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MOLECULAR DIAGNOSIS OF PROSTHETIC JOINT INFECTION. A REVIEW OF EVIDENCE

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Prosthetic joint infection (PJI) diagnosis includes several classes of verification. Among them, only a few have a stronger independent value, namely intraarticular purulence and communicating fistulas. Other diagnostic methods require careful test combinations, analysis, and interpretation. Molecular based techniques using the polymerase chain reaction (PCR) seem to be a promising PJI diagnostic modality due to its excellent sensitivity, specificity, positive predictive value, and speed. Most of the recent reviewers are in agreement that molecular diagnosis has enough potential for future application in orthopaedics even if there are only a few heterogeneous studies fully supporting this concept. Conversely, at least one study has been published with significantly worse results (sensitivity and specificity less than 0.75). The lack of supporting evidence in the published studies may be closely related to varying PCR laboratory procedures, inappropriate reference standards, and other methodological shortcomings among research centers. It is not yet justifiable to firmly include molecular methods into the present PJI diagnostic schemes. The orthopaedic community must await the results of well-organized ongoing studies before considering inclusion of molecular diagnostics as a PJI diagnostic method. The aim of this paper was to make a survey of current PJI molecular diagnostic techniques in orhopaedics.

INTRODUCTION

Prosthetic joint infection (PJI) is a serious complication of total joint arthroplasty that causes great morbidity in affected individuals and is a significant burden to any health care system[2, 4, 11, 15, 27, 31, 35, 56, 62]. The currently accepted frequency of PJI ranges from below 1 % to 5 %, with slightly higher figures following revision procedures[8, 26, 51, 71, 75]. In the United States, over 500,000 joint arthroplasties are performed annually, accordingly PJI affects a considerable faction of individuals regardless of its low frequency[62, 66]. Thus, the importance of understanding and advancing PJI diagnostics is of a special interest, especially since there are no universally accepted criteria[26, 31, 37, 54, 72].

ETIOLOGY AND CAUSATIVE AGENTS

Deep PJI can be defined as a successful intraarticular life strategy of microorganisms on both host and prosthetic surfaces as well as intracellularly[14, 16, 19, 79]. The ability of bacteria to colonize prosthetic materials has been evaluated in depth showing a positive association between prosthetic devices and facilitated bacterial establishment[1, 20, 22, 50, 52]. The bacteria most commonly isolated from PJIs include coagulase-negative staphylococci and *Staphylococcus aureus*, followed by polymicrobial infections, streptococci, Gram-negative bacilli and other minorities[9, 17, 25, 30, 67]. The clinical course of PJI sepsis is influenced by the host immune status, the "microbial safe zone" (i.e. prosthetic and inorganic surfaces), and bacteria-specific tactics for immune evasion[12, 19, 21, 23, 52, 67, 77].

CLINICAL PICTURE AND DIAGNOSIS

As is true for many infectious processes, early detection can often alter the natural course of the disease, and ultimately improve long-term outcomes for patients[3, 24, 60]. In an uncompromised host, invading bacteria are usually eliminated by the innate immune response. If this initial response fails to eradicate the offending bacteria, a mountable immune response may be provoked producing the characteristic signs and symptoms of infection. These may vary clinically from almost asymptomatic to superacute sepsis with erythema, edema, pain, effusions, local warmth, fever, and sinus tract formation[38, 64].

The clinical signs of infection can give rise to a high degree of suspicion, but alone cannot be relied upon for diagnosis and need not necessarily be present[78, 84]. Laboratory markers such as C-reactive protein, erythrocyte sedimentation rate, and white blood cell count are sensitive markers of inflammation and plausible infection, but they are unable to localize the exact site and have a low specificity[65, 78]. Recently, there has been much progress in PJI diagnostics using nuclear medicine techniques that offer excellent sensitivity, specificity, and accuracy[39, 55]. However, even with these diagnostic tools and a thorough understanding of the specific bacterial nature and pathogenicity, PJI diagnosis can be a difficult task, particularly in the face of late or chronic infections. This point is underscored by reports of PJIs that have been misdiagnosed and managed as aseptic loosening[6, 57, 74].

MICROBIAL DIAGNOSTICS

To date, the preferred tools for PJI diagnosis have been microbial methods. The traditional techniques (culture-dependent) rely on specimen retrieval from infected sources followed by inoculation on appropriate culture media. A series of species-specific biochemical or antibiotic susceptibility tests can then be performed to further characterize the infectious agents and help direct therapy[18, 85]. This practice is, however, plagued by sampling errors, inadequate quantities of vital bacteria retrieved, inappropriate transport, or fastidious organisms and may result in as many as 20 % of PJIs being culture negative[17, 53, 67, 71]. An additional drawback of traditional methods is the requirement for effective antibiofilm-oriented measures to increase the bacterial yield due to the predominantly biofilm-dependent nature of most PJIs[51, 74].

Molecular (culture-independent) diagnosis is based on the detection of microbial nucleic acids in clinical samples. A great advantage of this method is that it is able to detect a very small amount of genetic material in a retrieved specimen (theoretically only one DNA copy). Nevertheless, this feature can also be a source of false-positive results since even slight genetic contamination during sample retrieval, transport, or amplification can produce positive results. The aim of the current review is to present a clear and simple up-to-date assessment of PJI molecular diagnosis.

HISTORY OF POLYMERASE CHAIN REACTION

Methods using gene amplification and polymerase chain reaction (PCR) are based on an invention by Kerry B. Mullis (1985) who developed a technique to make a large number of DNA copies from an originally miniscule amount of DNA[47–49, 61]. Since then, many applications and protocols have been developed from the original prototype and have enabled an enormous expansion in the natural sciences and greatly contributed to medicine[63].

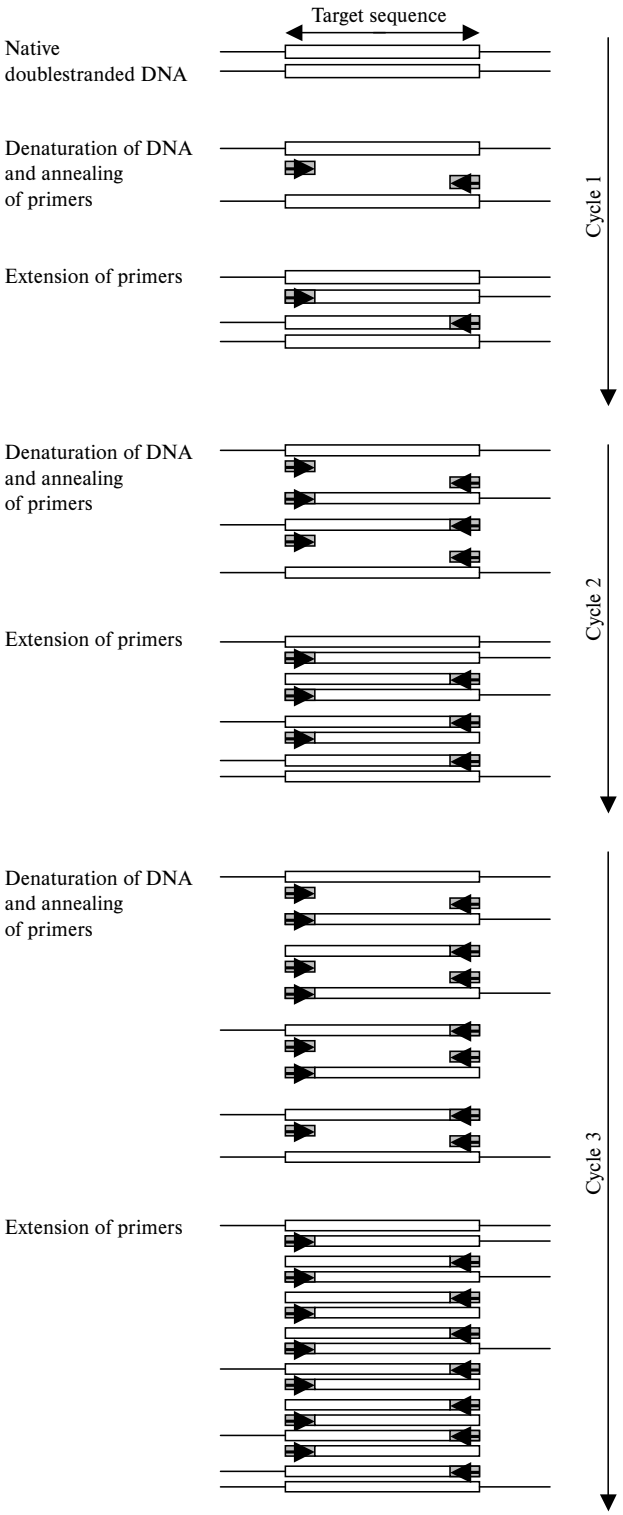

Fig. 1. Polymerase chain reaction (PCR). The strands of the doublestranded DNA are separated by thermal denaturation (90–96 °C) and subsequently cooled (50–65 °C) to allow primers to anneal specifically to the target region. Thermostable DNA polymerase is then used to extend the primers (72 °C) and duplicate the original target region. Each PCR reaction usually consists of 20–40 cycles and the target sequence is increasing exponentially during that time. After 20 cycles it is expected to be at 2^{20}-fold amplification level.

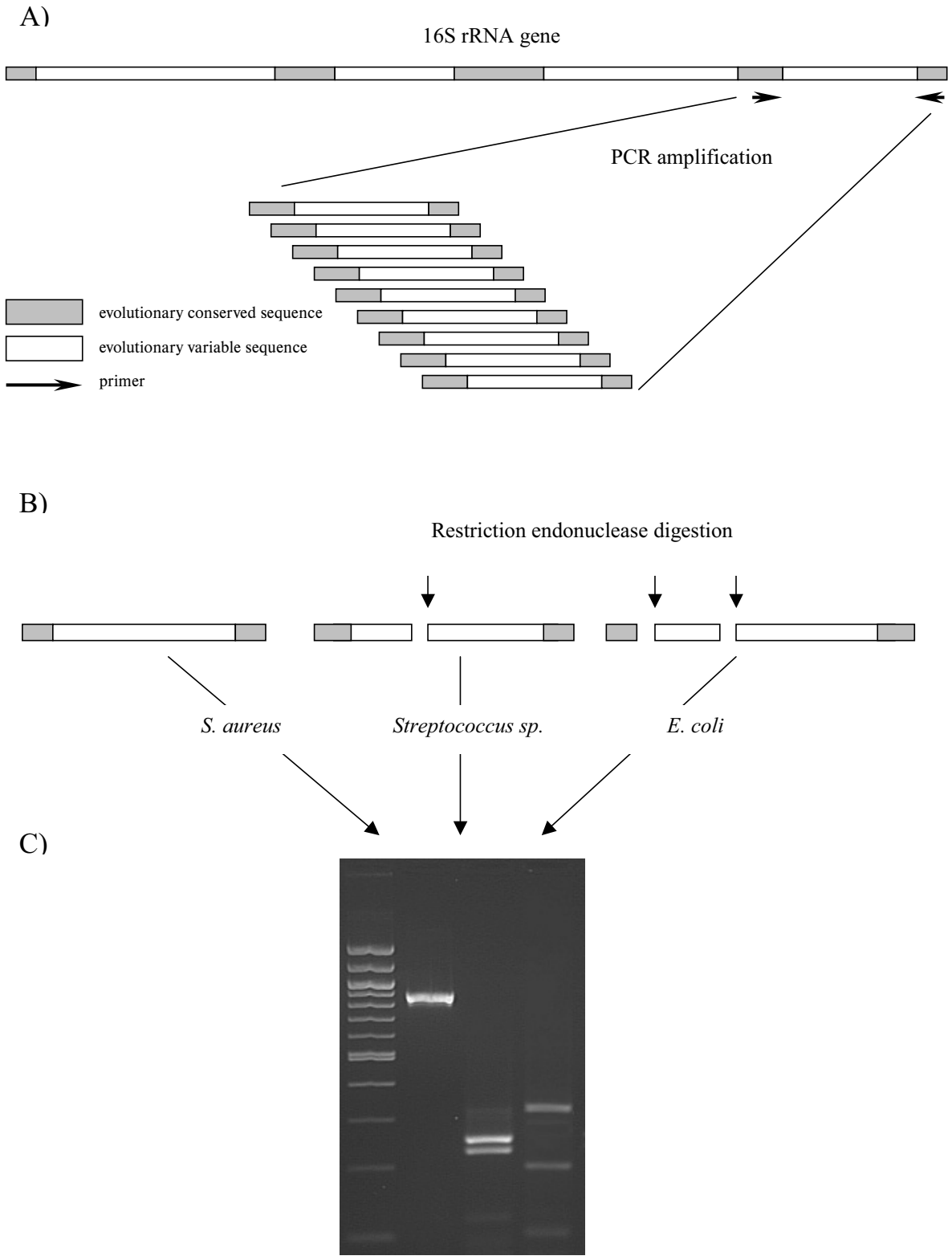

Fig. 2. Broad-range PCR detection of bacterial DNA and species identification using RFLP method. A) Amplification of the 16S rRNA sequence from primers targeted to the phylogenetically conserved areas of the gene allows broad range detection of various eubacteria in a single PCR. The resulting PCR products differ in nucleotide composition between bordering primer sequences. **B)** These differences can be recognized with specific-sequence-dependent restriction endonucleases (RE), which cleave amplified 16S rRNA sequences, producing species-specific restriction patterns. **C)** Agarose gel electrophoresis of RE digestion products. The amplified fragments of 16S rRNA genes of *Staphylococcus aureus* are not digested with RE, producing one fragment of the same molecular weight as an amplification product. Restriction treatment of streptococcal PCR products produce two DNA fragments of different molecular weights and *Escherichia coli* produces restriction patterns consisting of three different fragments.

Bacterial DNA or RNA can serve as targets for amplification and detection. PCR amplification of gene fragments unique to some explicit PJI pathogens (i.e. coagulase-negative staphylococci, *S. aureus*, *Streptococcus sp*., enterococci, Gram-negative bacteria, *Propionibacterium acnes, Serratia sp.*) has been widely established[10, 13, 34, 44, 45, 58]. The use of PCR for microbial diagnostics requires a strictly standardized protocol with a complicated structure of controls. Still, these methods have proven quite effective and have an even more promising future[83].

MOLECULAR IDENTIFICATION OF BACTERIA

Briefly, PCR is an *in vitro* technique for amplification of continuous, linear fragments of DNA within two specific short DNA sequences recognized by synthetic primer pairs during each PCR amplification cycle (Figure 1). The specific DNA amplification products are then characterized by their lengths and sequences that can be simply demonstrated using electrophoresis or hybridization with specific probes (Fig. 2).

Broad-range PCR is a useful assay to detect numerous eubacterial species. This assay relies on the existence of highly evolutionary conserved regions of the bacterial DNA coding for the 16S ribosomal RNA gene (16S rRNA). The amplification of the 16S rRNA sequence using primers targeted to the phylogenetically conserved gene allows for broad range detection of various eubacteria in a single PCR[82]. The resulting PCR products show the same base pair lengths but different nucleotide compositions between bordering primer sequences. Further analysis of these variable species-specific sequences enables the identification of specific microorganisms. This sequence analysis can be done using hybridization with probes, sequencing, or restriction fragment length polymorphism (RFLP) methods. Broad-range PCR with the abovementioned modifications has successfully detected the bacterial DNA from synovial fluid samples with different sensitivity for more than 17 bacterial species[69, 80].

MOLECULAR IDENTIFICATION OF ANTIBIOTIC RESISTANCE

The developing molecular diagnostic approaches based on PCR amplification can also detect genes associated with antibiotic resistance[29]. Thus, identification of resistance genes in clinical samples investigated by PCR enables modification of antibiotic regimens suggested for effective treatment. Specific primers for PCR detection have been successfully developed for: erythromycin resistance associated methylase genes *ermA*, *ermB, ermC*; macrolide transporter protein gene *mefA*; ATP-dependent macrolide efflux pump gene *msrA*; aminoglycoside modifying enzyme gene *Aac(6')-aph(2'')*; oxacillin resistance gene *mecA* coding a penicillin-binding protein 2a; penicillin resistance gene *blaZ* coding beta-lactamase; IMP-1 metallo-beta-lactamase gene (bla_{IMP}); vancomycin resistance gene (*vanA, vanB*)[32, 33, 43]. It is important to note that not all resistance mechanisms are linked to specific genes. For example, resistance to clarithromycin is associated with a point mutation in the 23S rRNA gene and is not detectable by PCR[40].

CURRENT STATE OF PJI MOLECULAR DIAGNOSTICS

Almost ten years after the inauguration of molecular diagnostics into the orthopaedic practice, these methods are still a question of research and discussion. Barrack and Wolfe as well as Spangehl et al. investigated the role of PCR for PJI diagnosis[5, 66]. They distinguished the advantageous accuracy, speed, low expense, and principle of PCR, and the potential disadvantages like false positive results, an interpretative error, and high demands for equipment as well as personnel. Bauer et al. recognized molecular diagnostics as highly sensitive methods with improved specificity and reduced time to diagnosis[6]. Trampuz et al. published a detailed analysis on several aspects of PJI diagnosis and strongly focused on molecular techniques, noting that newer amplification technologies as well as subsequent sequence analysis will most likely improve the statistical characteristics of these methods[71].

On the other hand, Masri et al. did not go into much depth about PCR methods as a diagnostic modality for PJI, but recognized that the methods hold promise for improved accuracy in spite of low specificity[46]. Windsor is more skeptical about the up-to-date molecular diagnostic scope and refers to PCR techniques as expensive and slow to aid intraoperative decision-making since they require two or more hours[68, 81].

LITERATURE REVIEW

A team from Thomas Jefferson University (Philadelphia, USA) was the first to establish a PCR technique to identify common prosthesis-related pathogens by targeting the 16S rRNA bacterial gene[36,41]. Their studies demonstrated a superior sensitivity and specificity of PCR for PJI detection. The pos itive predictive value in a sample of 20 total knee revisions was 100 %. In another group of 50 total knee arthroplasties, all culture positive specimens were congruently positive with PCR results, and the number of false positives was zero[42]. A shortcoming of these studies is the lack of a precise diagnostic standard since a single positive culture does not suffice. Trampuz et al. also criticized this study due to an unusual PCR result positive for *Candida sp*. characterized by a different target DNA sequence than bacterial 16S rRNA gene[71].

Tunney et al. examined 120 retrieved hip prostheses and tissue samples by 16S rRNA gene amplification using two different broad-range oligonucleotide primers[73]. To improve bacterial harvest, retrieved prostheses were lightly sonicated to disrupt adherent biofilms. According to their protocol, bacteria could be detected if they were

present in numbers greater than or equal to 10^4 CFU/ml. From their samples, bacterial DNA was detected in 72 % (85/118) of the sonicated materials while culture-based methods of tissue alone and tissue plus prosthesis were positive in only 4 % (5/120) and 22 % (26/120), respectively. Unfortunately, there are some inconsistencies in the study design.

A Czech study by Rozkydal et al. evaluated the benefit of 16S rRNA in a group of 32 patients with symptomatic total knee arthroplasties[59]. Based on their clinical presentations, laboratory tests, and imaging studies, the investigators distinguished between clinically clear PJI in 14 patients and non-septic diagnoses in 18 patients. Their PCR technique showed 100 % sensitivity for all clinically evident PJIs without any false positive results. Similarly, this study has also some weaknesses in its design, concerning a reference standard, a small number of positive cultures, and the lack of blinding[7].

Tarkin et al. performed a study aimed specifically at the molecular diagnosis of PJI caused by methicillin resistant *S. aureus* (MRSA)[70]. Tests were performed on 35 clinical samples retrieved at revision arthroplasty from 18 patients. A concordance between the culture-based methods and PCR was seen in 34 of 35 samples (97 %). Additionally, Tarkin et al. determined that the threshold for *mecA* PCR detection is influenced by the number of amplification cycles of the assay. However, the authors did note that they were unable to apply their results to individual therapeutic implications, since this aspect was not a part of their study.

van der Heijden et al examined the contribution of PCR for bacterial DNA detection in synovial fluid and tissue samples from patients with septic arthritis during antibiotic administration[76]. From six patients, all causative bacterial species were identified using broad range PCR, Gram staining, and culture. After 2–3 days of antibiotic therapy, cultures and Gram staining of retrieved samples were negative. However, PCR detected bacterial DNA in both the synovial fluid and tissue samples after 10 days in two patients, after 20 days in one patient, and after 22 days in another patient. Although this study was primarily oriented toward monitoring antibiotic therapy in a small group of patients, it demonstrated a potential downfall of culture-based techniques and a great advantage of PCR.

To date, one of the most consistent and knowledgeable orthopaedic reviews of PJI molecular diagnostics was given by Hoeffel et al[28]. Aside from the critical literary survey, the group tested their own PCR technique; the so-called genus focused primers, aimed at minimizing background DNA amplification and consequentially reducing false-positive results. In a preliminary group of 10 patients (21 samples) with suspected PJI, 20 of 21 samples had PCR results positive for bacterial DNA with excellent sensitivity (91 %) and specificity (100 %) when compared with culture methods. The authors then evaluated 108 samples from 63 patients and found amplifiable DNA in 102 samples (94 %). However, these figures were considered erroneous since only 11 patients (17 %) had PJI based on laboratory studies, culture, frozen section, and intraoperative evaluation. From the 11 patients, 7 were successfully recognized by PCR. Accordingly, when compared with culture methods, PCR sensitivity and specificity was 71% and 49%, respectively, and had a positive predictive value of 22% and a negative predictive value of 7 %. The authors concluded that the PCR methods should not serve as screening tests for PJIs and other musculoskeletal infections, but they could be useful to confirm infections, especially after initiating antibiotic treatment.

PERSPECTIVES OF MOLECULAR PJI DIAGNOSIS

Presently, PCR techniques are not routinely employed in orthopaedic practice to diagnose PJIs. With the development of well-equipped laboratories and increased enthusiasm among microbiologists and surgeons, the integration of PCR into modern orthopaedic practice may be expected. We believe that the future of PJI molecular diagnostics lies with real-time PCR and microarray assays (basic research, software), robotic assistance, fully automated devices (hardware), and well-performed clinical studies with promising results (sensitivity, accuracy, positive predictive values, etc.)[83].

CONCLUSION

According to some molecular biologists, there are three groups of orthopaedic surgeons with respect to molecular PJI diagnostic methods: those who accept the methods without criticism, those who completely ignore them, and those with a realist approach. Many molecular tools for bacterial DNA detection from clinical samples have been developed. One of the most significant contributions thus far has been amplification-based techniques (PCR) since some studies have confirmed their excellent sensitivity and specificity. Moreover, the majority of contemporary PCR techniques took less than 5 hours to complete which was significantly less than the 2–3 days required for routine cultures. However, there was notable heterogeneity in terms of procedure, detection threshold, true study controls, reference standards, and other aspects across the published studies testing the validity of PJI molecular diagnostics. Additionally, when compared to traditional microbiological approaches, PCR analysis is still hindered by higher costs, false-positive results and interpretative problems. Under these circumstances it is not justifiable to firmly include molecular methods into the present PJI diagnosis schemes. Nevertheless, we believe that PCR methods and results will be strongly considered in the near future and expect their incursion into the orthopaedic practice.

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