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Improved Diagnosis of Periprosthetic Joint Infection by Multiplex PCR of Sonication Fluid from Removed Implants[▽]

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The microbiological diagnosis of periprosthetic joint infection (PJI) is crucial for successful antimicrobial treatment. Cultures have limited sensitivity, especially in patients receiving antibiotics. We evaluated the value of multiplex PCR for detection of microbial DNA in sonication fluid from removed orthopedic prostheses. Cases of PJI in which the prosthesis (or part of it) was removed were prospectively included. The removed implant was sonicated, and the resulting sonication fluid was cultured and subjected to multiplex PCR. Of 37 PJI cases (17 hip prostheses, 14 knee prostheses, 4 shoulder prostheses, 1 elbow prosthesis, and 1 ankle prosthesis), pathogens were identified in periprosthetic tissue in 24 (65%) cases, in sonication fluid in 23 (62%) cases, and by multiplex PCR in 29 (78%) cases. The pathogen was detected in 5 cases in sonication fluid only (*Propionibacterium acnes* in all cases; none of these patients had previously received antibiotics) and in 11 cases by multiplex PCR only (all of these patients had previously received antibiotics). After exclusion of 8 cases caused by *P. acnes* or *Corynebacterium* species, which cannot be detected due to the absence of specific primers in the PCR kit, sonication cultures were positive in 17 cases and multiplex PCR sonication cultures were positive in 29 cases (59% versus 100%, respectively; $P < 0.01$). Among 19 cases (51%) receiving antibiotics, multiplex PCR was positive in all 19 (100%), whereas sonication cultures grew the organism in 8 (42%) ($P < 0.01$). Multiplex PCR of sonication fluid is a promising test for diagnosis of PJI, particularly in patients who previously received antibiotics. With modified primer sets, multiplex PCR has the potential for further improvement of the diagnosis of PJI.

Modern medicine has developed a variety of artificial devices to assist in the performance of physiological functions as short-term devices (e.g., catheters and fracture fixation hardware) or permanent devices (e.g., artificial cardiac valves, pacemakers, and prosthetic joints). Orthopedic devices are used for the treatment of degenerative joint disease (osteoarthritis) and bone fracture (10). They are increasingly implanted in the growing population of the elderly. The risk of periprosthetic joint infections (PJI) is additionally increasing due to longer resident time of the implant in the body, which can become infected from a distant infectious focus by hematogenous route at any time after implantation (29). Thus, the number of PJI is expected to increase steadily in the next decades.

The identification of the infecting microorganism is crucial for successful treatment of PJI. Currently, cultures of synovial fluid and intraoperative periprosthetic tissue represent the standard method for diagnosing PJI. With newer techniques, such as sonication of removed implants, the sensitivity has been significantly increased (18, 22). However, in a significant proportion of patients with PJI, the infecting microorganism remains unknown, particularly when patients had previously received antimicrobial treatment (1, 22). Molecular methods may improve the diagnosis for PJI due to high sensitivity and culture independence.

Several researchers have evaluated the role of PCR in the diagnosis of osteoarticular infections, including septic arthritis (8, 14, 15) and PJI (1a, 4, 5, 12, 17, 25). The value of PCR was mainly investigated in synovial fluid or periprosthetic tissue specimens, whereas sonication fluid was evaluated only recently (18, 26). The limitations of these studies are the use of a specific PCR, which is typically able to detect only a single microorganism, or the use of a broad-range (16S ribosomal DNA [rDNA]) PCR, which can detect previously unknown organisms but has lower sensitivity and specificity than specific PCR, requires subsequent sequencing for bacterial identification, and fails to detect mixed infections.

Herein, we investigated a novel approach for diagnosis of PJI combining two complementary diagnostic methods, namely, sonication of removed implants and multiplex real-time PCR of the resulting sonication fluid. The removed prostheses were sonicated as previously described (18, 22), and the resulting sonication fluid was cultured and subjected to molecular detection with a real-time multiplex PCR test (SeptiFast; Roche Diagnostics, Basel, Switzerland). This PCR kit was designed for detection and identification of the most common bacterial and fungal pathogenic species in blood (Table 1) (2, 11, 13, 16, 28) and has not yet been investigated for the diagnosis of PJI. We hypothesized that the combined approach of sonication and multiplex PCR of sonication fluid will improve the sensitivity and specificity for diagnosing PJI.

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TABLE 1. Microorganisms that can be detected by multiplex PCR (SeptiFast)

Group and organism
Gram-negative organisms
Escherichia coli
Klebsiella pneumoniae/oxytoca
Serratia marcescens
Enterobacter cloacae/aerogenes
Proteus mirabilis
Pseudomonas aeruginosa
Acinetobacter baumannii
Stenotrophomonas maltophilia
Gram-positive organisms
Staphylococcus aureus
Coagulase-negative staphylococci[a]
Streptococcus pneumoniae
Streptococcus spp.[b]
Enterococcus faecalis/faecium
Fungi
Candida albicans
Candida glabrata
Candida krusei
Candida tropicalis
Candida parapsilosis
Aspergillus fumigatus

[a] Includes *Staphylococcus hominis* subsp. *novobiosepticus*, *S. pasteuri*, *S. warneri*, *S. cohnii* subsp. *urealyticum*, *S. hominis* subsp. *hominis*, *S. lugdunensis*, *S. cohnii* subsp. *cohnii*, *S. capitis* subsp. *ureolyticus*, *S. capitis* subsp. *capitis*, *S. caprae*, *S. saprophyticus*, *S. saprophyticus* subsp. *saprophyticus*, *S. xylosus*, *S. epidermidis*, and *S. haemolyticus*.

[b] Includes *Streptococcus agalactiae*, *S. anginosus*, *S. bovis/S. gallolyticus*, *S. constellatus*, *S. cristatus*, *S. gordonii*, *S. intermedius*, *S. milleri*, *S. mitis*, *S. mutans*, *S. oralis*, *S. parasanguinis*, *S. pneumoniae*, *S. pyogenes*, *S. salivarius*, *S. sanguinis*, *S. thermophilus*, *S. vestibularis*, and *S. viridans.*

(Part of this research was presented at the European Congress of Clinical Microbiology and Infectious Diseases [ECCMID], Helsinki, Finland, 16 to 19 May 2009 [poster Nr.1828.].)

MATERIALS AND METHODS

Study design. A single-center prospective cohort study was conducted at the Schulthess Clinic, Zurich, Switzerland, a specialized orthopedic center with 150 beds and 7,500 surgical procedures performed per year, including primary implantations and revisions of joint prostheses. The study protocol was reviewed and approved by the institutional review board.

Study population. We prospectively included all consecutive patients aged 18 years or more from August 2008 through March 2009 with a prosthetic joint infection in whom the prosthesis or part of it (metal fixed or polyethylene mobile component) was removed at diagnosis of infection. The removed prosthesis was sent for sonication to determine the presence or absence of adherent microorganisms. Subjects were excluded if obvious contamination of an explanted component occurred in the operating room or fewer than two periprosthetic tissue specimens were collected for culture. Medical records were evaluated for the following: demographic characteristics; clinical, radiographic, laboratory, histopathological, and microbiological data; type of surgical management; antimicrobial therapy; and information about the primary arthroplasty and subsequent revisions (if any). According to the study protocol, patients received no perioperative antimicrobial prophylaxis before surgery in order to optimize the diagnostic yield of intraoperative cultures. Patients who had received an intravenous antibiotic for at least 24 h in the 10 days before surgery were defined as having received previous antimicrobial therapy.

Study definitions. PJI was considered if one of the following criteria was present (22, 23, 29): (i) visible purulence of a preoperative aspirate or intraoperative periprosthetic tissue (as determined by the surgeon), (ii) presence of a sinus tract communicating with the prosthesis, (iii) acute inflammation in intraoperative permanent periprosthetic tissue sections by histopathology (as determined by the pathologist), (iv) increased synovial fluid leukocyte count with >1,700 leukocytes/μl and/or >65% granulocytes (as described in reference 21), or (v) microbial growth in preoperative joint aspirate, intraoperative periprosthetic tissue, or sonication fluid of the removed implant. Low-virulence microorganisms, such as coagulase-negative staphylococci or *Propionibacterium acnes*, were considered pathogens, if at least one additional (culture-independent) criterion for PJI was fulfilled.

Synovial fluid and periprosthetic cultures. Synovial fluid was aspirated preoperatively at the discretion of the operating surgeon. The aspirate was transferred into a sterile vial containing no additives. For all patients, at least two intraoperative periprosthetic tissue specimens were retrieved from the bone-cement or bone-prosthesis interface with the most obvious inflammatory changes. Tissue specimens were collected in sterile vials and were individually homogenized in 3 ml Trypticase soy broth for 1 min using a mortar and pestle. The synovial fluid and tissue homogenate samples were inoculated in 0.1-ml aliquots onto aerobic and anaerobic sheep blood agar plates and in 1-ml aliquots into thioglycolate broth. The cultures were incubated at 35°C ± 1°C for 10 days. A terminal subculture was performed from all thioglycolate broth specimens on blood agar plates and incubated at 35°C ± 1°C for 5 more days. Each unique colony of isolated microorganisms was identified, and their antimicrobial susceptibility was tested using standard microbiological techniques.

Sonication fluid cultures. The removed prosthesis (or part of it) was aseptically removed in the operating room and transported to the microbiology laboratory in solid air-tight containers (Lock & Lock; Vetrag AG, Stäfa, Switzerland) within 48 h of removal. In the microbiological laboratory, sonication of the implant was performed as previously described (22). Briefly, 50 to 200 ml sterile Ringer solution (depending on the size of implant) was added to the container in a laminar airflow biosafety cabinet. The container with the implant was vortexed for 30 s, followed by sonication for 1 min (at a frequency of 40 ± 2 kHz and power density of 0.22 ± 0.04 W/cm^2), as determined by a calibrated hydrophone (type 8103; Brüel and Kjær, Naerum, Denmark). For sonication, ultrasound bath BactoSonic (Bandelin GmbH, Berlin, Germany) (www.bactosonic.info) was used. No differences in frequency or power density were observed at various locations within the ultrasound bath during the study period. The container was subsequently vortexed for an additional 30 s to remove any residual microorganisms and to homogeneously distribute them in the sonication fluid, which was plated in 0.1-ml aliquots onto aerobic and anaerobic sheep blood agar plates; 1 ml was inoculated in thioglycolate broth. All cultures were incubated at 35°C for 7 days and inspected daily for bacterial growth. Microorganisms on plates were enumerated (i.e., number of CFU/ml sonication fluid) and classified by using routine microbiological techniques.

Multiplex PCR assay. One milliliter of the sonication fluid was subjected to mechanical lysis and purification of DNA (SeptiFast lysis kit M^{GRADE}; Roche Diagnostics) according to the manufacturer's instructions. The lysed specimens were incubated for 1 h with a protease and chaotropic lysis buffer. Primer mix was introduced into each sample before amplification. The real-time PCR amplification of target DNA was performed in parallel reactions for Gram-positive bacteria, Gram-negative bacteria, and fungi using Hot Start Taq polymerase and the LightCycler instrument (LightCycler 2.0; Roche Diagnostics). The internal transcribed spacer (ITS) region was selected as the target region for bacterial and fungal species differentiation. Internal controls for the amplification step were included with each assay run.

Melting curve analysis was performed after completion of amplification with subsequent detection of PCR products by specific hybridization probes. The emitted fluorescence was simultaneously measured in four detection channels using a wavelength of 610 nm, 640 nm, 670 nm, and 705 nm. The data were automatically analyzed by the manufacturer's identification software program SIS (Roche Diagnostics) and reviewed manually for each detection channel. The crossing point of the amplification curve was recorded, and the emitted fluorescence signal was expressed as the *H* value, an integral of the fluorescence intensity and a semiquantitative measure of the initial quantity of target DNA. According to the manufacturer (Roche Diagnostics), the limit of detection in blood is 30 CFU/ml, except for coagulase-negative staphylococci, *Streptococcus pyogenes*, *Streptococcus agalactiae*, and *Candida glabrata*, for which the limit of detection is 100 CFU/ml (13, 14). A positive control and a negative control for multiplex PCR, supplied by the manufacturer, were included in each extraction series. All specimen handling preparations and the PCR setup were performed in a dedicated laminar airflow biosafety cabinet, equipped with an UV lamp, operated overnight. Dedicated equipment and unidirectional flow were used for the microbiological procedure.

Negative controls. Ten consecutive explanted prostheses from patients, who were diagnosed with mechanical (aseptic) loosening of the prosthesis and had no

TABLE 2. Characteristics of 37 patients with PJI

Characteristic	No. of patients or parameter value (%, unless range is specified)
Patient age, yr [median (range)]	71 (39–89)
Male	21 (57)
Location of PJI	
Hip	17 (46)
Knee	14 (38)
Shoulder	4 (11)
Ankle	1
Elbow	1
Type of removed implant for sonication	
Total prosthesis	26 (70)
Polyethylene mobile part	10 (27)
Joint spacer	1
Underlying joint disorder	
Osteoarthritis	26 (70)
Rheumatoid arthritis	7 (19)
Trauma	4 (11)
Type of arthroplasty	
Primary	10 (27)
Revision	27 (73)
Manifestation of infection according to surgery	
Early (<3 months)	6 (16)
Delayed (3 to 48 months)	25 (68)
Late (>48 months)	6 (16.2)
Time between the last surgical procedure and occurrence of infection, mo [median (range)]	7.5 (0.3–349)
Previous antimicrobial therapy received	19 (51)

history of previous infection, were included as controls. After the prosthesis was removed, it was subjected to sonication, followed by culture and multiplex PCR of sonication fluid, processed in the same way as prostheses from the study patients.

Statistical analysis. Comparisons of individual diagnostic tests were performed using the McNemar test. For mixed infections, a diagnostic test was considered positive if all infecting organisms were detected. Differences were considered significant when P values were <0.05. All calculations were performed using statistical software package SAS (version 8.2; SAS Institute Inc., Cary, NC). For graphic analysis, OriginPro software (version 8; Origin Lab Corp., Northampton, MA) was used.

RESULTS

Demographic and clinical characteristics of PJI. A total of 37 PJI cases were included in this study; the 37 cases included patients with hip ($n = 17$), knee ($n = 14$), shoulder ($n = 4$), ankle ($n = 1$), and elbow ($n = 1$) prostheses (Table 2). Three patients were included twice: two at the time of second debridement and one at the time of definite removal of the prosthesis after an initial salvage attempt. The median patient age at the time of infection was 71 years (range, 39 to 89 years), and 57% were males. The most common underlying joint disorder was osteoarthritis (70%), followed by rheumatoid arthritis (19%) and trauma (11%). Primary implantation was performed in 27% and revision surgery in 73% of the patients. Most infections (68%) occurred 3 to 24 months after surgery (delayed infections). The median time between the last surgical procedure and time of infection was 7.5 months (range, 0.3 to 349 months). Nineteen of the 37 (51%) patients had previously received antimicrobial therapy with a median duration of 9 days (range, 1 to 60 days).

TABLE 3. Comparison of periprosthetic tissue culture, sonication fluid culture, and multiplex PCR of sonication fluid in 37 cases of PJI

Infection type and microorganism	No. of episodes	No. of episodes with positive result by diagnostic test:		
		Periprosthetic tissue culture	Sonication fluid culture	Multiplex PCR of sonication fluid
Single microorganism	31	22	20	26
Staphylococcus aureus	9	5	5	9
Coagulase-negative staphylococci	11	9	8	11
Streptococcus mitis	1	1	1	1
Streptococcus agalactiae	1	1	1	1
Streptococcus dysgalactiae	1	1	0	1
Streptococcus gallolyticus	1	0	0	1
Streptococcus pneumoniae	1	0	0	1
Propionibacterium acnes	5	4	4	0
Candida albicans	1	1	1	1
Polymicrobial infection[a]	6	2	3	3
Total no. of episodes (%)	37 (100)	24 (65)	23 (62)	29 (78)

[a] Included coagulase-negative staphylococci, *Klebsiella pneumoniae*/*oxytoca* and *Enterococcus faecalis* ($n = 1$), *P. acnes* and coagulase-negative staphylococci ($n = 1$), *S. aureus* and coagulase-negative staphylococci ($n = 2$), *Corynebacterium* species and coagulase-negative staphylococci ($n = 1$), and *P. acnes* and *S. aureus* ($n = 1$).

Microbiology. Table 3 summarizes the microbiological findings of 37 cases of PJI. A single causative organism was found in 31 (84%) and a polymicrobial infection in 6 (16%) cases. Most monobacterial infections were staphylococcal infections. Methicillin resistance was detected in 7 of 11 (63%) episodes of coagulase-negative staphylococci (not including those from polymicrobial infections); none of the nine *Staphylococcus aureus* isolates causing infection was methicillin resistant. In addition, coagulase-negative staphylococci from two patients showed rifampin resistance (one patient with *Staphylococcus lugdunensis* infection who previously had a fistula and one patient with *S. aureus* infection in which no debridement was done 1 year ago and only ciprofloxacin and rifampin were given). None of the 10 implants from control patients showed growth in sonication fluid culture, and all were negative by multiplex PCR.

Comparison of culture and multiplex PCR. Among 37 cases of PJI, the causative organisms were identified in periprosthetic tissue culture in 24 (65%) cases, in sonication fluid culture in 23 (62%) cases, and by multiplex PCR in 29 (78%) cases (Table 3 and Fig. 1). In 21 cases (57%), the results of sonication fluid culture and multiplex PCR were concordant (positive results for 18 cases by both tests and negative results for 3 cases by both tests). Among 16 discordant cases, the causative organism was detected in 5 cases in sonication fluid culture only (*P. acnes* in all cases; none of these patients had previously received antibiotics) and in 11 cases by multiplex PCR only, including 4 *S. aureus* isolates, 3 coagulase-negative staphylococcal isolates, 2 *Streptococcus* species isolates, 1 *Klebsiella pneumoniae*/*oxytoca* isolate (part of polymicrobial infec-

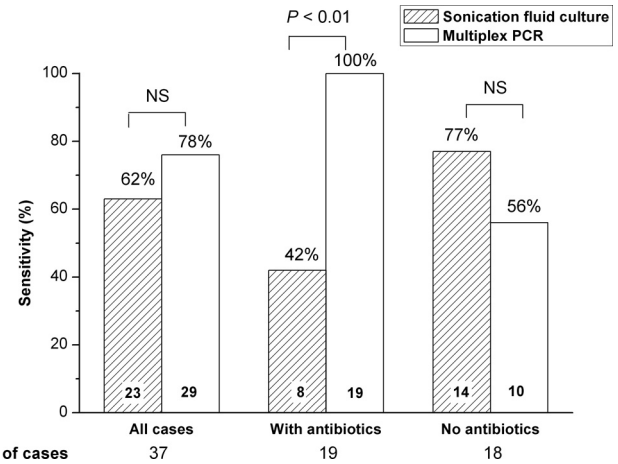

FIG. 1. Sensitivity of culture and multiplex PCR of sonication fluid. The sensitivity values are shown overall (all cases) and stratified according to patients who had received antimicrobial therapy previously ($n = 19$) and patients who had not received antimicrobial therapy ($n = 18$). Eight pathogens (7 *Propionibacterium acnes* and 1 *Corynebacterium* species) were missed by multiplex PCR due to lack of specific primers for these species. The value at the bottom of each bar indicates the number of PJI cases in that group, and the percentage above each bar indicates the sensitivity. NS, not significant.

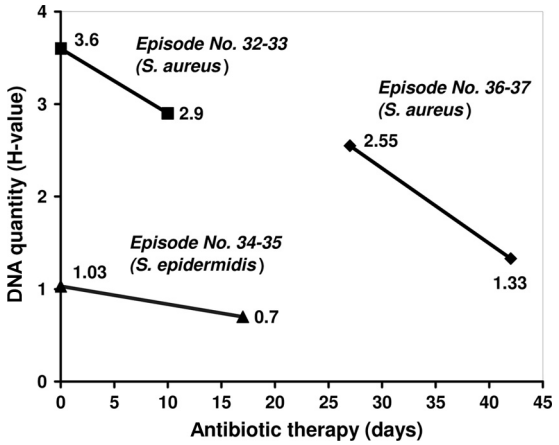

FIG. 2. Serial determinations of DNA quantity by multiplex PCR according to the duration of antibiotic treatment in three patients. The quantity of DNA by multiplex PCR is expressed as *H* values on the *y* axis. The infecting microorganism was *S. aureus* in 2 cases and *S. epidermidis* in 1 case. Note that the *H* value is a measure of DNA quantity, defined as the integral of the fluorescence intensity and a semiquantitative measure of the initial quantity of target DNA.

tion), and 1 *Streptococcus pneumoniae* isolate (all of these patients had previously received antibiotics).

Among the 8 cases in which the organism was missed by multiplex PCR (concordant results in 3 cases and discordant results in 5 cases), 7 were caused by *P. acnes* and 1 was caused by a *Corynebacterium* species (as a single organism or as part of a polymicrobial infection), as detected by sonication culture ($n = 2$) or periprosthetic tissue culture only ($n = 3$) or both ($n = 3$). After exclusion of 8 PJI cases caused by *P. acnes* or *Corynebacterium* species that cannot be detected by our multiplex PCR, the sonication culture detected the causative pathogen in 17 of 29 cases (59%) and multiplex PCR detected the causative pathogen in all 29 cases (100%) ($P < 0.01$).

Influence of previous antimicrobial therapy. Among 37 PJI cases, 19 of the patients (51%) had previously received antimicrobial therapy for a median duration of 9 days and 18 (49%) had received no antibiotics before diagnostic tests. Among 19 patients who had previously received antibiotics, sonication fluid cultures were positive in 8 cases (42%), compared to multiplex PCR, which gave positive results for all 19 (100%) ($P < 0.01$) (Fig. 1). In 18 patients who had not previously received antibiotic treatment, sonication fluid culture was positive in 14 cases (77%) and multiplex PCR was positive in 10 cases (56%). The 8 missed pathogens by multiplex PCR included 5 *P. acnes* isolates, 1 polymicrobial infection with *Corynebacterium* species, and 2 polymicrobial infections with *P. acnes* (Table 3).

Quantitative assessment of target DNA in sonication fluid. The DNA quantity (expressed as *H* value) showed no correlation with the CFU count in the sonication fluid (Table 4). However, in three patients for which serial testing of sonication fluid by multiplex PCR was available, a decrease of *H* value for the infecting microorganism was observed during antibiotic therapy (Fig. 2). The DNA of the initially infecting organism was identified up to 43 days after initiation of antimicrobial therapy.

DISCUSSION

Despite the fact that sonication of removed prostheses and subsequent culture of the sonication fluid has improved the sensitivity for detecting infection, a significant proportion of PJI still remains without identification of the organism causing the infection, especially in patients who previously received antimicrobial treatment (18, 22). Culture-independent (molecular) methods, such as PCR, have been proposed to improve the diagnosis of PJI.

Most commonly, specific or broad-range (16S rDNA) PCR was applied to synovial fluid or periprosthetic tissue (3–7, 9, 14, 17, 18, 26, 27). In a recent study, the sensitivities of specific PCR for detection of *P. acnes* and *Staphylococcus* spp. in sonication fluid were 89% and 97%, respectively (18). In contrast, broad-range PCR of tissue cultures of patients with PJI showed a sensitivity of only 50% (3). Unfortunately, contaminants detected with the broad-range PCR (false-positive results) belong to the same type of organisms as the microorganisms causing low-grade PJI, making the distinction of true-positive and false-positive PCR results difficult. For these reasons, PCR has not yet been integrated in the standard routine diagnostic procedure of PJI by most laboratories. With multiplex PCR, several disadvantages of the specific and broad-range PCR could be overcome. Up to now, multiplex PCR was used to investigate blood samples from patients with bloodstream infections and infective endocarditis (2, 11, 24). Another approach for molecular diagnosis of PJI used the messenger RNA-based reverse transcription-quantitative PCR using universal primers, which showed sensitivity equivalent to that of intraoperative cultures but exhibited detectable signals for 7 days after cultures turned negative (1a). To our knowledge, until this study, multiplex PCR had not yet been evaluated in periprosthetic tissue or sonication fluid samples.

TABLE 4. Characteristics of diagnostic results in 37 cases with PJI

Patient characteristics			Diagnostic result[a]						Antibiotic treatment before sonication		Comment
			Periprosthetic tissue culture		Sonication fluid culture		Multiplex PCR of sonication fluid				
Case no.[b]	Patient age (yr)	Implant type[c]	Organism	No. of positive samples/total no. of samples	Organism	Quantity (CFU/ml)	Organism	*H* value	Antibiotic(s)[d]	No. of days of treatment	
1	76	Knee (T)	No growth	0/6	No growth		*Streptococcus* spp.	0.85	Amc	6	*S. gallolyticus* in tissue cultures 6 days before
2	67	Knee (T)	No growth	0/9	CNS	>1,000	CNS	1.35	No	0	
			P. acnes	7/9	*P. acnes*	>1,000	Negative				
3	71	Hip (T)	*S. epidermidis*	2/7	CNS	>1,000	CNS	2.99	Ipm	14	
4	80	Hip (P)	*S. epidermidis*	5/7	CNS	20	CNS	1.27	Van-Rif	1	
5	49	Ankle (T)	*S. lugdunensis*	5/6	CNS	70	CNS	0.45	No	0	
6	86	Hip (T)	*S. aureus*	2/8	*S. aureus*	>1,000	*S. aureus*	2.01	Cli	32	
7	54	Hip (T)	*P. acnes*	4/9	*P. acnes*	In broth only	Negative		No	0	
8	84	Knee (P)	*S. agalactiae*	5/5	*S. agalactiae*	>1,000	*Streptococcus* spp.	0.35	Ipm-Rif	0	
9	80	Hip (T)	No growth	0/7	*E. faecalis*	>1,000	*E. faecalis*	0.90	Cip	2	*K. pneumoniae* in blood, hip, synovial fluid, and urine cultures 1 month before
			S. epidermidis	6/7	CNS	260	CNS	0.38			
			No growth	0/7	No growth		*K. pneumoniae*	0.41			
10	82	Shoulder (T)	*P. acnes*	5/6	*P. acnes*	200	Negative		No	0	
11	52	Hip (T)	*S. epidermidis*	5/6	CNS	>1,000	CNS	1.10	No	0	
			S. aureus	4/6	*S. aureus*	>1,000	*S. aureus*	1.4			
12	85	Hip (P)	*S. aureus*	3/7	No growth		*S. aureus*	1.78	Amc	3	
13	84	Hip (T)	*S. aureus*	1/5	*S. aureus*	In broth only	*S. aureus*	2.34	Flx	17	
14	63	Shoulder (T)	*S. epidermidis*	3/6	CNS	>1,000	CNS	2.07	No	0	
15	77	Hip (T)	*S. epidermidis*	1/6	CNS	30	CNS	1.59	No	0	
16	79	Hip (T)	*S. mitis*	4/10	*S. mitis*	850	*Streptococcus* spp.	1.86	Cro-Gen	2	Growth in blood cultures (all 6 samples)
17	78	Hip (T)	*S. epidermidis*	6/6	CNS	>1,000	CNS	1.96	No	0	
			Corynebacterium spp.	3/6	No growth		Negative				
18	39	Elbow (T)	*S. aureus*	6/6	*S. aureus*	>1,000	*S. aureus*	2.67	No	0	
			No growth	0/6	CNS	>1,000	CNS	0.70			
19	77	Knee (P)	No growth	0/4	No growth		*S. pneumoniae*	4.18	Cro	9	*S. pneumoniae* in knee synovial fluid and blood cultures 9 days before
20	68	Hip (P)	*S. dysgalactiae*	4/4	No growth		*Streptococcus* spp.	0.49	Amc	2	
21	72	Knee (T)	*S. aureus*	1/3	*S. aureus*	>1,000	*S. aureus*	3.5	Ipm	5	
22	89	Shoulder (P)	*P. acnes*	6/6	*P. acnes*	>1,000	Negative		No	0	
23	73	Hip (T)	*C. albicans*	1/9	*C. albicans*	20	*C. albicans*	1.94	No	0	*Candida albicans* PJI diagnosed 6 months before
24	61	Hip (T)	No growth	0/5	*S. aureus*	10	*S. aureus*	0.26	No	0	
25	59	Hip (T)	*S. epidermidis*	4/5	CNS	>1,000	CNS	2.10	No	0	
26	64	Hip (T)	*P. acnes*	2/5	No growth		Negative		No	0	
27	84	Hip (T)	*S. epidermidis*	6/6	No growth		CNS	1.82	Flx	28	
28	66	Knee (T)	*S. epidermidis*	5/5	CNS	>1,000	CNS	1.96	Amc	4	
29	57	Knee (T)	No growth	0/6	*P. acnes*	10	Negative		No	0	Acute inflammation in histopathology
30	70	Shoulder (P)	No growth	0/6	*P. acnes*	160	Negative		No	0	
			S. aureus	5/6	No growth		*S. aureus*	0.26			
31	71	Knee (T)	*S. epidermidis*	2/5	No growth		CNS	1.64	Van	4	
32	58	Knee (P)	*S. aureus*	5/5	*S. aureus*	>1,000	*S. aureus*	3.60		0	
33		Knee (P)	No growth	5/5	No growth		*S. aureus*	2.90	Flx-Rif	10	
34	82	Knee (P)	*S. epidermidis*	5/5	CNS	In broth only	CNS	1.03	No	0	
35		Knee (T)	No growth	0/5	No growth		CNS	0.71	Van-Rif	17	
36	55	Knee (T)	No growth	0/3	No growth		*S. aureus*	2.55	Ipm-Rif	27	*S. aureus* cultured 27 days before
37		Knee (S)	No growth	0/2	No growth		*S. aureus*	1.35	Ipm-Rif	42	

[a] CNS, coagulase-negative staphylococci. "In broth only" denotes growth only in thioglycolate medium.
[b] Cases 32 amd 33 are the same patient, cases 34 and 35 are the same patient, and cases 36 and 37 are the same patient.
[c] The location of the implant is shown first. The specific part of the implant is shown in parentheses as follows: (P), polyethylene mobile part; (T), total prosthesis; (S), spacer consisting of polymethyl methacrylate impregnated with vancomycin.
[d] Anc, amoxicillin-clavulanate; Ipm, imipenem; Van, vancomycin; Rif, rifampin; Cli, clindamycin; Cip, ciprofloxacin; Flx, floxacillin; Cro, ceftriaxone; Gen, gentamicin.

In this study, the multiplex PCR of sonication fluid samples showed the potential for improved diagnosis of PJI. The sensitivity of multiplex PCR was better than the sensitivity of sonication fluid cultures (78% versus 62%), particularly in patients who had previously received antibiotic therapy (100% versus 42%; $P < 0.01$). Seven of 8 false-negative PCR results were caused by *P. acnes*, and one false-negative PCR result was caused by a *Corynebacterium* species, which cannot be detected by the multiplex PCR used, due to the absence of specific primers for this organism in the PCR kit. When *P. acnes* or *Corynebacterium* species were excluded from the analysis, all 19 infecting microorganisms were detected by multiplex PCR. *P. acnes* is a common pathogen in PJI, especially in shoulder prosthetic joint disease (18, 20). We found all *P. acnes* isolates in either periprosthetic tissue or synovial fluid cultures. A prolonged incubation time (10 to 14 days) of periprosthetic tissue samples and sonication fluid is mandatory to optimize the detection of this pathogen by culture (19).

Interestingly, we were able to show that the microbial DNA density (represented as *H* value) decreases with antimicrobial treatment but remains positive for up to 43 days of treatment. This provides the opportunity to detect the pathogen despite previous antibiotic treatment, a common clinical situation. With additional molecular tests, specific resistance genes, such as the genes conferring resistance to methicillin, quinolones, and rifampin, can be detected in addition. This information is crucial for efficient and targeted antimicrobial therapy in negative cultures. Interestingly, no correlation between the bacterial density in sonication fluid and the DNA quantity was observed. This observation could be the result if some microorganisms were killed by sonication (despite reduced acoustic energy used for this purpose) but the DNA was not affected. The sensitivity of the sonication fluid culture may be improved by an additional centrifugation step of the fluid and cultivation of the sediment with concentrated bacteria only. Importantly, a positive PCR result cannot be used for evaluation of treatment efficacy, unless serial determinations over time are performed and available. Nevertheless, this feature may be an advantage in PJI where etiological diagnosis is needed for treatment if conventional cultures were negative.

In conclusion, multiplex PCR of sonication fluid can improve the diagnosis of PJI, particularly among patients who had previously received antibiotic therapy. All undetected organisms by PCR were *P. acnes* or *Corynebacterium* species, which cannot be detected due to the absence of specific primers in the PCR kit. We suggest that the specific primer set be modified to include the most common organisms causing PJI, including low-virulence pathogens, such as *P. acnes*, *Corynebacterium* species, *Finegoldia magna*, and *Peptostreptococcus* species. The potential of multiplex PCR in the diagnosis of PJI is especially high in patients who had previously been exposed to antibiotics and have a high probability of false-negative cultures. Additional laboratory and clinical studies with multiplex PCR are needed to define the appropriate primer design, processing procedure, and proper patient selection for further optimization of the diagnosis of PJI.

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