



## ■ INFECTION

# Detecting the presence of bacterial RNA by polymerase chain reaction in low volumes of preoperatively aspirated synovial fluid from prosthetic joint infections

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**Aims**

Preoperative diagnosis is important for revision surgery after prosthetic joint infection (PJI). The purpose of our study was to determine whether reverse transcription-quantitative polymerase chain reaction (RT-qPCR), which is used to detect bacterial ribosomal RNA (rRNA) preoperatively, can reveal PJI in low volumes of aspirated fluid.

**Methods**

We acquired joint fluid samples (JFSs) by preoperative aspiration from patients who were suspected of having a PJI and failed arthroplasty; patients with preoperative JFS volumes less than 5 ml were enrolled. RNA-based polymerase chain reaction (PCR) and bacterial culture were performed, and diagnostic efficiency was compared between the two methods. According to established Musculoskeletal Infection Society (MSIS) criteria, 21 of the 33 included patients were diagnosed with PJI.

**Results**

RNA-based PCR exhibited 57.1% sensitivity, 91.7% specificity, 69.7% accuracy, 92.3% positive predictive value, and 55.0% negative predictive value. The corresponding values for culture were 28.6%, 83.3%, 48.5%, 75.0%, and 40.0%, respectively. A significantly higher sensitivity was thus obtained with the PCR method versus the culture method.

**Conclusion**

In situations in which only a small JFS volume can be acquired, RNA-based PCR analysis increases the utility of preoperative puncture for patients who require revision surgery due to suspected PJI.

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**Keywords:** Prosthetic joint infection, RNA, Polymerase chain reaction, Aspirated synovial fluid, Low volume

**Article focus**

■ This study surveyed the value of reverse transcription-quantitative polymerase chain reaction (RT-qPCR) in revealing PJI with low volumes of aspirated fluid preoperatively.

**Key messages**

■ The diagnostic efficiency between RNA-based polymerase chain reaction (PCR) and bacterial culture was compared, and a significantly higher sensitivity was

obtained with the PCR method versus the culture method.

**Strengths and limitations**

■ In the present study, we analyzed cases in which less than 5 ml of a joint fluid sample (JFS) was obtained preoperatively and evaluated a PCR-based method for preoperative detection that is less dependent on the sample volume and concentration of bacteria.

■ The small number of samples (21) used in this study is a limitation.

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## Introduction

Prosthetic joint infection (PJI) is a serious complication of arthroplasty. The incidence rate of PJI for primary arthroplasty is 0.5% to 3.0%, whereas that following revision surgery is between 4.0% and 6.0%.<sup>1,2</sup>

Because different treatment strategies are used for patients with suspected PJI (including PJI and aseptic loosening) who require revision surgery, a clear preoperative diagnosis of suspected PJI is ideal. However, imaging methods of preoperative diagnosis have weak sensitivity and low specificity;<sup>3</sup> serological inflammatory tests are highly sensitive<sup>4</sup> but have low specificity.<sup>5,6</sup> Preoperative joint fluid aspiration and culture are indicated if an infection is suspected due to the results of the above tests.<sup>7</sup> Therefore, the utility of preoperative aspiration culture has been frequently questioned because of its high false-negative rate, which can be caused by microbe-related factors, e.g. a paucity of bacteria in the joint fluid, highly fastidious bacterial growth, the biofilm nature of the PJI, or the effects of primary antibiotic therapy.<sup>8,9</sup> Furthermore, as conventional culture usually requires 3 ml to 5 ml of sample, joint fluid sample (JFS) volumes from preoperative punctures can be insufficient,<sup>10</sup> resulting in an even lower positive rate. Accordingly, it is necessary to improve diagnostic performance when using low-volume JFS from preoperative aspiration.

Detection of 16S ribosomal RNA (rRNA) by polymerase chain reaction (PCR) might increase the detection rate of microorganisms in PJIs with small amounts of sample.<sup>11,12</sup> Most methods rely on bacterial DNA detection, which leads to higher sensitivity compared to culture methods.<sup>13–15</sup> However, the false-positive rate of DNA-based PCR is higher than that of culture,<sup>16</sup> and exogenous bacterial DNA that originates from percutaneous aspiration may account for this issue. Studies have shown that the instability of RNA under natural conditions can reduce the false-positive rate caused by exogenous contamination in simulated joint infections.<sup>17</sup> Moreover, clinical studies have reported that the sensitivity of RNA-based PCR is the same as that of bacterial culture while exhibiting a lower false-positive rate.<sup>18–20</sup> Regardless, the samples used in these studies were acquired intraoperatively and were greater in volume than those collected preoperatively, which may have enhanced sensitivity. Thus, it remains to be determined whether diagnostic efficacy can be improved by RNA-based PCR detection of bacteria in low volumes of JFS acquired from preoperative aspiration.

As a volume of 4 ml to 5 ml JFS is usually needed for routine culture,<sup>21,22</sup> suspected PJI patients with preoperative aspiration JFS volumes of less than 5 ml were enrolled in this study. The diagnostic efficiencies of RNA-based PCR and bacterial culture using preoperative joint fluid were compared to investigate whether RNA-based real-time quantitative PCR can improve the accuracy of PJI

diagnosis relative to bacterial culture when using low volumes of preoperatively aspirated synovial fluid from suspected PJI cases.

## Methods

**Patient selection and sample collection.** The patients included in this study were chosen from those who underwent revision surgery in our hospital due to suspected PJI between January 2014 and December 2016. The inclusion standards were as follows: based on symptoms, medical history, and clinical data, patients suspected of having PJI and who finally received revision surgery. A diagnosis of PJI was made according to Musculoskeletal Infection Society (MSIS) criteria for PJI. One of the following must be met for diagnosis of PJI: a sinus tract communicating with the prosthesis; a pathogen is isolated by culture from two separate tissue or fluid samples obtained from the affected prosthetic joint; four of the following six criteria exist: 1) elevated ESR and CRP (ESR > 30 mm/hour; CRP > 10 mg/l); 2) elevated synovial fluid white blood cell (WBC) count (> 3,000 cells/ $\mu$ l); 3) elevated synovial fluid neutrophil percentage (> 65%); 4) presence of purulence in the affected joint; 5) isolation of a microorganism in one periprosthetic tissue or fluid culture; 6) more than five neutrophils per high-power field in five high-power fields observed from histological analysis of periprosthetic tissue at  $\times$ 400 magnification. JFS obtained by puncture before revision, with a total sample volume of less than 5 ml and more than 2 ml (the minimum volume for both culture and PCR methods). The exclusion standards were as follows: incomplete laboratory and clinical information available; contaminated specimens or specimens suspected of being contaminated; specimens obtained from patients with infections in other body parts that might influence the accuracy of detection. For each included patient, medical history, demographic characteristics, physical signs, serological inflammatory markers, WBC count and polymorphonuclear (PMN)% of JFS, intraoperative findings, and culture results were recorded. Patients who met the standard described by the MSIS were designated as having PJI.<sup>23</sup> All preoperative aspirations and revision surgeries were performed under standard conditions with written informed patient consent. The study was approved by the Ethics Committee of the First Affiliated Hospital of Fujian Medical University, Fuzhou, China (Ethics Number: [2014] 047). According to described guidelines, the preoperative aspiration procedures were conducted in a sterile operating room.<sup>24</sup> After draping with a sterile towel, a sterile needle was used to aspirate the joint cavity, without local anaesthesia, to acquire a JFS. The extracted joint fluid from each subject was divided into two parts: 1 ml to 3 ml was used for culture and cell counting; and the residual 0.5 ml to 1 ml was reserved for RNA extraction and detection.

**Preoperative culture.** Since only the cases with a preoperative sample volume of less than 5 ml were involved in this study, the sample was preferentially injected into a BACTEC Peds Plus bottle (Becton-Dickinson GmbH, Heidelberg, Germany), which requires a smaller volume of joint fluid (1 ml to 3 ml) for culture.<sup>25,26</sup> Except for two cases, whose infection was secondary to periodontal disease, anaerobic infection was suspected and the BACTEC Anaerobic/F bottle (Becton-Dickinson GmbH) was used (coincidentally, the amount of joint fluid obtained in these two cases was 5 ml; after PCR, 4 ml was still used for culture). Furthermore, to allow growth of fastidious bacteria, the time for culture was extended from the traditional length of one week to two weeks.<sup>27</sup>

**Preoperative RNA-based PCR.** Preoperatively obtained JFS was added to an RNA protection reagent (76163; Qiagen, Valencia, California, USA) immediately after collection and then centrifuged and lysed according to the same method used to obtain DNA. RNeasy Mini Kit (74102; Qiagen) was used to extract total RNA. Prior to RNA elution, the samples were treated with DNase to remove DNA contamination.

In line with the manufacturer's instructions, 1  $\mu$ g of total RNA was reverse-transcribed using random primers. After obtaining complementary DNA (cDNA) samples, quantitative polymerase chain reaction (qPCR) was performed in line with standard protocols using a SYBR Green Kit (RR820A; Takara, Dalian, China) in an Applied Biosystems (ABI) 7500 PCR instrument. Briefly, 1  $\mu$ l of cDNA was combined with 19  $\mu$ l of reaction mixture containing primers at 0.5  $\mu$ M and 0.5X SYBR Green mixture. We used the consensus primer pair to amplify the bacterial 16S rRNA gene (primers are listed in Supplementary Table i). The PCR cycling conditions were as follows: 50°C for ten minutes, 95°C for five minutes, and 40 cycles of 95°C for ten seconds and 60°C for 30 seconds. Sterile synovial fluid was used as a negative control. As a positive control, a standard strain of *Escherichia coli* was added to sterile synovial fluid at a concentration of 10<sup>10</sup> CFU/ml. A two-cycle difference from the sterile baseline was considered a detectable positive result. Amplification products were collected into 1.5 ml Eppendorf tubes and prepared for sequencing using the ExoSAP-IT reagent (Affymetrix, Santa Clara, California, USA). Sequencing was performed using the BigDye Terminator method with a 3730xl Genetic analyzer (Applied Biosystems, Foster City, California, USA). The sequences generated were compared against those in the National Center for Biotechnology Information (NCBI) GenBank database.<sup>28</sup>

**Intraoperative culture.** Samples were taken at multiple sites of suspected infection, including necrotic and purulent tissues and sites of bone erosion (five samples from at least three sites for each patient). After collection, tissues were ground for one minute in brain-heart infusion broth with a mortar and

pestle, and the homogenate was cultured on aerobic and anaerobic sheep-blood agar plates (BD Diagnostic Systems, Heidelberg, Germany). Each intraoperatively removed prosthesis was placed into a sterile box with 200 ml to 400 ml of Ringer's solution (Caiyou Industry, Shanghai, China), to make sure the prosthesis was totally immersed in liquid. The box was vortex-mixed for 30 seconds, subjected to sonication (40 kHz, 25°C to 37°C) for ten minutes, and then vortex-mixed again for 30 seconds. After that, the sonicate fluid was centrifuged at 3,000 g for ten minutes, the supernatant was discarded, and 10 ml of the sediment was injected into a BACTEC Plus culture bottle (Becton-Dickinson GmbH, Heidelberg, Germany). Joint fluid samples obtained during operation were cultured using the same method for the same incubation time used for preoperative samples. We combined the results of synovial fluid, tissue, and prosthesis sonication fluid culture to get a comprehensive positive bacterial culture result.

**Statistical analysis.** All statistical calculations (sensitivity, specificity, accuracy, positive and negative predictive values (PPV and NPV), positive likelihood ratio (LR+), and confidence intervals (CI)) were performed using SPSS v.23.0 (IBM, Armonk, New York, USA). These values were further evaluated with the McNemar test (sensitivity and specificity) and chi-squared test (accuracy, PPV, and NPV). The statistical significance threshold was set at  $p = 0.05$ .

## Results

**Analysis of included cases.** In line with the inclusion standard, 42 patients qualified for our study. Among these, six with incomplete clinical data and three with suspected cases of specimen contamination during transfer were excluded. Therefore, 33 patients were ultimately included in our study. In line with MSIS PJI diagnostic criteria, 21 cases were diagnosed with PJI (72.3% of the included cases), including six males and 15 females aged 49 to 88 years (mean 63.9 years (SD 6.5)); 16 patients underwent hip arthroplasty and five underwent knee arthroplasty. A total of 12 cases were diagnosed as non-PJI, including three males and nine females aged 53 to 77 years (mean 67.6 years (SD 9.3)); nine patients underwent hip arthroplasty and three underwent knee arthroplasty. The causes for the previous arthroplasties are provided in Supplementary Table ii.

**Comparison of preoperative aspiration culture and PCR.** Analyses of preoperatively aspirated JFSs via culture and PCR showed a sensitivity, specificity, PPV, NPV, accuracy, and LR+ of 28.6%, 83.3%, 75.0%, 40.0%, 48.5%, and 1.71, respectively, for the preoperative culture method and 57.1%, 91.7%, 92.3%, 55.0%, 69.7%, and 6.86, respectively, for the RNA-based PCR method. The sensitivity of the PCR technique was notably superior to that of the culture technique for detecting bacteria from preoperatively aspirated JFS ( $p < 0.05$ ). However, no significant difference

between the two techniques was found for specificity, NPV, PPV, accuracy, or LR+ value (Supplementary Table iii).

**Microorganisms detected by culture and PCR.** Of the 21 cases diagnosed as PJI, preoperative low-volume JFS culture (all samples < 5 ml) identified six positive cases (28.6% sensitivity) and two false positives (83.3% specificity), whereas intraoperative sample culture identified 17 positive cases (81.0% sensitivity) and no false positives (100% specificity). The sensitivities of both preoperative culture and PCR were significantly lower than that of intraoperative culture ( $p < 0.05$ ). The type and frequency of infecting organisms in culture and PCR were analyzed, and the results are shown in Supplementary Table iv. *Staphylococcus epidermidis* was found to be the most common microorganism (Supplementary Table iv). PCR amplicon sequencing provided poor resolution for species-level identification of coagulase-negative staphylococci (CoNS). Five *Staphylococcus* species, three *Staphylococcus aureus* species, and two CoNS species were identified by sequencing, confirming that *Staphylococcus* was the predominate infecting organism. Interestingly, although intraoperative culture showed better diagnostic efficiency than did RNA-based PCR, the latter method did detect bacteria in two false-negative cultures (Supplementary Tables v and vi, cases 3 and 4).

**Utility of RNA-based PCR in cases of false diagnosis by culture.** We observed several instances of missed diagnosis or misdiagnosis among the preoperative sample cultures, as presented in Supplementary Tables v and vi. For cases 1 and 2, the results of preoperative serological inflammatory tests were above the threshold, the puncture fluid was not purulent, and the total sample volume was approximately 5 ml (4 ml was used for culture and WBC counting; 1 ml was used for RNA-based PCR). The WBC count and PMN% were below the cutoff values in case 1 but above the values in case 2. The results of preoperative culture were negative in both cases. Definite PJI diagnosis was made for cases 1 and 2 by intraoperative culture, which revealed infections with *Bacteroides fragilis* and *E. coli*, respectively, and preoperative RNA-based PCR was positive in both cases. For cases 3, 4, and 5, the patients experienced joint swelling and pain after the primary arthroplasty and were treated with antibiotics for three days, one week, and four weeks, respectively, before synovial fluid was obtained. Therefore, although the results for serological inflammatory markers, WBC counts, and PMN% were above threshold values, the results of pre- and intraoperative culture were negative. During each operation, a large amount of pus was found in the joint cavity, the results of intraoperative pathology were positive, and PJI diagnosis was confirmed according to MSIS minor criteria. Preoperative RNA-based PCR results were positive for cases 3 and 4 and negative for case 5. All five patients described above underwent standard two-stage revision, with cement spacers inserted at the first stage. In case 6, PJI was suspected one year after primary

arthroplasty due to joint pain and high skin temperature. The results of synovial puncture fluid culture suggested an infection with *Bacillus subtilis*, but other indicators did not support PJI, including RNA-based PCR. Ultimately, infection was excluded by intraoperative findings, and a one-stage revision was performed for aseptic loosening.

## Discussion

A previous meta-analysis of PJI diagnosis in 34 studies indicated that preoperative hip and knee joint fluid culture has 65% to 78% sensitivity and 93% to 97% specificity.<sup>9</sup> Unlike these previous studies, we only analyzed cases in which less than 5 ml of a JFS was obtained preoperatively. According to our results, among 21 cases of definite PJI eight positive cases were identified by preoperative joint fluid culture; the cultures revealed a spectrum of bacteria commonly found in musculoskeletal infections, but the sensitivity of this method was not as high as that described in previous studies.<sup>29,30</sup> Interestingly, the sensitivity of intraoperative culture (including analysis of tissue, joint fluid, and sonicated fluid) was greatly increased. This discrepancy is likely related to the specimen volume. Positive culture results usually require multiple samples, thus requiring greater volume, especially when diagnosing infections caused by fastidious bacteria.<sup>31</sup> Therefore, we evaluated a PCR-based method for preoperative detection that is less dependent on the sample volume and concentration of bacteria. Because the puncture approach has a great risk of introducing DNA from exogenous bacteria, a higher false-positive rate is obtained when employing DNA-based PCR.<sup>32</sup> Furthermore, we have compared these two methods in a previous study, and demonstrated that the specificity of RNA-based PCR was superior to that of DNA-based PCR, while the sensitivity was similar.<sup>20</sup> In this study, the joint fluid was limited; if we performed a DNA-based PCR additionally, the bacterial cultures would be affected due to low volumes of samples. Therefore, we utilized only RNA-based PCR to detect bacteria in joint fluid obtained before surgery.<sup>20</sup> The results revealed higher specificity and sensitivity of RNA-based PCR than those of culture for low-volume preoperatively aspirated samples, although the sensitivity of RNA-based PCR was not as high as that of intraoperative sample culture.

We identified several false-negative and false-positive results based on preoperative joint fluid culture (Supplementary Tables v and vi). One explanation for the negative culture results for cases 1 and 2 is that an insufficient amount of joint fluid was obtained before surgery; because additional specimens were obtained during surgery, a definitive PJI diagnosis was established by intraoperative culture. RNA-based PCR was accurate in cases 1 and 2, despite their low sample volumes. Antibiotics were applied preoperatively in cases 3, 4, and 5, with normal results in serological inflammation tests and negative culture results for both pre- and intraoperative samples.

Nonetheless, PJI was confirmed based on typical patient symptoms, observation of pus-filled joint cavities, and examination of frozen intraoperative samples. RNA-based PCR showed positive results for cases 3 and 4 and a negative result for case 5, which may be related to the duration of preoperative antibiotic use. In cases 3 and 4, antibiotics were used for less than one week; in case 5, antibiotics were used for more than four weeks. Studies have indicated that 16S rRNA can play a role as an indicator of cell activity,<sup>33</sup> and that it cannot be detected one week after antibiotic treatment has blocked bacterial growth.<sup>34</sup> Therefore, RNA-based PCR detection is more sensitive than bacterial culture for patients who have used antibiotics before surgery; regardless, there are time windows in which false-negative diagnoses are possible. In case 6, a false-positive result was obtained with preoperative bacterial culture, although RNA-based PCR did not. One possible explanation is that exogenous RNA introduced by puncture was degraded during the extraction process, highlighting another possible advantage of RNA-based PCR compared to DNA-based PCR and culture.

In our study, we used universal primers to identify the presence of bacteria, although these primers do not permit species discrimination. Consequently, we sequenced the amplified PCR products to identify the organisms, but consistent with a previous report this method was limited to recognizing CoNS at the genus level.<sup>30</sup> Although the 16S rRNA sequence is highly conserved, it also harbours variable regions. As studies using rRNA-based reverse transcription-quantitative polymerase chain reaction (RT-qPCR) methods to discover hypervariable regions of 16S rRNA have been conducted to distinguish among different *Staphylococcus* species,<sup>19,35</sup> designing species-specific primers for bacterial identification at the level of species may be possible in following studies.<sup>36</sup>

Because our study was a single-centre study and the observed PJI occurrence was low, the number of specimens acquired was relatively small, which may lead to bias. In addition, for two patients different culture bottles from the same company were used, which may also cause bias. Future multicentre trials with larger numbers of cases are warranted to confirm our findings.

In this study, the majority of the limited volume of joint fluid obtained before revision surgery was used for inoculating bacterial culture, whereas only 1 ml of each specimen was used for PCR analysis. Therefore, RNA-based PCR is compatible with bacterial culture methods. It is thus recommended that RNA-based PCR be performed when preoperatively aspirated joint fluid is limited, and when patients have not been using antibiotics for an extended period (less than one week). Further work is warranted to improve the efficiency of PCR for preoperative diagnosis of PJI. In future work, we will use a more stable RNA extraction system, and stricter operation in specimen transportation and PCR process to reduce the risk of contamination. Additionally, we will

use primers for a broader bacterial spectrum to increase the diagnostic efficiency of PCR.

### Supplementary Material



Tables showing the primers used in this study, clinical data of patients with suspected infections, a comparison of diagnostic efficiency between culture and polymerase chain reaction, the organisms of prosthetic joint infection detected by culture and polymerase chain reaction, and typical cases misdiagnosed by preoperative culture.

### References

1. **Bozic KJ, Ries MD.** The impact of infection after total hip arthroplasty on hospital and surgeon resource utilization. *J Bone Joint Surg Am.* 2005;87-A(8):1746-1751.
2. **Kurtz SM, Lau E, Schmier J, Ong KL, Zhao K, Parvizi J.** Infection burden for hip and knee arthroplasty in the United States. *J Arthroplasty.* 2008;23(7):984-991.
3. **Tigges S, Stiles RG, Roberson JR.** Appearance of septic hip prostheses on plain radiographs. *AJR Am J Roentgenol.* 1994;163(2):377-380.
4. **Schinsky MF, Della Valle CJ, Sporer SM, Paprosky WG.** Perioperative testing for joint infection in patients undergoing revision total hip arthroplasty. *J Bone Joint Surg Am.* 2008;90-A(9):1869-1875.
5. **Laiho K, Mäenpää H, Kautiainen H, et al.** Rise in serum C reactive protein after hip and knee arthroplasties in patients with rheumatoid arthritis. *Ann Rheum Dis.* 2001;60(3):275-277.
6. **Saleh A, George J, Faour M, Klika AK, Higuera CA.** Serum biomarkers in periprosthetic joint infections. *Bone Joint Res.* 2018;7(1):85-93.
7. **Trampuz A, Hanssen AD, Osmon DR, Mandrekar J, Steckelberg JM, Patel R.** Synovial fluid leukocyte count and differential for the diagnosis of prosthetic knee infection. *Am J Med.* 2004;117(8):556-562.
8. **Barrack RL, Jennings RW, Wolfe MW, Bertot AJ.** The Coventry Award. The value of preoperative aspiration before total knee revision. *Clin Orthop Relat Res.* 1997;345:8-16.
9. **Qu X, Zhai Z, Wu C, et al.** Preoperative aspiration culture for preoperative diagnosis of infection in total hip or knee arthroplasty. *J Clin Microbiol.* 2013;51(11):3830-3834.
10. **Partridge DG, Winnard C, Townsend R, Cooper R, Stockley I.** Joint aspiration, including culture of reaspirated saline after a 'dry tap', is sensitive and specific for the diagnosis of hip and knee prosthetic joint infection. *Bone Joint J.* 2018;100-B(6):749-754.
11. **Bémer P, Plouzeau C, Tande D, et al; Centre de Référence des Infections Ostéo-articulaires du Grand Ouest (CRIOGO) Study Team.** Evaluation of 16S rRNA gene PCR sensitivity and specificity for diagnosis of prosthetic joint infection: a prospective multicenter cross-sectional study. *J Clin Microbiol.* 2014;52(10):3583-3589.
12. **Janz V, Schoon J, Morgenstern C, et al.** Rapid detection of periprosthetic joint infection using a combination of 16s rDNA polymerase chain reaction and lateral flow immunoassay: A Pilot Study. *Bone Joint Res.* 2018;7(1):12-19.
13. **Tarkin IS, Henry TJ, Fey PI, Iwen PC, Hinrichs SH, Garvin KL.** PCR rapidly detects methicillin-resistant staphylococci periprosthetic infection. *Clin Orthop Relat Res.* 2003;414(414):89-94.
14. **Fenollar F, Roux V, Stein A, Drancourt M, Raoult D.** Analysis of 525 samples to determine the usefulness of PCR amplification and sequencing of the 16S rRNA gene for diagnosis of bone and joint infections. *J Clin Microbiol.* 2006;44(3):1018-1028.
15. **Moojen DJF, Spijkers SNM, Schot CS, et al.** Identification of orthopaedic infections using broad-range polymerase chain reaction and reverse line blot hybridization. *J Bone Joint Surg Am.* 2007;89-A(6):1298-1305.
16. **Mariani BD, Martin DS, Levine MJ, Booth RE Jr, Tuan RS.** The Coventry Award. Polymerase chain reaction detection of bacterial infection in total knee arthroplasty. *Clin Orthop Relat Res.* 1996;331(331):11-22.
17. **Birmingham P, Helm JM, Manner PA, Tuan RS.** Simulated joint infection assessment by rapid detection of live bacteria with real-time reverse transcription polymerase chain reaction. *J Bone Joint Surg Am.* 2008;90-A(3):602-608.
18. **Lee MS, Chang WH, Chen SC, et al.** Molecular diagnosis of periprosthetic joint infection by quantitative RT-PCR of bacterial 16S ribosomal RNA. *Scientific World Journal.* 2013;2013:950548.
19. **Bergin PF, Doppelt JD, Hamilton WG, et al.** Detection of periprosthetic infections with use of ribosomal RNA-based polymerase chain reaction. *J Bone Joint Surg Am.* 2010;92-A(3):654-663.

20. Fang XY, Li WB, Zhang CF, et al. Detecting the Presence of Bacterial DNA and RNA by Polymerase Chain Reaction to Diagnose Suspected Periprosthetic Joint Infection after Antibiotic Therapy. *Orthop Surg*. 2018;10(1):40-46.
21. Société de Pathologie Infectieuse de Langue Française (SPILF); Collège des Universitaires de Maladies Infectieuses et Tropicales (CMIT); Groupe de Pathologie Infectieuse Pédiatrique (GPIP); Société Française d'Anesthésie et de Réanimation (SFAR); Société Française de Chirurgie Orthopédique et Traumatologique (SOFOT); Société Française d'Hygiène Hospitalière (SFHH); Société Française de Médecine Nucléaire (SFMN); Société Française de Médecine Physique et de Réadaptation (SOFMER); Société Française de Microbiologie (SFM); Société Française de Radiologie (SFR-Rad); Société Française de Rhumatologie (SFR-Rhu). Recommendations for bone and joint prosthetic device infections in clinical practice (prosthesis, implants, osteosynthesis). *Med Mal Infect*. 2010;40(4):185-211.
22. Osmon DR, Berbari EF, Berendt AR, et al; Infectious Diseases Society of America. Executive summary: 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.
23. Parvizi J, Zmistowski B, Berbari EF, et al. New definition for periprosthetic joint infection: from the Workgroup of the Musculoskeletal Infection Society. *Clin Orthop Relat Res*. 2011;469(11):2992-2994.
24. Della Valle C, Parvizi J, Bauer TW, et al; American Academy of Orthopaedic Surgeons. American Academy of Orthopaedic Surgeons clinical practice guideline on: the diagnosis of periprosthetic joint infections of the hip and knee. *J Bone Joint Surg Am*. 2011;93-A(14):1355-1357.
25. Hughes HC, Newnham R, Athanasou N, Atkins BL, Bejon P, Bowler ICJW. Microbiological diagnosis of prosthetic joint infections: a prospective evaluation of four bacterial culture media in the routine laboratory. *Clin Microbiol Infect*. 2011;17(10):1528-1530.
26. Hughes JG, Vetter EA, Patel R, et al. Culture with BACTEC Peds Plus/F bottle compared with conventional methods for detection of bacteria in synovial fluid. *J Clin Microbiol*. 2001;39(12):4468-4471.
27. Schwotzer N, Wahl P, Fracheboud D, Gautier E, Chuard C. Optimal culture incubation time in orthopedic device-associated infections: a retrospective analysis of prolonged 14-day incubation. *J Clin Microbiol*. 2014;52(1):61-66.
28. No authors listed. National Center for Biotechnology Information. <https://www.ncbi.nlm.nih.gov> (date last accessed 20 August 2019).
29. Borde JP, Häcker GA, Guschl S, et al. Diagnosis of prosthetic joint infections using UMD-Universal Kit and the automated multiplex-PCR Unyvero i60 ITI(®) cartridge system: a pilot study. *Infection*. 2015;43(5):551-560.
30. Gomez E, Cazanave C, Cunningham SA, et al. Prosthetic joint infection diagnosis using broad-range PCR of biofilms dislodged from knee and hip arthroplasty surfaces using sonication. *J Clin Microbiol*. 2012;50(11):3501-3508.
31. Gandhi R, Silverman E, Courtney PM, Lee GC. How Many Cultures are Necessary to Identify Pathogens in the Management of Total Hip and Knee Arthroplasty Infections? *J Arthroplasty*. 2017;32(9):2825-2828.
32. Newsome T, Li BJ, Zou N, Lo SC. Presence of bacterial phage-like DNA sequences in commercial Taq DNA polymerase reagents. *J Clin Microbiol*. 2004;42(5):2264-2267.
33. Aellen S, Que YA, Guignard B, Haenni M, Moreillon P. Detection of live and antibiotic-killed bacteria by quantitative real-time PCR of specific fragments of rRNA. *Antimicrob Agents Chemother*. 2006;50(6):1913-1920.
34. González-Escalona N, Fey A, Höfle MG, Espejo RTA, A Guzmán C. Quantitative reverse transcription polymerase chain reaction analysis of *Vibrio cholerae* cells entering the viable but non-culturable state and starvation in response to cold shock. *Environ Microbiol*. 2006;8(4):658-666.
35. Tsang STJ, McHugh MP, Guerendiain D, et al. Underestimation of *Staphylococcus aureus* (MRSA and MSSA) carriage associated with standard culturing techniques: One third of carriers missed. *Bone Joint Res*. 2018;7(1):79-84.
36. Chakravorty S, Helb D, Burday M, Connell N, Alland D. A detailed analysis of 16S ribosomal RNA gene segments for the diagnosis of pathogenic bacteria. *J Microbiol Methods*. 2007;69(2):330-339.

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#### Author contributions

- B. Yang: Designed the study.
- X. Fang: Wrote the manuscript.
- Y. Cai: Performed the study.
- Z. Yu: Carried out the English translation.
- W. Li: Collected the data.
- C. Zhang: Edited the English language.
- Z. Huang: Analyzed the data.
- W. Zhang: Integrated the data.
- Bin Yang, Xinyu Fang, and Yuanqing Cai contributed equally to this work.

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- None declared

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#### Ethical review statement

- The study was approved by the Ethics Committee of the First Affiliated Hospital of Fujian Medical University, Fuzhou, China (Ethics Number: [2014] 047).

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