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Prosthetic Joint Infection Diagnosis Using Broad-Range PCR of Biofilms Dislodged from Knee and Hip Arthroplasty Surfaces Using Sonication

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Periprosthetic tissue and/or synovial fluid PCR has been previously studied for prosthetic joint infection (PJI) diagnosis; however, few studies have assessed the utility of PCR on biofilms dislodged from the surface of explanted arthroplasties using vortexing and sonication (i.e., sonicate fluid PCR). We compared sonicate fluid 16S rRNA gene real-time PCR and sequencing to culture of synovial fluid, tissue, and sonicate fluid for the microbiologic diagnosis of PJI. PCR sequences generating mixed chromatograms were decatenated using RipSeq Mixed. We studied sonicate fluids from 135 and 231 subjects with PJI and aseptic failure, respectively. Synovial fluid, tissue, and sonicate fluid culture and sonicate fluid PCR had similar sensitivities (64.7, 70.4, 72.6, and 70.4%, respectively; $P > 0.05$) and specificities (96.9, 98.7, 98.3, and 97.8%, respectively; $P > 0.05$). Combining sonicate fluid culture and PCR, the sensitivity was higher (78.5%, $P < 0.05$) than those of individual tests, with similar specificity (97.0%). Thirteen subjects had positive sonicate fluid culture but negative PCR, and 11 had negative sonicate fluid culture but positive PCR (among which 7 had prior use of antimicrobials). Broad-range PCR and culture of sonicate fluid have equivalent performance for PJI diagnosis.

With the aging population and associated musculoskeletal diseases, the number of knee and hip arthroplasties performed is steadily rising. The growing number of arthroplasties has been proportionally accompanied by a rise in the number of prosthetic joint infection (PJI) and aseptic failure (AF) cases. PJI is associated with high morbidity and economic burden (4); furthermore, some subacute and chronic PJI cases may masquerade as AF. PJI management includes surgery (often involving multiple procedures) and long-term antibiotic treatment and follow-up (4). Accurate diagnostic methods that differentiate PJI from AF are required for proper management.

Besides ascertainment for the presence of PJI, microbiologic characterization of PJI is necessary to direct appropriate treatment. Current methods for detection of organisms associated with PJI lack sensitivity and specificity. Seven to 39% of PJI cases are culture negative (2, 25). Culture-negative PJI is associated with prior antimicrobial use (15) or the presence of fastidious, biofilm-associated organisms. Such cases pose a challenge in PJI management. Recent studies have demonstrated that vortexing and sonication of the prosthesis with culture of the resultant sonicate fluid form a valuable diagnostic strategy for the diagnosis of hip and knee PJI (25). However, culture is affected by prior use of antimicrobials, and despite the use of sonication, there remain culture-negative PJI cases. Evaluation of new testing methods is warranted to attempt to increase the sensitivity of microbiologic diagnosis of PJI.

PCR might be more sensitive than culture for the diagnosis of PJI because it can detect DNA from fastidious and nonculturable bacteria and might not be as affected by prior antibiotic use as is culture (24). Additionally, it may permit faster identification and management of infected cases. The limited numbers of studies using PCR for the diagnosis of PJI have shown mixed results (8, 28). While some have shown higher sensitivity than conventional culture (16, 28), others have shown lower sensitivity (8, 10). Furthermore, most studies have used PCR of tissue or synovial fluid, with few evaluating PCR of sonicate fluid (1, 27).

PCR targeting the 16S rRNA gene with sequencing of the amplified product detects a range of bacteria, as this target is universally present in bacteria. Despite the potential benefits of broad-range PCR, there are possible limitations. Some 16S rRNA gene PCR primers cross-react with human DNA, compromising specificity when clinical specimens are tested directly (11). Also, this approach does not indicate whether a polymicrobial or monomicrobial infection is present; in the former circumstance, sequence analysis may be uninformative (i.e., due to overlapping electropherogram peaks) or misleading (i.e., due to missed detection of minority species). Kommedal et al. addressed these limitations with a computer algorithm (RipSeq Mixed; iSentio, Bergen, Norway) that analyzes mixed bacterial sequences (13, 14). They also assessed the performance of 16S rRNA gene primers and their cross-reactivity with human DNA. Primers which amplify the V3-V4 hypervariable region of the 16S rRNA gene (studied herein) show no apparent cross-reactivity with human DNA (13, 14).

We evaluated broad-range PCR for the diagnosis of PJI from

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sonicate fluid assayed with the aforementioned primers followed by sequencing of amplified product, and we used RipSeq software to analyze mixed sequences.

MATERIALS AND METHODS

Study population. Three hundred seventy-nine study subjects whose explanted hip or knee arthroplasty components were submitted to the Mayo Clinic (Rochester, MN) clinical microbiology laboratory for implant vortexing and sonication and sonicate fluid culture along with periprosthetic tissue culture between April 2006 and May 2011 were studied. Sonicate fluid left over after culture was frozen at −70°C for subsequent testing. Samples were excluded if there was obvious contamination of the prosthesis during the prosthesis resection, transport, or processing in the microbiology laboratory. Subject demographics, location and type of arthroplasty, clinical presentation, laboratory results, imaging findings, histopathology, and duration of antibiotic use prior to surgery were recorded. This study was approved by the Mayo Clinic Institutional Review Board.

Case definition. PJI was defined as the presence of at least one of the following: (i) synovial fluid or periprosthetic purulence, (ii) sinus tract communicating with the prosthesis, and (iii) periprosthetic tissue histopathology showing acute inflammation (25). AF was defined as prosthesis failure not meeting these criteria. Prior antimicrobial use was defined as antibiotic receipt in the 4 weeks preceding surgery.

Nonculture tests and synovial fluid analysis. Erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) evaluations were performed on specimens collected prior to surgery, with values of >30 mm/h and >10 mg/ml, respectively, considered abnormal. Arthrocentesis was performed preoperatively, with synovial leukocyte count and differential determined by microscopy. A synovial fluid leukocyte count and neutrophil percentage of >1,700 cell/μl and >65%, respectively, for knees (23) and >3,000 cells/μl and >80%, respectively, for hips (21) were considered abnormal.

Tissue, synovial fluid, and sonicate fluid cultures. A minimum of two periprosthetic tissue specimens were collected per subject. Tissues were homogenized with 3 ml of brain heart infusion broth for 1 min, and the homogenate was inoculated onto aerobic and CDC anaerobic sheep blood agar and incubated at 35 to 37°C in 5 to 7% CO_2 and anaerobically, respectively. A thioglycolate broth (BD Diagnostic Systems, Sparks, MD) was also inoculated. Incubation time varied throughout the study period. Aerobic plates were incubated for 2 days from April 2006 to April 2008 and for 4 days thereafter. Anaerobic and broth cultures were incubated for 7 days until 18 April 2011 and for 14 days thereafter. Anaerobic incubation duration was extended based on a 2011 publication showing an increased rate of recovery of *Propionibacterium* species (5). A tissue culture was considered positive when the same organism was isolated from ≥2 tissue samples.

Synovial fluid was inoculated into a Bactec Peds Plus/F bottle and incubated in a Bactec 9240 instrument (BD Diagnostic Systems) for 5 days (if more than 1 ml was received) or onto aerobic blood agar, chocolate agar, and CDC anaerobic 5% blood agar and into thioglycolate broth (if less than 1 ml was received). The last was incubated as indicated for tissue cultures. Growth of any organism in synovial fluid was considered positive.

The explanted prostheses were collected aseptically in the operating room and placed into an autoclaved 1-liter polypropylene wide-mouthed container (Nalgene, Lima, OH). Sonication cultures were performed as previously described (19, 20). Briefly, 400 ml of Ringer's solution was added to the container and vortexed for 30 s. The container was subjected to sonication in a Bransonic 5510 ultrasound bath (Branson, Danbury, CT) for 5 min at a frequency of 40 kHz. The container was vortexed for another 30 s. Fifty-milliliter aliquots of sonicate fluid were centrifuged at 3,150 × *g* for 5 min and concentrated to 0.5 ml. A total of 0.1 ml was inoculated onto a sheep blood agar plate, which was incubated aerobically for 4 days, and a CDC anaerobic 5% blood agar plate, which was incubated anaerobically for 14 days. Growth of ≥20 CFU/10 ml was considered significant.

16S rRNA gene real-time PCR. An aliquot of 1 ml of concentrated sonicate fluid was thawed and centrifuged at 5,000 × *g* for 5 min. Genomic DNA was extracted from a 100-μl pellet using a QIAamp UCP Pathogen minikit (QIAamp Inc., Valencia, CA) by following the manufacturer's instructions. The extracted DNA was eluted to a volume of 100 μl.

Real-time PCR using the LightCycler 2.0 instrument (Roche Molecular Diagnostics, Indianapolis, IN) and LightCycler FastStart DNA Master SYBR green I reaction mix (Roche Applied Science, Indianapolis, IN) was used to target the V3-V4 region of the 16S rRNA gene. The following primers were used: forward, 5′-CGG-CCC-AGA-CTC-CTA-CGG-GAG-GCA-GCA-3′, and reverse, 5′-GCG-TGG-ACT-ACC-AGG-GTA-TCT-AAT-CC-3′. Five microliters of extracted DNA was added to a PCR mixture that consisted of 15 μl of FastStart SYBR green master mix, 4.5 mM $MgCl_2$ (total), and 0.5 μM each primer. Real-time PCR was performed using the following cycling parameters: 10 min of 95°C preincubation for *Taq* DNA polymerase activation and DNA denaturation, followed by 40 cycles of 95°C for 10 s, 68°C for 10 s, and 72°C for 30 s. Samples were processed in batches of 20. Each run included a positive DNA extraction control (10^3 CFU/ml *Escherichia coli*), a negative DNA extraction control (Ringer's solution), and a master mix control (water).

Amplification products were spun out of the capillary into a 1.5-ml Eppendorf tube and prepared for sequencing using ExoSAP-IT (Affymetrix, Santa Clara, CA). Sequencing was performed using the BigDye Terminator method with a 3730*xl* Genetic analyzer (Applied Biosystems, Foster City, CA). The generated sequences were compared against those of the National Center for Biotechnology Information's (NCBI) GenBank database. Identity of ≥99.0% between the query sequence and the GenBank database with a difference of >0.4% between species was used for identification to the species level. Identities of >97.0% and <99.0% were considered for identification to the genus level. Mixed sequences were visually inspected using Sequencher 4.7 (Gene Code, Ann Arbor, MI) or ChromasPro (Technelysium Pty. Ltd., Brisbane, QLD, Australia) software to set an appropriate *y* axis cutoff and trim the sequence. The mixed sequences were then analyzed using the RipSeq Mixed web-based software and database (iSentio, Bergen, Norway), as previously described (13, 14).

A positive result was determined by the value of the crossing point (CP; point at which DNA amplification reaches the fluorescent threshold). Since the average cycle number at which amplification occurred in negative controls was 30 cycles, a positive sample was defined as having a CP of <26 cycles. Using this cutoff, signal from DNA contamination of reagents and materials, such as Ringer's solution, was well differentiated from positive results.

Inclusiveness was confirmed using 159 bacteria collected from the site of infection in subjects with PJI. By spiking negative sonicate fluid with clinical isolates of *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Escherichia coli*, the analytical sensitivity was determined to be 10^3 to 10^4 CFU/ml using a CP of <26 cycles as a cutoff.

Specimens were assayed for the human β-globin gene to assess for PCR inhibition. Briefly, real-time PCR of the DNA extracts was performed using the LightCycler Control Kit DNA primers (which amplify a 110-bp fragment of the β-globin gene) and the LightCycler FastStart DNA Master SYBR green I reaction mix (Roche Applied Science, Indianapolis, IN). The kit includes a positive control; master mix alone served as a negative control. The β-globin gene was detected in DNA extracts from all 366 specimens.

Statistical analysis. Sensitivities and specificities of each of the microbiology tests were compared to the "gold standard," the PJI definition, using McNemar's test for related proportions. The binomial exact method was used to calculate 95% confidence intervals. Independent proportions were compared using chi-square, while means and medians for continuous variables were compared using Student's *t* test and the Mann-Whitney test, respectively. *P* values of <0.05 were considered statistically significant. One hundred thirty-eight cases of PJI would have been re-

quired to detect a 15% difference in sensitivity between sonicate fluid culture and PCR with a power of 80%. A receiver operating characteristic (ROC) curve was also constructed to assess the optimal CP for the PCR assay. Statistical analyses were performed using SPSS software (version 19) and MedCalc (version 12.1.4.0).

RESULTS

Four sonicate fluids from polyethylene liners alone and nine from duplicate subjects with multiple submitted prostheses were excluded. A total of 366 prostheses from unique subjects were analyzed, of which 135 were associated with PJI and 231 with AF. The mean subject age was 66 years (range, 24 to 92 years), and 183 subjects (50%) were men. There were 130 hip and 236 knee arthroplasties. Osteoarthritis was the most common underlying joint pathology. The median prosthesis ages at the time of arthroplasty revision or resection were 22 months (range, 0 to 317 months) for PJI and 51 months (range, 0 to 459 months) for AF ($P < 0.001$). The majority of PJIs occurred more than a year after the index surgery (76%). The following surgical procedures were performed: resection ($n = 144$), partial resection ($n = 1$), revision ($n = 202$), and partial revision ($n = 19$). Sixty-nine subjects received antimicrobials within 4 weeks preceding implant removal. Baseline characteristics were similar between subjects with PJI and AF, except that the PJI group had higher white blood cell counts, erythrocyte sedimentation rates, C-reactive protein levels, and synovial fluid leukocyte and neutrophil counts, more preoperative antimicrobial use, diabetes, and resection arthroplasties, younger implants, and shorter timer time to implant failure than the AF group (Table 1).

Fifty-nine subjects had positive synovial fluid cultures (55 PJI and 4 AF), 98 had positive tissue cultures (95 PJI and 3 AF), 102 had positive sonicate fluid cultures (98 PJI and 4 AF), and 100 had positive PCR results (95 PJI and 5 AF). Five subjects had isolated positive synovial fluid cultures (2 PJI and 3 AF), seven had isolated positive tissue cultures (4 PJI and 3 AF), four had isolated positive sonicate fluid cultures (2 PJI and 2 AF), and six had isolated positive sonicate fluid PCR results (3 PJI and 3 AF). The sensitivities of synovial fluid culture, tissue culture, sonicate fluid culture, and sonicate fluid PCR were 64.7, 70.4, 72.6, and 70.4%, respectively (Table 2), with no statistically significant difference among tests. The specificities of synovial fluid culture, tissue culture, sonicate fluid culture, and PCR were 96.9, 98.7, 98.3, and 97.8%, respectively, with no statistically significant difference among tests. We initially constructed a ROC curve to determine the CP of sonicate fluid PCR that would provide the optimal sensitivity and specificity for the diagnosis of PJI. The area under the curve was 0.87. This analysis suggested using a CP cutoff value of 27.59 cycles, with which PCR had a higher sensitivity (80.0 versus 72.6%; $P = 0.013$) but a lower specificity (90.9 versus 98.3%; $P < 0.001$) than sonicate fluid culture. Due to the false-positive percentage of 9.1% using this CP cutoff, we used a CP cutoff of <26 cycles, trading sensitivity for specificity. By combining tissue culture, sonicate fluid culture, and PCR, a higher sensitivity was obtained (83.0%; $P < 0.001$) than with each individual test, but with a lower specificity (95.7%, $P < 0.05$) than with either tissue or sonicate fluid culture alone. When sonicate fluid culture and PCR were evaluated in combination, the sensitivity was statistically higher (78.5%; $P < 0.05$) than with the other tests, with no statistical difference in specificity (97.0%; $P > 0.05$).

Forty subjects who met the study definition of PJI had negative PCR results, of which 52.5% had negative results by all assessed methods. Using a CP cutoff of <27.59 cycles, 37.5% of them would have had a positive PCR. Unusual organisms were cultured from three subjects with PJI (*Mycobacterium abscessus*, *Candida albicans*, and *Candida glabrata*). Among subjects with AF, five had positive PCR results. These patients were blindly evaluated by an infectious diseases expert (J.M.S.), who, after reviewing the clinical and laboratory findings, considered two cases as PJI and one case as possible PJI, even though they did not meet the study criteria for PJI.

The overall concordance (positive or negative) and concordance at the genus level between sonicate fluid culture and PCR were 93 and 90%, respectively. The amplified V3-V4 hypervariable region provided poor resolution for species-level identification of certain organisms, such as streptococci and coagulase-negative *Staphylococcus* species (CoNS), limiting reporting to the genus level. Eight subjects with polymicrobial infections had partial concordance between PCR and sonicate fluid culture, mainly because PCR did not detect some organisms detected by sonicate fluid culture (Table 3). Only one subject had discrepant results, with *Propionibacterium acnes* isolated from sonicate fluid culture and PCR positive for a *Streptococcus* sp. This subject had an acute presentation with a purulent joint, more consistent with a streptococcal than a *P. acnes* infection.

Eleven subjects had negative sonicate fluid cultures and positive sonicate fluid PCR results (Table 4). Of these, seven had received antimicrobials, three had either tissue or synovial fluid cultures concordant with the sequencing results, and one (case 430) had discrepant tissue culture (CoNS) and PCR (*Enterococcus* sp.) results.

A microorganism was isolated from sonicate fluid for 13 subjects (11 PJI and 2 AF) with negative PCR results (Table 5). Ten had concordant positive tissue cultures (≥2 samples positive), and one (case 236) had a *C. albicans* infection (not targeted by PCR). Eleven had negative sonicate fluid PCR, but with a CP between 26.01 and 29 cycles; amplified product sequencing yielded concordant findings with sonicate fluid cultures.

Sixty-nine subjects (54 PJI and 15 AF) had received antimicrobials within 4 weeks of the index procedure. In these subjects, the sensitivity and specificity of tissue culture, sonicate fluid culture, and PCR were 75.9 and 93.3%, 75.9 and 93.3%, and 72.2 and 80%, respectively, and there was no difference in sensitivity between sonicate fluid culture and PCR ($P = 0.54$). In this subgroup, when using a CP cutoff of <27.59 cycles, PCR sensitivity was higher (87%), with no loss of specificity (80%).

Compared to tissue culture, PCR had an overall concordance of 90.2%. In the PJI group, 81 had positive results by both tissue culture and PCR, of which 71 had concordant PCR results to the genus level, 9 had partial concordance (PCR did not detect some pathogens identified by culture), and 1 had discordant tissue culture (CoNS) and PCR (*Enterococcus* sp.) results. PCR missed 14 subjects with PJI who had positive tissue cultures, including an *M. abscessus* and a *C. albicans* infection. Eight of these subjects had positive sonicate culture in concordance with the tissue culture. PCR detected a pathogen in 14 with PJI and negative tissue cultures. Nine had concordant sonicate fluid cultures, and seven had a history of prior antimicrobial use. In the AF group, five had positive PCR results with negative tissue cultures, of which three had prior antimicrobial use.

The most common organisms isolated in culture were CoNS, followed by *S. aureus*, streptococci, and *P. acnes*. Twenty-one subjects (5.7%) had polymicrobial results by either tissue culture,

TABLE 1 Clinical characteristics of study subjects[a]

Characteristic	Subjects with PJI ($n = 135$)	Subjects with AF ($n = 231$)	P value[c]
Age, mean in yrs (range)	66 (31–92)	66 (24–91)	0.870
No. (%) of subjects that were male	73 (54.1)	110 (47.6)	0.318
No. (%) with comorbidities			
Diabetes	30 (22.2)	30 (13.0)	**0.032**
Immunosuppressants	4 (3.0)	2 (8.6)	0.061
Other	5 (3.7)	2 (8.6)	0.114
No. (%) with underlying joint pathology			
Osteoarthritis	101 (74.8)	169 (73.2)	0.832
Bone fracture	18 (13.3)	33 (14.3)	0.912
Inflammatory arthropathy	7 (5.2)	17 (7.4)	0.548
Osteonecrosis	3 (2.2)	9 (3.9)	0.564
Other	6 (4.4)	3 (1.3)	0.133
No. (%) with implant			
Knee	86 (63.7)	150 (64.9)	0.906
Hip	49 (36.3)	81 (35.1)	0.906
Age of the implant, in mo, median (range)	22 (0–317)	51 (0–459)	**<0.001**
Time to clinical failure,[b] in mo, median (range)	7 (0–316)	453 (0–453)	**<0.001**
Nonculture tests			
WBC (10^9/liter), mean (SD)	8.1 (3.2)	7.2 (2.6)	**0.012**
ESR (mm/h), mean (SD)	45.1 (30.6)	17.2 (17.2)	**<0.001**
CRP (mg/liter), mean (SD)	50.2 (53.7)	11.8 (23.6)	**<0.001**
No. (%) of subjects for whom arthrocentesis was performed	89 (66.0)	134 (58.0)	0.161
Synovial fluid leukocytes (10^3/μl), median (range)	20 (0.1–1,071)	0.7 (0.1–125)	**<0.001**
Synovial fluid neutrophil percentage, median (range)	89 (2–100)	15 (0–96)	**<0.001**
No. (%) for whom synovial fluid culture was performed	85 (63.0)	129 (56.0)	0.229
No. (%) of subjects with surgical procedure			
Revision	13 (9.6)	208 (90.0)	**<0.001**
Resection	121 (89.6)	23 (10.0)	**<0.001**
Partial resection	1 (0.7)	0 (0.0)	0.837
No. of periprosthetic tissue samples taken, mean (SD)	4.2 (1.3)	3.2 (0.9)	**<0.001**
No. with PJI definition/total no. of subjects (%)			
Acute inflammation on histopathology	81/103 (78.6)	0/231 (0.0)	
Intraoperative purulence	106 (78.5)	0 (0.0)	
Presence of sinus tract	35 (25.9)	0 (0.0)	
No. (%) with prior use of antimicrobials	54 (40.0)	15 (6.5)	**<0.001**

[a] All percentages are in relation to the number of subjects with PJI or AF unless otherwise indicated. WBC, white blood cell count; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; PJI, prosthetic joint infection; AF, aseptic failure.
[b] Time from the implant surgery to onset of symptoms.
[c] Bold values are statistically significant.

TABLE 2 Comparison of microbiologic tests studied[a]

Test	% Sensitivity	% Specificity	% Accuracy
Tissue culture	70.4 (64.5–76.3)	98.7 (97.2–100)	88.3 (84.2–92.4)
Sonicate fluid culture	72.6 (66.8–78.4)	98.3 (96.6–100)	88.8 (84.7–92.9)
Sonicate fluid PCR (CP < 26 cycles)	70.4 (64.5–76.3)	97.8 (95.9–99.7)	87.7 (83.5–91.9)
Combination of tests above	83.0 (78.2–87.8)	95.7 (93.1–98.3)	91.0 (87.3–94.7)
Sonicate fluid culture plus PCR (CP < 26 cycles)	78.5 (73-2-83.8)	97.0 (94.8–99.2)	90.2 (86.4–94.0)
Synovial fluid culture	64.7 (56.5–72.9)	96.9 (93.9–99.9)	84.1 (77.8–90.4)
Sonicate fluid PCR - higher cutoff (CP < 27.59 cycles)	80.0 (74.8–85.2)	90.9 (87.2–94.6)	86.8 (82.5–91.3)

[a] Values in parentheses are 95% confidence intervals. CP, crossing point.

sonicate fluid culture, or PCR (Table 3). All subjects with polymicrobial findings, except one (case 84), had PJI. These subjects were more likely to have a sinus tract than subjects with monomicrobial results (52.4 versus 7%; $P = 0.04$). Fourteen had positive PCR results with mixed chromatograms that were analyzed using RipSeq, providing partial or complete identifications of the multiple organisms (Table 3).

DISCUSSION

Implant sonication culture has been shown to improve the diagnosis of PJI; however, despite its application, pathogens are not

TABLE 3 Subjects with polymicrobial results from tissue culture, sonicate fluid culture, and/or 16S rRNA real-time PCR with sequencing[a]

Case	Result(s) of:				Prior antimicrobial(s)
	Tissue culture	Sonicate culture	PCR	Other tests	
84	*Propionibacterium acnes*[b]	*Clostridium perfringens*, *P. acnes*	Negative	Normal ESR/CRP	
175	*Staphylococcus aureus*, *Finegoldia magna*, GPB	*S. aureus*, *F. magna*	*Staphylococcus* sp., *F. magna*[c]	Sinus tract present Synovial fluid culture: *S. aureus*	Trimethoprim-sulfamethoxazole
192	CoNS, *Paecilomyces* sp.[b]	CoNS, GPB	CoNS	Sinus tract present Prior blood cultures: CoNS, *Candida* sp., *Pseudomonas* sp.	
226	CoNS	Negative	CoNS, *P. acnes*[c]	Sinus tract present	Trimethoprim-sulfamethoxazole, levofloxacin
238	*Escherichia coli*, CoNS, GPB[b]	*E. coli*, CoNS, GPB	*E. coli*/*Shigella* spp., *Corynebacterium* sp.[c]	Sinus tract present	Levofloxacin
250	Negative	Negative	CoNS, *P. acnes*, *S. aureus*[c]	Chronic sinus tract present	Fusidic acid
264	*S. aureus*, CoNS	*S. aureus*	*S. aureus*	Sinus tract present	Cephalexin
266	*Enterococcus* sp., CoNS	*Enterococcus* sp., CoNS	*Enterococcus* sp., CoNS[c]	Synovial fluid culture: *Enterococcus* sp., CoNS	
279	*Actinomyces odontolyticus*, *Prevotella melaninogenica*, *Enterococcus* sp., anaerobic Gram-negative coccus, anaerobic Gram-positive coccus, viridans group streptococcus, *Abiotrophia*/*Granulicatella* sp.	*A. odontolyticus*, *P. melaninogenica*, *Enterococcus* sp., *Veillonella* sp., *F. magna*, viridans group streptococcus, *Abiotrophia*/*Granulicatella* sp., *Pandoraea norimbergensis*	*P. melaninogenica*	Multiple sinus tracts present	Unknown antibiotic
280	CoNS[b]	Negative	*Streptococcus* sp., *Gemella* sp.[c]		
287	*Actinomyces europaeus*, anaerobic Gram-positive coccus,[b] *Enterococcus faecalis*, *S. aureus*	*S. aureus*, *Peptostreptococcus* sp., *E. faecalis*	*S. aureus*[c]	Chronic sinus tracts present. Synovial fluid culture: *A. europaeus*, *E. faecalis*	
300	Negative	CoNS, *Staphylococcus lugdunensis*	Negative	Previous PJI with *Klebsiella* sp. and *P. acnes* Blood cultures: *S. lugdunensis*	Moxifloxacin/rifampin
331	CoNS, *F. magna*	CoNS, *F. magna*	*Corynebacterium* sp., *F. magna*, CoNS[c]	Synovial fluid culture: CoNS, *S. lugdunensis*	
335	*S. aureus*, CoNS	*S. aureus*, CoNS	*S. aureus*, CoNS[c]		
337	*S. aureus*, *Pseudomonas aeruginosa*, CoNS, GPB, *F. magna*	*S. aureus*, *Pseudomonas* sp., *F. magna*, *Actinomyces neuii*	*Pseudomonas aeruginosa*, *Peptoniphilus* sp.[c]	Sinus tract present	Levofloxacin
369	GPB, *S. aureus*	Negative	Negative	Sinus tract present	Cefazolin
392	CoNS	CoNS, *P. acnes*	CoNS[c]	Synovial fluid culture: CNS	
396	CoNS[b]	*Enterococcus* sp., CoNS	*Enterococcus* sp., CoNS[c]	Synovial fluid culture: *E. faecalis*	
417	*S. aureus*, GPB, CoNS, *Dolosigranulum pigrum*	*S. aureus*, GPB, CoNS	CoNS[c]		Cephalexin
428	CoNS[b]	*Enterococcus* sp., CoNS	*Enterococcus* sp.[c]	Synovial fluid culture: *Enterococcus* sp.	
429	*Enterococcus* sp., GPB	*Enterococcus* sp., *S. aureus* (<20 CFU/10 ml)[d]	*Enterococcus* sp.	Sinus tract present Synovial fluid culture: *Enterococcus* sp.	

[a] ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; CoNS, coagulase-negative *Staphylococcus* species; GPB, Gram-positive bacillus resembling *Corynebacterium* sp. Underlined case indicates aseptic failure.
[b] Isolated from a single tissue sample and considered negative by definition.
[c] Mixed sequence identified by RipSeq.
[d] Considered negative by definition.

isolated in all cases using culture. Some authors have proposed that molecular tests may provide a higher diagnostic yield than culture-based techniques, especially for subjects with previous exposure to antimicrobials (8, 16, 28). Bacterial DNA has been identified in synovial fluid of subjects with septic arthritis several days after starting antimicrobial treatment (despite negative cultures) (29). However, the various results reported from different studies on the use of PCR for the diagnosis of PJI have made PCR a technique not yet widely accepted in clinical practice. The sensitivity and specificity of broad-range PCR from synovial fluid and/or tissue for the diagnosis of PJI have been reported to be between 50 to 92% and 65 to 94%, respectively (8, 16, 28).

Few studies have evaluated the use of molecular methods on sonicate fluid from explanted prostheses for the diagnosis of PJI. Tunney et al. evaluated PCR of sonicate fluid from hip prostheses (27). Bacterial DNA was detected in prosthesis sonicates by conventional broad-range PCR in all culture-positive samples and in a further 64% of culture-negative samples. However, in that study, PJI was not well defined and clinical data and histopathology were not provided for all subjects. Achermann et al. evaluated a real-time multiplex PCR test for the diagnosis of PJI from sonicate fluid (1). PCR was numerically but not statistically more sensitive than sonicate fluid culture (78 versus 62%); among the 19 subjects who had received antibiotics prior to the resection arthroplasty,

TABLE 4 Bacteriological findings for subjects with positive 16S rRNA real-time PCR results and negative sonicate fluid cultures[a]

Case	Result(s) of: Sonicate fluid culture (CFU/10 ml)	Sequence analysis of amplified DNA	Other tests	Prior antimicrobial(s)
74	*Staphylococcus aureus* (<20)[b]	*S. aureus*	Tissue and synovial fluid culture: *S. aureus*	Vancomycin
214	GBS (<20)[b]	*Streptococcus* sp.	Synovial fluid culture: GBS	Penicillin, ceftriaxone
217	*Escherichia coli* (<20)[b]	*E. coli*/*Shigella* sp.	Tissue culture: *E. coli*	
226		CoNS, *Propionibacterium acnes*	Tissue culture: CoNS	Trimethoprim-sulfamethoxazole, levofloxacin
250	CoNS (<20)[b]	CoNS, *P. acnes*, *S. aureus*		Fusidic acid
280		*Streptococcus* sp., *Gemella* sp.	Tissue culture: CoNS[c]	
299		*Streptococcus* sp.	↑ ESR and CRP	Vancomycin
321		*Klebsiella pneumoniae*		
379		*Serratia marcescens*	Normal ESR and CRP, synovial leukocytes: 3,134 cells/μl	Cefazolin, vancomycin
397		*Streptococcus* sp.		Penicillin, clindamycin
430		*Enterococcus* sp.	Tissue and synovial fluid culture: CoNS	

[a] GBS, group B streptococcus; CoNS, coagulase-negative staphylococcus; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein. Underlined cases indicate aseptic failure.
[b] Considered negative by definition.
[c] Single positive tissue culture which was considered negative by definition.

PCR detected the pathogen in 100% of cases, compared to 42% with culture. A prior study by our group showed that a different broad-range PCR assay from that studied herein, performed on sonicate fluid, had a lower sensitivity than sonicate fluid culture (42 versus 77%), with equal specificity (99%) (18).

In the current study, there was no statistical difference in the proportion of subjects with PJI detected by sonicate fluid PCR or culture. PCR was negative for 13 subjects who had positive sonicate cultures, including one *C. albicans* infection, which is not detectable by our assay. The majority of these cases were negative by PCR because of the low CP cutoff applied; sequence analysis correctly identified (to the genus level) 11 of these. If a higher CP cutoff (<27.59 cycles) had been applied, PCR would have been more sensitive than sonicate fluid culture (80 versus 72.6%), but it would also have been less specific (90.9 versus 98.3%). For this reason, we opted to apply a CP cutoff (<26 cycles) that provided a high specificity. Decreasing the false-positive rate will result in fewer patients unnecessarily subjected to antimicrobial treatment and possible subsequent surgeries. However, we realize that with this approach we may not be able to microbiologically diagnose some PJI cases, precluding targeted antimicrobial treatment, and, moreover, that this approach may miss some occult cases of PJI.

Eleven subjects had positive PCR results with negative sonicate fluid cultures, of which seven had a history of antimicrobial use prior to the arthroplasty resection. We performed a subgroup analysis of those who had prior use of antimicrobials, and there

TABLE 5 Bacteriological results for subjects with positive sonicate fluid cultures and negative 16S rRNA real-time PCR[a]

Case	Result(s) of: Sonicate fluid culture	PCR sequencing result[b]	Other tests	Prior antimicrobial(s)
73	Viridans group streptococcus	*Streptococcus* sp.	↑ CRP	
84	*Clostridium perfringens*, *Propionibacterium acnes*[d]		Tissue culture: *P. acnes*[c]	
126	*Staphylococcus epidermidis*	CoNS	Tissue and synovial fluid culture: CoNS	Levofloxacin
236	*Candida albicans*		Tissue and synovial culture: *C. albicans*	Fluconazole
255	*Enterococcus faecium*	*Enterococcus* sp.	Tissue culture: *E. faecium*	Vancomycin
289	CoNS	CoNS	Tissue and synovial fluid cultures: CoNS	
291	CoNS	CoNS	Tissue culture: CoNS	Clindamycin, moxifloxacin
300	CoNS, *Staphylococcus lugdunensis*	*Staphylococcus* sp.	Blood cultures: *S. lugdunensis*	Moxifloxacin, rifampin
342	CoNS	CoNS	Synovial fluid culture: CoNS	Vancomycin, rifampin
360	CoNS	CoNS	Tissue and synovial fluid cultures: CoNS	
378	CoNS	CoNS	Tissue culture: CoNS	
412	CoNS	CoNS	Tissue culture: CoNS[c]	
440	*Pseudomonas aeruginosa*	*Pseudomonas* sp.	Tissue and synovial fluid culture: *P. aeruginosa*	Ciprofloxacin

[a] CoNS, coagulase-negative staphylococcus; CRP, C-reactive protein. Underlined cases indicate aseptic failure.
[b] Crossing point ≥ 26, which was considered a negative PCR result.
[c] Single positive tissue culture, which was considered negative by definition.
[d] Reverse primer has a mismatch against *P. acnes* in position 3 from the 3′ end; this may explain the negative PCR for this subject.

was no difference in the number of pathogens identified by either PCR or culture. However, when we analyzed only subjects who had received antimicrobials within 2 weeks of the index surgery ($n = 34$), there was a trend toward higher sensitivity for PCR than for sonicate fluid culture (73.3 versus 66.7%; $P = 0.727$). In contrast to the study by Tunney et al., the number of subjects with AF and positive culture and/or PCR results was small (6.5%), suggesting that low-grade infection of the implant not detected by current diagnostic strategies might not be a significant cause of the AF. These cases may represent PJI with positive intraoperative cultures only, according to the classification of Tsukayama et al. (26).

A drawback of most studies of diagnostic tests for PJI is the lack of a standardized definition of PJI. The Musculoskeletal Infection Society recently published a new definition for PJI (17), which takes into account ESR and CRP levels, synovial fluid leukocyte count and neutrophil percentage, and periprosthetic tissue or synovial fluid culture. If we had applied this new definition to our data, we would have obtained similar results (i.e., 122 PJI and 244 AF cases). The sensitivities of synovial fluid culture, periprosthetic tissue culture, sonicate fluid culture, and PCR (CP cutoff < 26 cycles) would have been 68.8, 80.3, 78.7, and 77.0%, respectively ($P > 0.05$). The specificities of synovial fluid culture, periprosthetic tissue culture, sonicate fluid culture, and PCR (CP cutoff $<$ 26 cycles) would have been 97, 100, 97.5, and 97.5%, respectively ($P > 0.05$). Using a higher CP ($<$27.59 cycles), PCR would have had a sensitivity similar to that of tissue culture (85.2%) but higher than that of sonicate or synovial fluid culture ($P = 0.02$), with lower specificity (89.9%) than those of the other tests studied ($P < 0.05$).

Broad-range PCR is a very sensitive method, prone to false-positive results from contaminating DNA. *Taq* DNA polymerases and other PCR reagents may be contaminated with bacterial DNA (22). Strategies to eliminate spurious bacterial DNA include treatment of reagents with gamma irradiation (6, 9), UV irradiation (6), restriction endonucleases (6), DNase I (6), or heat-labile double-strand-specific DNase (Biotec Marine Biochemicals, Tromsø, Norway) (6); reported results have been variable. During assay development, we tried all these strategies and found that, with one exception, they were ineffective and/or caused PCR inhibition. Heat-labile double-strand-specific DNase was the only method that was effective and did not inhibit PCR. Despite improvement in performance of PCR assay reagents, the Ringer's solution used for implant sonication contained background DNA, resulting in false-positive PCR results. Since we were unable to completely control contamination of the already-sonicated samples, we used a detection threshold to control false-positive results. Clarke et al. encountered a high false-positive rate (29%) using a sensitive 16S rRNA PCR (10 bacteria per cm^3 of sample) in subjects undergoing primary arthroplasties (7). Tunney et al. used a 16S rRNA PCR assay with a limit of detection of 10^4 CFU/ml in failed arthroplasties; this limit of detection is similar to ours (27). The authors suggested that with this high limit of detection, the likelihood of false-positive results was low; however, the study design limited evaluation of assay specificity.

The evaluated microbiologic tests had limited sensitivity and/or specificity when used individually. We explored the possibility of combining sonicate fluid culture and PCR and in doing so were able to obtain higher sensitivity, with specificity similar to those of the individual tests. Although this could be implemented in a clinical laboratory, a cost-benefit analysis should be performed to justify the added cost of PCR.

Although polymicrobial PJIs are not frequent, their evaluation with broad-range PCR can be challenging. Mixed chromatograms are a shortcoming of broad-range PCR, and one usually has to resort to cloning or high-performance liquid chromatography to analyze mixed sequence results (24). Although our PCR assay was able to detect all PJI cases with polymicrobial infections, and the analysis of the mixed chromatograms was facilitated by the use of RipSeq Mixed, some organisms were still not detected by PCR. This is possibly due to competition of DNA targets for primers, favoring the most abundant targets.

False-positive results continue to limit the application of broad-range PCR in the diagnosis of PJI. Target-specific PCR and/or reverse transcriptase PCR (RT-PCR) to detect (messenger or ribosomal) RNA instead of DNA (which may be less prone to false positivity [3]) warrants further study.

Although PCR had a sensitivity similar to that of culture, PCR-based techniques have the advantage over culture of a faster turnaround time, which could have practical application in clinical practice. Kobayashi et al. developed a real-time broad-range PCR assay along with a rapid nucleic acid extraction method for the intraoperative diagnosis of PJI (12). Combined with rapid DNA extraction, PCR could theoretically become a point-of-care test for patients undergoing revision arthroplasty. Culture usually has a turnaround time of 18 to 24 h or more, compared to our 4-h PCR protocol (0.5 h for sonication of the removed implant, 2.5 h for DNA extraction, and 1 h for PCR), which yields a positive or negative result. However, sequencing of the amplified DNA can take up to 24 h.

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