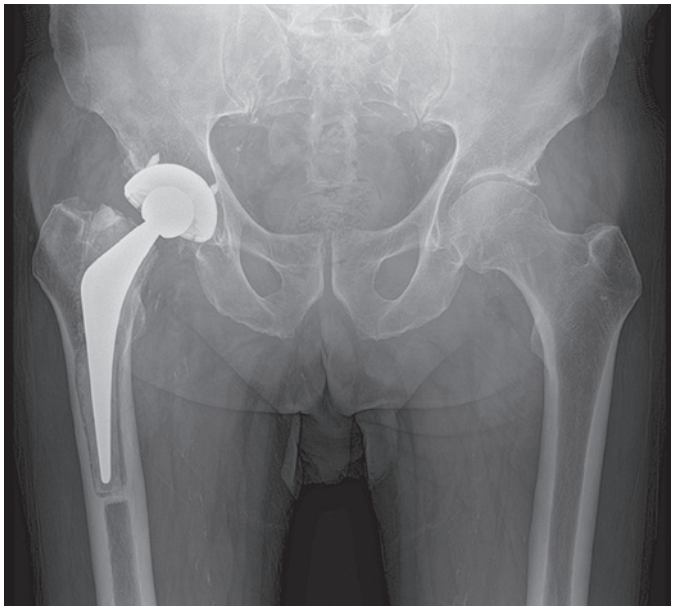

Fig. 3. **Radiographic imaging of a loose THA in anteroposterior view (patient No. 25). *Coagulase-negative Staphylococcus* was detected exclusively through PCR.**

In the culture examination of native periprosthetic fluid, pathogens were detected in 8 out of 26 samples using both standard and extended culture methods ($p < 0.001$). In the culture examination of the sonicate from the endoprosthesis, infection was detected in 9 cases using the standard culture

Table 2. **Overview of cultivation and PCR results for the presented cases, including diagnostic laboratory and radiographic examinations**

PATIENT NO.	TYPE OF PROCEDURE	CRP	LEUKOCYTES	D-DIMERS	PREVIOUS ANTIBIOTICS	RADIOGRAPHIC SIGN OF INFECTION	BACTERIA	CULTIVATION	CULTIVATION AFTER SONIFICATION	PCR	PCR AFTER SONICATION
1	THA	23	6,8	–	+	+	*Staphylococcus coag. neg.*	+	+	+	+
2	TKA	11	5,2	–	+	–	*Staphylococcus coag. neg.*	–	–	+	+
3	THA	42	11,1	–	+	+	*Staphylococcus aureus*	+	+	+	+
4	THA	31	8,6	–	+	+	*Pseudomonas aeruginosa*	+	+	+	+
5	TKA	15	9	+	+	+	*Staphylococcus coag. neg.*	–	–	–	+
6	TKA	18	7,2	+	+	–	*Staphylococcus coag. neg.*	–	–	+	+
7	THA	26	5	–	+	–	*Streptococcus pyogenes*	–	+	+	+
8	THA	41	13,3	+	+	+	*Staphylococcus coag. neg.*	+	+	+	+
9	TKA	40	7,4	–	+	–	*Staphylococcus aureus*	–	–	+	+
10	TKA	24	6,7	+	+	+	*Pseudomonas aeruginosa*	–	–	+	+
11	TKA	22	9,8	+	+	–	*Pseudomonas aeruginosa*	–	–	–	+
12	TKA	10	6,1	–	+	+	*Staphylococcus coag. neg.*	–	–	+	+
13	THA	18	7	+	+	+	*Staphylococcus coag. neg.*	+	+	+	+
14	TKA	37	7,6	+	+	–	*Streptococcus pyogenes*	–	+	+	+
15	THA	46	13,5	–	+	+	*Staphylococcus aureus*	+	+	+	+
16	TKA	38	12	+	+	–	*Salmonella enterica*	–	–	–	+
17	TKA	15	6,1	–	+	–	*Staphylococcus coag. neg.*	–	–	+	+
18	THA	13	5,5	–	+	+	*Staphylococcus coag. neg.*	–	–	+	+
19	TKA	20	6,6	–	+	–	*Staphylococcus coag. neg.*	–	–	+	+
20	THA	36	9,7	–	+	–	*Pseudomonas aeruginosa*	–	–	–	+
21	TKA	41	11,5	+	+	–	*Streptococcus pyogenes*	+	+	+	+
22	TKA	35	12,1	+	+	–	*Staphylococcus aureus*	–	–	–	+
23	TKA	17	8,8	–	+	+	*Staphylococcus coag. neg.*	–	–	+	+
24	TKA	25	9,2	+	+	–	*Pseudomonas aeruginosa*	–	–	–	+
25	THA	11	5,3	–	+	+	*Staphylococcus coag. neg.*	–	–	+	+
26	THA	27	7,9	–	+	–	*Escherichia colli*	+	+	+	+

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method, 8 of which matched the positive samples from the native material culture, and 1 was newly diagnosed. An additional positive sample was identified during the extended culture of the sonicate. In total, 10 positive samples were diagnosed using the sonicate culture with both standard and extended methods ($p = 0.157$) (Fig. 3).

DISCUSSION

The authors evaluated the efficacy of sonication in improving the detection of PJI, particularly in low-grade chronic cases where standard diagnostic methods might fail. Given the challenges of accurately diagnosing PJI, especially when traditional cultures of synovial fluid and periprosthetic tissue fall short, the study introduced sonication followed by PCR analysis as a potential solution. By using ultrasonic waves, this method dislodges bacteria from biofilms on prosthetic devices, allowing for a more effective analysis of the synovial fluid or periprosthetic tissue through PCR. The study compared the outcomes of standard PCR with sonication-assisted PCR. This approach aimed to enhance diagnostic sensitivity and specificity, thereby improving patient outcomes by more reliably identifying infections.

Numerous researchers, including Liu, Rodriguez, and Mannaerts, have previously explored the use of sonication on explanted prostheses, consistently demonstrating an improvement in the sensitivity of both traditional culture methods and PCR, with results comparable to those presented in this study (9, 10, 16, 18). Conversely, studies by authors such as Renz, Puges, and Akgün have reported that additional sonication followed by PCR analysis of sonicate fluid did not yield a significantly higher sensitivity (1, 16, 17). This discrepancy may be attributed to both pre-analytical and analytical phases of testing. In the pre-analytical phase, factors such as the method of storing explanted implants in transport media, ensuring their hermetic sealing during transport, and the speed of transport to the laboratory must be carefully considered. In the analytical phase, attention must be given to the processing of the sonicate in the microbiology laboratory and the determination of the bacterial or molecular load that is considered positive within the test parameters. Despite these challenges, the authors observed an increase in the number of positive samples using both sonicate culture and PCR diagnostics compared to traditional tissue culture and native fluid PCR analysis.

In our samples that were tested positive for microbial agents solely after the additional sonication process, we identified *coagulase-negative Staphylococcus* in 3 cases, *Pseudomonas aeruginosa* in 2 cases, and *Streptococcus pyogenes* in 1 case. Based on literature, commonly identified pathogens include *coagulase-negative staphylococci*, such as *Staphylococcus epidermidis*, which are often missed due to their biofilm-forming abilities. *Staphylococcus aureus*, including methicillin-resistant strains (MRSA), and *Pseudomonas aeruginosa*, known for its virulence and resistance mechanisms, are also frequently detected through this method (5, 16). Other notable pathogens include *Escherichia coli*, *Enterococcus*, and various anaerobic organisms, highlighting the importance of comprehensive pathogen identification in PJI management (18, 21, 26). The combination of sonication and PCR not only increases the sensitivity of detection but also enables quicker results, facilitating timely treatment interventions (20).

The limitations of this study include the relatively small sample size of 26 patients, which may limit the generalizability of the findings. Additionally, the study was conducted at a single center, potentially introducing selection bias and limiting the applicability of the results to other populations. The study focused on a specific cohort with suspected mitigated PJI, which may not reflect the broader spectrum of PJI cases. Moreover, while sonication followed by PCR improved detection in some cases, the study did not assess the long-term clinical outcomes associated with this diagnostic approach, nor did it compare the cost-effectiveness of sonication-assisted PCR with standard diagnostic methods. Finally, the study did not explore the potential for false-positive results or the impact of various sonication parameters on the accuracy of pathogen detection. On the contrary, the prospective design allowed a more systematic and controlled assessment of sonication's effectiveness in enhancing PJI detection. Furthermore, the patient selection criteria were strictly defined, ensuring a focused and reliable assessment.

CONCLUSIONS

The findings of this study indicate that the integration of sonication with PCR markedly enhances the detection of PJI, especially in instances where standard PCR techniques may be insufficient, such as in low-grade chronic infections. This improved detection capability facilitates more precise diagnoses, potentially leading to better patient outcomes by identifying elusive pathogens and thereby mitigating the risk of prosthesis failure. ■

Institutional Review Board Statement

This study was conducted according to the guidelines of the Declaration of Helsinki. Consent was not deemed necessary by the ethics committee due to study design, which was based on routine clinical data.

Informed Consent Statement

Informed consent was obtained from all subjects involved in the study.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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