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ORIGINAL PAPER

Advancing Diagnostic Techniques for Implant-Related Infections: the Synergistic Effect of Sonication and Dithiothreitol

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ABSTRACT

Purpose: *Implant-related infections represent a significant complication contributing to increased morbidity and mortality. Determining the microbial agent causing the infection is crucial for successful treatment. Despite the incidence of periprosthetic joint infections (PJIs) rising over time, no diagnostic test with 100% sensitivity is available to identify these infections accurately. The present study aims to determine whether combining sonication with Dithiothreitol (DTT) enhances the accuracy and sensitivity in diagnosing implant-related infections.*

Methods: *Specifically, the present study included 30 patients, who underwent implant removal due to suspicion of infection. Implants were divided into two segments: one was processed using the sonication method and the other one by combining DTT and sonication.*

Results: *The mean +/-SD was 81.17 +/- 67.53 CFU/mL for the sonication group and 109.7 +/-62.78 CFU/mL for the combination group.*

Conclusion: *The results of our study indicate that the combination of DTT and sonication increases the colony count by about 28.53 CFU/mL, which enhances the possibility of detecting orthopaedic implant associated infections.*

Keywords: implant-related infection, colonies, sonication, Dithiothreitol.

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INTRODUCTION

Implant-related infections, although uncommon, are the main reason for the failure of orthopedic surgeries, including both arthroplasty and internal fixation (1). Infections associated with orthopedic materials are among the most serious complications, leading to prolonged hospitalization, long-term antimicrobial therapy and additional surgeries. These further increase the risk of complications, morbidity and mortality (2).

Newer surgical techniques, prophylactic antimicrobial treatments, improved biomaterials and infection prevention measures within the operating room have significantly reduced the frequency of infections. The incidence of periprosthetic infections ranges from 0.3 to 1.9% after total arthroplasty and can reach up to 4% after revision surgeries (3, 4).

Determining the microbial agent causing the infection is crucial for treatment success. The culture of the material is the classic method for isolating the responsible microorganism, with its main disadvantage being the high rates of false-negative results (7-39%) (5, 6). The issue can arise due to previous antimicrobial treatment, fungal infections, specific types of infections, or the ability of microbes to form biofilm on the surface of orthopedic materials (7).

Sonication of the orthopaedic materials is identified as the most reliable method for isolating microorganisms involved in forming the biofilm, facilitating the diagnosis of infections (8). Dithiothreitol (DTT), a chemical compound used in microbiology, has been shown to reduce biofilm formation *in vitro* (9). Recent studies have also demonstrated its effectiveness in breaking down biofilms and detaching microbes, thereby enabling their further isolation by culture, identification and testing for sensitivity to appropriate antimicrobial agents (10).

The present study aims to determine whether the combination of sonication and DTT increases the accuracy and sensitivity of diagnosing implant-related infections. ❑

MATERIALS AND METHODS

A prospective study, including 30 patients who underwent implant removal due to suspected infection, was performed in our institution from January 2017 to July 2022. More specifically, suspicion of infection was based on the presence of raised levels of two markers of inflammation or fistula: C-reactive protein (CRP) or erythrocyte sedimentation rate (ESR). The hardware was removed during surgery and divided into two segments for each patient. Each segment was placed into a separate sterile container and then the two containers were transported to the laboratory, where they were processed within six hours. The first implant segment was processed using the sonication method and the second one by the newly tested method of combining DTT and sonication.

This study included 30 subjects (19 males and 11 females) with an average age of 51 +/- 12.82 years. The implants consisted of pins from 10 patients, six plates with screws, screws from eight patients, two nails, two implants of arthrodesis and two cups and stems of total hip arthroplasties. After removing the implants, they were sent to the microbiology department. A comparison was made between the results obtained from sonication and the combining (DTT + sonication) technique.

In the sonication method, a low-frequency and low-intensity ultrasound was performed using a specialized ultrasonic water bath (BactoSonic, Bandelin, Berlin, Germany), as described by Trampuz *et al* (11). Small-sized implants were fully immersed in a container with Ringer's solution or 0.9% NaCl, while larger implants were covered by approximately 80–90% with one of the aforementioned solutions. Subsequently, the containers with implants were vortexed for 30 s, followed by sonication at a frequency of 35–40 kHz for one minute. After sonication, an additional vortexing step was performed for 30 seconds. This was followed by quantitative inoculation with 100 µL of sonication fluid in a blood agar plate for aerobic culture at 37 °C for five days, in a chocolate agar plate in 5% CO_2 for 10 days, and in a Brucella agar plate for anaerobic culture at 37°C for 15 days. Furthermore, 3 mL of ultrasound fluid was added to thioglycolate broth at 37°C. If no growth was observed on the plates, the thioglycolic broth was further subcultured onto blood and Brucella agar plates. The drills were inspected for microbial growth daily (12). All samples were handled in a bio-safety chamber to prevent contamination. In the second container, an alternative approach

was performed to dislodge bacteria from infected implants. Initially, the implant was immersed in a phosphate-buffered solution of DTT concentration 6.48 mM (0.1% w/v DTT in phosphate-buffered saline) and stirred mechanically for 15 min at room temperature. After that, the abovementioned sonication method was performed in the second segment as well. A quantitative culture of the solution was then performed in similar conditions, as mentioned before for ultrasound fluid. Macroscopically, different microbial colonies were isolated, identified and tested for antimicrobial susceptibility by using VITEK Compact (Biomerieux, Marcy l'Etoile, France). Sonication or sonication and DTT fluid cultures were considered positive if at least five colonies grew on agar plates after 24 hours of incubation for aerobes and fungi, and up to 14 days of incubation for anaerobes. Samples with suspected contamination were excluded from the study.

Demographic and epidemiological data were recorded for all patients. Informed consent was obtained from all participants included in the study. All procedures were performed in compliance with the ethical standards of the institutional research committee (protocol code: 15239 and date of approval: 18 December 2017). ❑

RESULTS

Our study aimed to determine whether the combination of DTT and sonication increases the accuracy in diagnosing implant-related infections.

The two methods were compared using paired t-tests. Differences were considered significant when p values were equal to or less than 0.05. All calculations were performed using the statistical software package SPSS version 16.0 (SPSS Chicago, IL, USA).

Finally, a total of 30 patients (19 males and 11 females) with an average age of 51 +/- 12.82 years were enrolled in the present study. Patients' demographics are summarised in Table 1.

Paired t-test revealed a significant difference between the two methods ($p<0.05$). Regarding colony count, the mean+/-SD was 81.17 +/- 67.53 CFU/mL for the sonication group 109.7 +/-62.78 CFU/mL for the combination group.

The results of our study indicate that the combination of the two methods increases the colony count by approximately 28.53 CFU/mL, demonstrating a significant enhancement in detecting microorganisms.

N (%)	30
Age (years, mean ± SD)	51 +/- 12.82
Gender (male/female)	19/11

TABLE 1. Patients' demographic data

Microorganism	Number of samples
Staphylococcus epidermidis	13
Staphylococcus aureus	9
Staphylococcus haemolyticus	5
Staphylococcus capitis	2
Enterococcus faecalis	1

TABLE 2. Microorganisms isolated in the present study

The following microorganisms were identified: *Staphylococcus epidermidis* in 13 samples (43.33%), *Staphylococcus aureus* in nine (30%), *Staphylococcus haemolyticus* in five (16.67%), *Staphylococcus capitis* in two (6.67%) samples and *Enterococcus faecalis* in one sample (3.33%). All infections were monomicrobial (Table 2). In two patients, sonication yielded negative results, while the combination of the two techniques lead to positive results, demonstrating the added diagnostic value of combining these methods. This underscores the value of using multiple diagnostic approaches to improve detection accuracy and ensure more reliable identification of implant-related infections. ❑

DISCUSSION

Orthopaedic procedures using implants have increased nowadays, with a rise in implant-associated infections being seen too (6, 7). Accurate identification of the causative agent is important for treatment success. Relying solely on clinical evaluation alone is often inefficient for diagnosing implant-related infection, making specific microbiological methods essential. The success rate of treating prosthetic infections can be significantly enhanced with early diagnosis.

In cases where there has been previous antibiotic therapy, fungal infection, a low-grade agent, or the presence of biofilm drive in false negative results of a tissue culture (13). Although sonication has some well-known limitations, it remains the gold standard because biofilm disruption *in vitro* is appropriate for diagnosing periprosthetic joint infections. Due to its disad-

vantages, an alternative method that is reliable and readily available is highly desirable.

A study by Wu *et al* reported that DTT could inhibit biofilm formation (13). Drago *et al* showed that DTT and sonication were evaluated on different parts of the implants, in patients with aseptic loosening and periprosthetic joint infection (14). Dithiothreitol demonstrated similar specificity to sonication but higher sensitivity. Using distinct techniques for different parts of an arthroplasty assumes that the infection in both components involves the same bacteria in both qualitative and quantitative terms. This approach may lead to missed information and potentially overlook differences in the microbial populations affecting each component.

In a recent study, a comparison between sonication and DTT was performed at the same implant and the analysis of the two methods suggested that DTT holds comparable efficacy to sonication in detecting infection (15). In another study, the authors processed some infected materials with both DTT and sonication. Their results indicated that the mean colony counts were comparable between the two techniques (16). In contrast to the aforementioned findings, a recent study compared the effectiveness of EDTA and DTT, demonstrated that sonication was superior to the chemical methods for some common microorganisms (17). A meta-analysis by Tsikopoulos *et al* did not establish any significant difference between the diagnostic potential of sonication and the chemical-based biofilm methods (18). Moreover, there is no evidence that DTT affects bacterial viability when used at the concentration typically employed in clinical diagnostics (0.1%) (16, 18).

Increasing CFU (Colony Forming Units) through a diagnostic method is important for several reasons: it allows for more accurate, reliable and detailed information about the presence and characteristics of microorganisms in a sample. This is vital for diagnostics, treatment, safety, and research purposes. In some samples, microorganisms may be present in low concentrations, making direct detection difficult. By culturing the sample and allowing microorganisms to grow and form colonies, even small numbers of microorganisms can be detected. Early diagnosis of enhanced sensitivity can lead to earlier detection of infections, which is crucial for timely treatment and intervention.

Based on our findings, combining these two methods enhances the detection of colonies, leading to a greater possibility of diagnosing implant-associated infections. The reason for this is that the combination ensures a more efficient breakdown of the biofilm and more cultures are created. Therefore, in a material with low microbial load, sonication alone would yield negative results, while the combination of the two methods would allow diagnosis to be done. ❑

CONCLUSION

Our study demonstrates that integrating DTT and sonication with specific bacterial broths represents a promising strategy for improving the detection of biofilm-related infections on orthopedic prosthetic devices. This combined approach significantly enhances bacterial growth and viability, leading to more accurate and reliable diagnoses. By leveraging the strengths of both DTT and sonication, along with the added benefits of bacterial enrichment, it is essential to conduct additional studies across multiple institutions to validate these promising results further and ensure their broader applicability and reliability across diverse clinical settings. ❑

Conflicts of interest: none declared.

Ethical approval: All data utilized in this manuscript was collected after informed consent was given for anonymized data collection and use was given by patients. Local ethical approval was established before the collection of data.

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