Sonication to improve the yield in culture-negative peri-prosthetic joint infection

Noha Tharwat Abou El-Khier, Samah Sabry El-Kazzaz, Adham Elgeidi and Abd El Rhman Elganainy

Medical Microbiology & Immunology Department, Faculty of Medicine, Mansoura University, Al Mansurah, Egypt; Orthopaedics and Traumatology Department, Faculty of Medicine, Mansoura University, Al Mansurah, Egypt

ABSTRACT

Peri-prosthetic joint infection (PJI) is a catastrophic complication, after joint arthroplasty. Culture-negative PJI presented 7–39% of PJI cases at most health-care centers. We aimed to investigate the diagnostic accuracy of sonication fluid culture (SFC) with the respective peri-prosthetic tissue cultures (PTC). Also, to evaluate the efficacy of sonication in PTC-negative PJI. Peri-prosthetic tissue specimens were subjected to microbiological culture and histo-pathological examination. Retrieved prosthesis components were sonicated. SFC was examined. One hundred and seventy patients had PJI and 95 were aseptic failures. SFC was positive in 120 patients (70.5%). For PTC, 80 of 170 samples (47.1%) were positive; all reveal the same bacterial species with the corresponding SFC. In 50 patients, both PTC and SFC cultures of the prosthesis were negative. There were 40 negative PTC samples proved to be positive by SFC, 39 displayed Staphylococcus epidermidis, and only one sample yielded Propionibacterium acne. Biofilm formation was detected in 22 (56.4%) out of the 39 S. epidermidis isolates. The overall sensitivity of SFC compared to PTC was (70.6% versus 47.1%). SFC represents a sensitive, accurate, easy diagnostic strategy representing increased sensitivity contrasted with PTC. S. epidermidis, although an important commensal, is considered the most significant pathogen in the context of PJI.

Introduction

Peri-prosthetic joint infection (PJI) represent a major therapeutic challenge after total joint arthroplasty [1,2]. Although PJI is a relatively rare complication with an incidence of 1–3% for primary arthroplasty [3–5], it may bring about multiple surgical procedures, prolonged antibiotics courses which constitutes a considerable economic burden on patients, surgeons, clinics, health-care systems, and community all together, besides a mortality rate of up to 7% [6]. PJI stand-out amongst the most widely recognized reasons for revision hip and knee arthroplasties [7]. The diagnosis and management of PJI remain an enigma as it is crucial to discrepant between septic and aseptic failure since management is entirely dissimilar [1,8].

Despite the existence of definite signs of infection, cultures may be negative in 7–39% of PJI cases. This occurs due to many factors; patchy distribution of infection, low inoculum of infection, the lack of sensitivity and specificity of standard culture methods because of sampling limitations (i.e. excessive cauterization), the presence of non-recoverable biofilm-embedded bacteria on the prosthetic material, fastidious
organisms, and the prior antibiotic therapies [9,10].

The principal problem in the management of PJI is that microorganisms form biofilms to protect themselves from environmental influences [11]. Numerous diverse foreign materials are frequently implanted in arthroplasty surgery, e.g. bone cement, polyethylene compounds, and diverse metal composites. These biomaterials are remote bodies that deliver surfaces for bacteria to adhere and consequently biofilm formation [12]. Biofilm offers defensive barrier to organisms, bringing about resistance to antimicrobials and host immune responses [13–15]. It is troublesome for the antibiotics and the immune system to eradicate the bacterial cells embedded in the biofilm. The bacteria in biofilms usually provoke less inflammatory response than the planktonic bacterial cells, which makes it increasingly hard to isolate; thus, the diagnosis of these infections necessitates techniques unlike those used in conventional microbiology workrooms [16,17].

Biofilm disruption procedures to ‘mobilize’ the microorganism have been portrayed. The sonication method of the retrieved prosthesis is a procedure performed to expand the detectability of microorganisms. Nevertheless, not all centers are well appointed to implement sonication of the retrieved prostheses constraining the utilization of this procedure. The advantages of sonication incorporate the simplicity, reproducibility, and ability to yield viable microorganisms that can be subjected to antimicrobial susceptibility testing. Likewise, the sonicate fluid normally yields a high number of organisms, which may help in discrimination of contaminated prostheses [18,19].

The objective of present study was to compare sonication with the more established microbiological peri-prosthetic tissue cultures (PTC), in diagnosis of PJI and to validate the usefulness of sonication to improve yield in cases with PTC-negative PJI.

Subjects and methods

This is a case–control study of 265 patients with a history of arthroplasty (knee or hip) and were admitted to Mansoura University Hospital (MUH), Mansoura, Egypt for staged revision surgery (due to Persistent pain and/or loosening of the prosthesis) over 4 years period between September 2014 to August 2018. Patients comprised 135 males and 130 females with age range from 38 to 72 years (mean 58.4 years). Two hundred and six patients had hip prosthesis and 59 patients with knee prosthesis. Patients with malignancies or patients with chronic inflammatory conditions (e.g. rheumatoid arthritis) were not enrolled. We also excluded patients when there was an obvious contamination of a removed prosthesis occurred in the operating room, the implant did not fit the container provided or less than three Peri-prosthetic tissue specimens were submitted for PTC. Receipt of antimicrobial agents during the 14 days before removal of the prosthesis or Peri-operative antimicrobial prophylaxis were also considered as exclusion criteria.

Under complete aseptic conditions, blood samples were withdrawn from all patients within 2 h preoperatively. White blood cell count (WCC), C-reactive protein (CRP) and erythrocyte sedimentation rates (ESR) were assessed.

Six Peri-implant tissue samples from the bone–cement/bone–prosthesis interface, from capsule and from soft tissues with most observable inflammatory changes were collected intraoperatively. Two formalin preserved, paraffin-embedded samples were sent for histopathological assessment, and other samples were collected into sterile surgical containers and instantly sent to the microbiology laboratory for PTC.
Peri-prosthetic tissue samples were homogenized in 3 ml Tryptase soy broth using mortar and pestle for 1 min. Aliquots of 0.1 ml of homogenate were inoculated onto aerobic blood agar, chocolate agar, MacConkey agar (Oxoid), and anaerobic blood agar (Remel). Aerobic cultures were incubated at 37°C for 3 days, examined daily. Anaerobic cultures were incubated in an anaerobic jar at 35°C for 14 days. Identification of the isolates was done according to the standard microbiological methods [20]. A positive culture was defined as growth of the same organism in two or more tissue specimens, except for Staphylococcus aureus (S. aureus), for which one positive specimen was needed.

Isolates retrieved from hip and knee prostheses by sonication protocol as described previously by Vergidis et al. [21]. Each of the explanted prostheses was placed in a sterile container and 400 ml of ringer lactate solution was added to it before being submitted to the microbiology laboratory. It was handled within 6 h of removal. The container was vortexed during 30 s, sonicated for 1 min at 40 kHz, and vortexed for 30 s at room temperature. The resulting sonication fluid was concentrated by centrifugation at 2,000 x g for 20 min and plated in aliquots of 0.1 ml onto aerobic and anaerobic blood agar plates and processed and identified in the same manner as PTC. A positive SFC was defined as growth of at least 20 colony forming unit (CFU) from any plate.

Biofilm production of Staphylococcus epidermidis (S. epidermidis) isolates was assessed by Congo red agar (CRA) method as previously described by Freeman et al. [22]. Isolates with black colonies were considered biofilm-producers, and those with red colonies were considered non-biofilm producers.

Enrolled patients were assigned into two groups; Septic (PJJ) and aseptic loosening of prosthesis groups according to diagnostic criteria of PJ proposed by Musculoskeletal Infection Society (MSIS) (Table 1) [23]. Absence of any of these criteria was defined as aseptic loosening or an implant failure.

| Major criteria | A sinus tract communicating with the joint, OR Two positive PTC with phenotypically identical organisms |
| Minor criteria | Serum ESR > 30 mm/h AND CRP > 1 mg/dL |
| | Synovial fluid WBC > 3000 OR ++ change on leukocyte esterase test strip |
| | Synovial fluid PMNL percentage > 80% |
| | Positive histologic analysis of peri-prosthetic tissue (>5 neutrophils/HPF in 5 HPFs (x 400)) |
| | A single positive culture |

**Ethical approval**

The protocol of this study was accepted by Institutional Review Board (IRB), Faculty of Medicine, Mansoura University; code number: R/16.12.81.

**Statistical analysis**

Data were processed and analyzed using IBM SPSS statistics (version 22). Categorical data were expressed in form of count and percent while continuous data were expressed in form of mean ±SD. Two-by-two contingency tables were used to calculate the sensitivities, specificities, positive predictive values (PPV) and negative predictive values (NPV) of the different culture methods. Ninety-five percent confidence intervals (95% CI) were calculated as exact binomial confidence intervals. McNamara`s test of paired proportions was used to compare between sensitivities of the SFC and PTC methods. Probability levels ≤0.05 were considered statistically significant.

**Results**

Two hundred and sixty-five patients (135 males, 130 females) were admitted for staged revision surgery after knee or hip arthroplasties. The age
of enrolled patients ranged from 38 to 72 years (Mean: 58.4 years). Demographic data and results of preoperative inflammatory markers (ESR, WCC, and CRP) prior to explantation are shown in Table 2. Of the enrolled patients, PJI was definitely diagnosed based on the MSIS criteria in 170 patients out of 265 (64.2%), while the others 95 of 265 (35.8%) were diagnosed as aseptic failure of the prostheses. SFC was positive in 120 patients (70.5%). The isolates were: 57 S. epidermidis (42 methicillin-resistant), 26 S. aureus (9 methicillin-resistant), 14 Escherichia coli, 9 Pseudomonas aeruginosa, 9 Proteus mirabilis, and 6 Klebsiella pneumoniae, one Serratia marcescens, one Enterococcus faecalis, and one P. acnes. In four of 120 (3.3%) infected implants, mixed infections were found. For PTC, 80 of 170 samples (47.1%) were positive, all reveal the same bacterial species with the corresponding SFC. All positive PTC were also confirmed by the SFC (Table 3). In 40 PTC-negative samples that were proved to be positive by sonication, 39 revealed S. epidermidis, and only one sample showed P. acnes. For the 39 S. epidermidis isolates we assessed biofilm production by CRA method; biofilm formation was detected in 22 (56.4%) out of the 39 S. epidermidis isolates, which were evidenced by their rough black colonies. The remaining 17 (43.6%) isolates were non-biofilm producers since they formed smooth red colonies. Table 4 demonstrates the sensitivity of the SFC versus the PTC in study patients. The global sensitivity of SFC was 70.6% (95% CI 63.13% to 77.32%), and that of PTC was 47.1% (95% CI 39.37% to 54.85%, P-value <0.001). Moreover, SFC had the same specificity as PTC (100%).

Discussion

Being a huge economic and personal burden, PJIs recently dragged the researchers’ attention. Several diagnostic modalities were developed to identify the pathogen. These modalities ranged from laboratory tests like CRP and IL-6 to radiological studies like bone scan passing by histopathological and microbiological studies of intraoperative samples.

Several studies have shown that histopathology is more sensitive than other diagnostic methods. Nevertheless, it has the drawback that it does not reveal the causative pathogen, and consequently, the proper antibiotic treatment could not be achieved. Contrariwise, PTC provide the optimal antibiotic. However, culture-negative PJI occurs in

| Table 2. Characteristics of the enrolled patients. |
|---------------|------------------|------------------|---------|
| Characteristics | Patients with PJI (n = 170) | Patients with aseptic loosening (n = 95) | P value |
| Demographic data | | | |
| Age | 58.3 | 57.6 | 0.07 |
| Sex | 3.4 | 2.3 | |
| Male | 95 (55.9%) | 40 (42.1%) | 0.03 |
| Female | 75 (44.1%) | 55 (57.9%) | |
| Arthroplasty | | | |
| Knee | 38 (22.4%) | 21 (22.1%) | 0.9 |
| Hip | 132 (77.6%) | 74 (77.9%) | |
| Inflammatory markers | | | |
| ESR (mm/hour) | | | |
| Mean | 98 | 20 | 0.0001 |
| SD | 30.2 | 5.6 | 0.0001 |
| WCC (cell x 10^9/L) | | | |
| Mean | 13.2 | 5.6 | 0.0001 |
| SD | 1.8 | 2.1 | |
| CRP (mg/L) | | | |
| Mean | 60.9 | 17.1 | 0.0001 |
| SD | 51.2 | 9.3 | |
7–39% of PJI cases at most health-care centers, a reality that frequently misdirects the diagnosis [24,25].

The precise diagnosis of PJI is difficult because of biofilm-forming ability of some bacteria on the surface of the prostheses. Moreover, when the bacteria encased in a biofilm, it becomes recalcitrant to antibiotic therapy and to immune surveillance. In addition, biofilm masks the organism and leads to false-negative cultures [26].

The best available option to dislodge bacteria colonizing the retrieved prosthesis is the sonication. The use of ultrasound to dislodge bacteria attached to prostheses was included in the ISO (International Standardization Organization) standards. This procedure comprises low-frequency ultrasound waves going through the fluid around the prosthesis and making zones of high and low pressure. Bacteria are freed from the surface of the prosthesis when microscopic bubbles that are formed during the low-pressure stage collapse during the high-pressure stage. The liquid encompassing the prosthesis with the liberated bacteria can be submitted for culture [27].

Our study results showed a statistically significant difference between sensitivities of SFC and PTC; SFC of the extracted orthopedic prostheses were more sensitive than PTC (70.6% and 47.1%, respectively, p < 0.001). This was in agreement with a study conducted by Piper and his colleagues (2009) [28] who reported that SFC was more sensitive than PTC (66.7% versus 54.5%, p = 0.046). In meta-analysis by Liu et al., it has been shown that SFC was valuable for the diagnosis of PJI and it was more sensitive than traditional PTC [29].

In 40 patients with negative PTC, explant SFC identified microorganisms responsible for infections, supporting the speculation, that sonication increases the possibility of bacterial isolation in culture-negative PJsIs. This was in accordance with former studies [30,31]. Moreover, the higher sensitivity of SFC, ranging from 67% to 91%, as

| Culture results | Number of patients | Number of isolates | Isolated microorganisms (number of patients) |
|-----------------|--------------------|--------------------|---------------------------------------------|
| SFC +, PTC+     | 80                 | Monomicrobial      | Staphylococcus aureus (26)                    |
|                 |                    |                    | Staphylococcus epidermidis (18)              |
|                 |                    |                    | Escheriecha coli (12)                        |
|                 |                    |                    | Pseudomonas aeruginosa (8)                   |
|                 |                    |                    | Proteus mirabilis (7)                        |
|                 |                    |                    | Klebsiella pneumoniae (4)                    |
|                 |                    |                    | Serrata marcescens (1)                       |
|                 |                    | Polymicrobial      | Pseudomonas aeruginosa and Klebsiella pneumoniae (1) |
|                 |                    |                    | Proteus mirabilis and Escheriecha coli (1)    |
|                 |                    |                    | Escheriecha coli and Enterococcus faecalis (1) |
|                 |                    |                    | Proteus mirabilis and Klebsiella pneumoniae (1) |
|                 |                    | Monomicrobial      | Staphylococcus epidermidis (39)              |
|                 |                    |                    | Propionibacterium acne (1)                  |
| SFC+, PTC-      | 40                 | Monomicrobial      | Staphylococcus aureus (39)                   |
| SFC-, PTC+      | 0                  | Monomicrobial      | Staphylococcus epidermidis (18)              |
| SFC-, PTC-      | 50                 | Monomicrobial      | Staphylococcus aureus (26)                   |

SFC; sonicate fluid culture; PTC: peri-prosthetic tissue cultures

| Table 3. Comparison of SFC and PTC in patients with PJI (n = 170). |
|--------------------|---------------|---------------|
| Culture results    | Number of patients | Number of isolates |
| SFC +, PTC+        | 80             | Monomicrobial  |
| SFC+, PTC-         | 40             | Monomicrobial  |
| SFC-, PTC+         | 0              | Monomicrobial  |
| SFC-, PTC-         | 50             | Monomicrobial  |

| Table 4. Sensitivity, Specificity, PPV, NPV of SFC versus PTC. |
|-----------------|--------------|--------------|
| SFC              | PTC           |
| Sensitivity*     | 70.59%       | 47.06%       |
| Specificity      | 100%         | 100%         |
| PPV              | 100%         | 100%         |
| NPV              | 65.52%       | 51.35%       |

*Sensitivity, Specificity, Positive Predictive Value, Negative Predictive Value of SFC versus PTC.

*P Value < 0.001
opposed to PTC, has been confirmed by many previous studies [17,30–33]. In contrast, Kempthorne et al. [34] have reported that SFC is less sensitive than PTC; this may be explained by the lack of histopathological examination that has underestimated the true incidence of PJI. In addition, Van Diek et al. [35] previously screened for infections in revision surgery and reported that SFC is less sensitive than PTC. Underestimation of SFC sensitivity might be due to the difference in the CFU cutoff used for a positive SFC, and mis-classification bias of the type of infection.

Sonication methodology is straightforward, reproducible and can be easily applied in most microbiology research facilities. Sonication dislodges bacteria from the implant permitting them to be cultured, without distressing their viability [16]. Nevertheless, while sonication classically harvests higher bacterial count, false-positive results should be considered, which could be attributed to contamination of the explanted prosthesis at the operating room or during transportation to the laboratory. However, at least 20 CFU per plate [28] was used as a cutoff value in this study to define a positive SFC and thus reducing the risk of false-positive results.

In this study, the CRA test as a qualitative method detected 43.6% isolates (17/39) as biofilm producers, which was quite similar to Ruzicka et al. who reported biofilm production in 64 out of 147 (43.5%) isolates by CRA method. Arslan and Ozkardes/Satorres and Alcara´z also reported biofilm formation with CRA test in 38.5% and 41.3% of Staphylococci isolates, respectively [36–38].

Generally, the distribution of bacterial isolates recovered in our study are similar to those conveyed in the previous studies, that the S. aureus and S. epidermidis represent nearly 50% of all PJIs [10,16,39].

Matching with our results, about 80% of S. epidermidis isolates are methicillin-resistant S. epidermidis (MRSE) according to previous epidemiological studies. Contrary to our results, reports showed that approximately 60% of S. aureus invasive infections were due to methicillin-resistant strains (MRSA) [10].

There are some limitations in our study; first, the relatively small number of the enrolled patients. Second, only hip and knee arthroplasties were included. Third, despite intraoperative peri-prosthetic tissue samples were gathered according to surgeon’s judgment with most evident inflammatory changes; there is possibly the tissue picked is not infected. Fourth, detection of bacterial DNA by molecular techniques was not applied and this might have resulted in our failure to detect non-cultivable or highly fastidious bacteria. Fifth, Patients who received antibiotics within 2 weeks preoperatively were not enrolled. Therefore, we did not have the chance to survey the impact of this parameter on the comparison between SFC and PTC.

Conclusions

Sonication of removed arthroplasty components appeared to improve microbiologic diagnosis of PJI. SFC represents a simple, delicate, and sensitive diagnostic methodology contrasted with PTC. Taking into consideration that PTC are sometimes negative in patients with PJIs, we recommend the addition of sonication protocol to the regular work-up of patients with assumed PJI to potentially improve the yield of ordinary culture methods, consequently, reducing the number of culture-negative PJIs. S. epidermidis, although an important commensal, could be considered as the most significant pathogen in the context of PJI. Screening for biofilm is recommended for retrieved orthopedic prostheses.

Our conclusions requisite confirmation in a larger cohort of patients including all other joint arthroplasties and different orthopedic implants.
Summary table

What is known about this subject:
- The precise diagnosis of PJI is difficult because of biofilm-forming ability of some bacteria on the surface of the prosthesis.
- Despite the existence of definite signs of infection, PTC may be negative in 7–39% of PJI cases.
- Sonication dislodges bacteria from the implant permitting them to be cultured, without distressing their viability.

What this paper adds:
- SFC represents a simple, delicate, and sensitive diagnostic methodology contrasted with PTC.
- Sonication of removed arthroplasty components appeared to improve microbiologic diagnosis of culture-negative PJI.

Disclosure statement

No potential conflict of interest was reported by the authors.

ORCID

Noha Tharwat Abou El-Khier http://orcid.org/0000-0003-4978-0987

References

[1] Buttaro MA, Tanoira I, Comba F, et al. Combining C-reactive protein and interleukin-6 may be useful to detect periprosthetic hip infection. Clin Orthop Relat Res. 2010;468:3263–3267.
[2] Miyamae Y, Inaba Y, Kobayashi N, et al. Quantitative evaluation of periprosthetic infection by real-time polymerase chain reaction: a comparison with conventional methods. Diagn Microbiol Infect Dis. 2012;74:125–130.
[3] Phillips JE, Crane TP, Noy M, et al. The incidence of deep prosthetic infections in a specialist orthopaedic hospital: a 15-year prospective survey. J Bone Joint Surg Br. 2006;88:943.
[4] Kurtz SM, Lau E, Schmier J, et al. Infection burden for hip and knee arthroplasty in the United States. J Arthroplasty. 2008;23:984–991.
[5] Jamsen E, Varonen M, Huhtala H, et al. Incidence of prosthetic joint infections after primary knee arthroplasty. J Arthroplasty. 2010;25:87.
[6] Poultides LA, Liaropoulos LL, Malizos KN. The socioeconomic impact of musculoskeletal infections. J Bone Joint Surg Am. 2010;92:e13.
[7] Ong KL, Kurtz SM, Lau E, et al. Prosthetic joint infection risk after total hip arthroplasty in medicare population. J Arthroplasty. 2009;24(6):105.
[8] Wetters NG, Berend KR, Lombardi AV, et al. Leucocyte esterase reagent strips for the rapid diagnosis of periprosthetic joint infection. J Arthroplasty. 2012;27(8):8–11.
[9] Berbari EF, Marculescu C, Sia I, et al. Culture-negative prosthetic joint infection. Clin Infect Dis. 2007;45(9):1113–1119.
[10] Reznicke J, Hewlett A. Diagnostic and treatment considerations for prosthetic joint infections: sonication and new gram-positive agents. Curr Treat Options Infect Dis. 2015;7:335–341.
[11] Yang L, Liu Y, Wu H, et al. Combating biofilms. FEMS Immunol Med Microbiol. 2012;65:146–157.
[12] Arciola CR, Campoccia D, Ehrlich GD, et al. Biofilm-based implant infections in orthopaedics. Adv Exp Med Biol. 2015;830:29–46.
[13] Darouiche RO, Dhir A, Miller AJ, et al. Vancomycin penetration into biofilm covering infected prostheses and effect on bacteria. J Infect Dis. 1994;3:170.
[14] Brady RA, Leid JG, Camper AK, et al. Identification of Staphylococcus aureus proteins recognized by the antibody-mediated immune response to a biofilm infection. Infect Immun. 2006;74(6):3415–3426.
[15] Bjarnsholt T, Kirketerp-Moller K, Jensen PO, et al. Why chronic wounds will not heal: A novel hypothesis. Wound Repair Regen. 2008;16(1):2–10.
[16] Trampuz A, Piper KE, Jacobson MJ, et al. Sonication of removed hip and knee prostheses for diagnosis of infection. N Engl J Med. 2007;357:654–663.
[17] Esteban J, Gómez-Barrena E, Cordero J, et al. Evaluation of quantitative cultures from sonicated retrieved orthopaedic implants in the diagnosis of orthopaedic infection. J Clin Microbiol. 2008;46(2):488–492.
[18] Della Valle C, Parvizi J, Bauer TW, et al. Diagnosis of periprosthetic joint infections of the hip and knee. J Am Acad Orthop Surg. 2010;18(12):760–770.
[19] Parvizi J, Ghanem E, Menashe S, et al. Periprosthetic infection: what are the diagnostic challenges? J Bone Joint Surg Am. 2006;88(4):138–147.
[20] Roberts L. Specimen collection and processing. In: Mahon CR, Lehman DC, Manuselis G, editors. Textbook of diagnostic microbiology e-book 4th ed., Elsevier Health Sciences. Chapt. 6: 2007. 111–125.
[21] Vergidis P, Greenwood-Quaintance KE, Sanchez-Sotelo J, et al. Implant sonication for
the diagnosis of prosthetic elbow infection. J Shoulder Elbow Surg. 2011;20:1275–1281.

[22] Freeman DJ, Falkiner FR, Keane CT. New method for detecting slime production by coagulase negative staphylococci. J Clin Pathol. 1989;42:872–874.

[23] Parvizi J, Gehrke T. International consensus group on periprosthetic joint infection. definition of periprosthetic joint infection. J Arthroplasty. 2014;29:1331.

[24] Osmon DR, Berbari EF, Berendt AR, et al. Diagnosis and management of prosthetic joint infection: clinical practice guidelines by the infectious diseases society of America. Clin Infect Dis. 2013;56(1):1–10.

[25] Tohtz SW, ¨uller MM, Morawietz L, et al. Validity of frozen sections for analysis of peri-prosthetic loosening membranes. Clin Orthop Relat Res. 2010;468(3):762–768.

[26] Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. Science. 1999;284(5418):1318–1322.

[27] Gómez-Barrena E, García-Rey E. Sonication of removed implants in the infected total knee arthroplasty. In: Rodríguez-Merchán E, Oussedik S, editors. The infected total knee arthroplasty (pp 117–121). Cham: Springer; 2018.

[28] Piper KE, Jacobson MJ, Cofield RH, et al. Microbiologic diagnosis of prosthetic shoulder infection by use of implant sonication. J Clin Microbiol. 2009;47(6):1878–1884.

[29] Liu H, Zhang Y, Li L, et al. The application of sonication in diagnosis of periprosthetic joint infection. Eur J Clin Microbiol Infect Dis. 2016;36(1):1–9.

[30] Tani S, Lepetsos P, Stylianakis A, et al. Superiority of the sonication method against conventional periprosthetic tissue cultures for diagnosis of prosthetic joint infections. Eur J Orthop Surg Tr.2018;28(1):51–57.

[31] Sebastian S, Malhotra R, Sreenivas V, et al. Sonication of orthopaedic implants: A valuable technique for diagnosis of prosthetic joint infections. J Microbiol Methods. 2018;146:51–54.

[32] Puig-Verdie L, Alentorn-Geli E, Gonzalez-Cuevas A, et al. Implant sonication increases the diagnostic accuracy of infection in patients with delayed, but not early, orthopaedic implant failure. Bone Joint J. 2013;95-B:244–249.

[33] Dapunt U, Lehner B, Burckhardt I, et al. Evaluation of implant sonication as a diagnostic tool in implant-associated infections. J Appl Biomater Funct Mater. 2014;12:135–140.

[34] Kemphorne JT, Ailabouni R, Raniga S, et al. Occult infection in aseptic joint loosening and the diagnostic role of implant sonication. Biomed Res Int. 2015;2015.

[35] Van Diek FM, Albers CGM, Van Hooff ML, et al. Low sensitivity of implant sonication when screening for infection in revision surgery. Acta Orthop. 2017;88(3):294–299.

[36] Ruzicka F, Hola V, Votava M, et al. Biofilm detection and clinical significance of Staphylococcus epidermidis isolates. Folia Microbiol (Praha). 2004;49(5):596–600.

[37] Satorres SE, Alcara´z LE. Prevalence of icaA and icaD genes in Staphylococcus aureus and Staphylococcus epidermidis strains isolated from patients and hospital staff. Cent Eur J Public Health. 2007;15(2):87–90.

[38] Arslan S, Ozkardes F. Slime production and antibiotic susceptibility in staphylococci isolated from clinical samples. Memorias Do Instituto Oswaldo Cruz. 2007;102(1):29–33.

[39] Zimmerli W, Trampuz A, Ochsner PE. Prosthetic-joint infections. N Engl J Med. 2004;351:1645–1654.