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Role of Universal 16S rRNA Gene PCR and Sequencing in Diagnosis of Prosthetic Joint Infection

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The etiological diagnosis of prosthetic joint infection (PJI) requires the isolation of microorganisms from periprosthetic samples. Microbiological cultures often yield false-positive and false-negative results. 16S rRNA gene PCR combined with sequencing (16SPCR) has proven useful for diagnosing various infections. We performed a prospective study to compare the utility of this approach with that of culture to diagnose PJI using intraoperative periprosthetic samples. We analyzed 176 samples from 40 patients with PJI and 321 samples from 82 noninfected patients using conventional culture and 16SPCR. Three statistical studies were undertaken following a previously validated mathematical model: sample-to-sample analysis, calculation of the number of samples to be studied, and calculation of the number of positive samples necessary to diagnose PJI. When only the number of positive samples is taken into consideration, a 16SPCR-positive result in one sample has good specificity and positive predictive value for PJI (specificity, 96.3%; positive predictive value, 91.7%; and likelihood ratio [LR], 22), while 3 positive cultures with the same microorganism are necessary to achieve similar specificity. The best combination of results for 16SPCR was observed when 5 samples were studied and the same microorganism was detected in 2 of them (sensitivity, 94%; specificity, 100%; and LR, 69.62). The results for 5 samples with 2 positive cultures were 96% and 82%, respectively, and the likelihood ratio was 1.06. 16SPCR is more specific and has a better positive predictive value than culture for diagnosis of PJI. A positive 16SPCR result is largely suggestive of PJI, even when few samples are analyzed; however, culture is generally more sensitive.

Prosthetic joint infection (PJI) affects 1 to 2% of patients with a joint prosthesis and is associated with severe complications, high morbidity, and increased hospital costs (23, 29). The distinction between aseptic loosening and infection is frequently difficult and has obvious clinical consequences.

PJI diagnosis frequently relies on a combination of clinical manifestations, imaging techniques, histological examination of intraoperative specimens, isolation of microorganisms from joint fluid or tissue, and, more recently, microbiological culture of implants after sonication (4, 9, 21).

Conventional microbiological cultures are sometimes associated with false-negative results (4, 9, 21), mainly because of antimicrobial treatment (4). In addition, a significant number of cultures obtained from noninfected patients during surgery prove to be falsely positive and misleading (6).

Molecular techniques overcome some of the limitations of conventional microbiology in several clinical entities (13, 15, 18, 28). Although many studies have been performed, the role of molecular techniques in the diagnosis of PJI remains unclear. In addition, these techniques have not been extensively tested in the routine of a clinical microbiology laboratory (10, 11, 14, 17, 19, 24, 26).

The aim of this study was to prospectively compare the utility of a universal 16S rRNA gene PCR followed by sequencing (16SPCR) with that of microbiological culture of samples taken during surgery for the diagnosis of PJI.

MATERIALS AND METHODS

The study was performed in a tertiary 1,500-bed hospital attending to an urban and rural population of 715,000 inhabitants. The clinical microbiology and infectious diseases department processes more than 200,000 clinical specimens per year.

Patients and samples. The study population comprised 122 adult patients undergoing prosthetic joint replacement due to suspected infection or aseptic loosening. Patients were enrolled prospectively from June 2004 to October 2007.

Surgeons were requested to obtain at least 5 biopsy specimens from different sites and 1 sample of joint fluid during surgery. However, to evaluate the utility of microbiological culture or PCR on the basis of our clinical-histopathological "gold standard," we studied patients with more than 1 sample sent for analysis. Only samples received under conditions adequate for molecular analysis (no transport medium or saline solution added) were included.

Definitions. Preoperative suspicion of PJI was based on clinical, imaging, or laboratory data (4, 5, 29).

PJI was considered proven in the presence of at least one of the following (9, 23): purulence in the synovial fluid or around the prosthesis, acute inflammation detected in the histological examination of periprosthetic tissue, or a sinus tract communicating with the prosthesis. Inflammation was defined as previously described (3). Infection was ruled out when none of these criteria were fulfilled or when antibiotics were not administered and no infection was detected for at least 1 year after surgery.

To interpret microbiological results, patients with less than 3 intraop-

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erative samples analyzed were defined as true positives when the same microorganism (considering biotype and antibiotype) was isolated from preoperative joint fluid samples and some of the intraoperative samples. In patients with more than 3 intraoperative samples, we defined true positive as the isolation of the same microorganism in 3 or more specimens, as recommended by Atkins et al. (1), and in the preoperative samples, when taken.

A false-positive result was defined as the detection of a microorganism (contaminant) by culture or PCR in a sample from a patient considered to be noninfected or the detection of microorganisms not considered true positives according to the previous definition. True-negative results were defined as no microorganisms or no amplification by PCR obtained in any of the samples from a noninfected patient. False-negative results were defined as no microorganisms isolated or no amplification by PCR in samples from patients considered as having PJI.

A microorganism was considered causative when present in 3 or more samples, as described previously (1). In patients with less than 3 samples analyzed, the results of preoperative cultures were also taken into consideration.

An infectious diseases consultant reviewed the clinical records independently of the microbiological results to classify patients as infected or not.

Microbiological methods. Biopsy specimens were aseptically disrupted in sterile mortar with saline solution. Aliquots of 100 μl of synovial fluid were cultured on sheep blood, chocolate, and brucella agar for 2 days at 37°C in air, in 5% CO_2, and anaerobically, respectively. Chocolate and brucella agar plates were reincubated for up to 7 days. Samples were also cultured in brain heart infusion broth for 10 days and subcultured when turbid. Gram stain was performed on all samples.

The bacteria isolated were identified and susceptibility testing was performed using MicroScan panels (Siemens Healthcare Diagnostic, West Sacramento, CA) and conventional microbiological procedures according to CLSI recommendations (7).

Molecular methods. All PCRs were performed in parallel with cultures and blind to culture results.

DNA was extracted from 100 mg of tissue or 500 μl of synovial fluid using the QIAmp DNA minikit method (Qiagen Ltd., West Sussex, United Kingdom) according to the manufacturer's recommendations. In each run, a negative control with nuclease-free UV-treated water was included in every 10 test samples.

The 16S rRNA gene was amplified using conventional PCR with primers fD1 (forward, 5′-AGAGTTTGATCCTGGCTCAG-3′) and rP2 (reverse, 5′-ACGGCTACCTTGTTACGACTT-3′), producing an amplicon of approximately 1,500 bp (27). The PCR was made in 50 μl of a reaction mixture consisting of 0.25 μM each primer, 0.2 mM deoxynucleoside triphosphates (Roche Diagnostics, Mannheim, Germany), 1.5 mM $MgCl_2$, 5 μl of 10× *Taq* buffer, 1.5 units of AmpliTaqLD DNA polymerase (Applied Biosystems Inc., Foster City, CA), and 5 μl of DNA at a 10-fold dilution. PCR was performed in a GeneAmp PCR system 9700 (Applied Biosystems Inc.) with a preincubation step of 94°C for 5 min and 40 cycles at 94°C for 1 min, 60°C for 1 min, and 72°C for 1.5 min, followed by a final extension step of 72°C for 10 min. Each analysis included a positive control prepared from a PCR-negative biopsy specimen spiked with 10^4 CFU/mg of a laboratory *Gordonia sputi* strain and extracted in the same way as the samples.

To detect PCR inhibitions, the human β-globin gene was amplified for each sample with primers βGloF (forward, 5′-GAAGAGCCAAGGACAG GTAC-3′) and βGloR (reverse, 5′-GGAAAATAGACCAATAGGCAG-3′) in the same run and under the same PCR conditions. PCR results were considered valid if all the controls were negative or positive, as appropriate, and the β-globin gene was detected in all samples.

DNA sequencing reactions. The first 500 bp of the 16S rRNA gene was sequenced with primers fD1 and E533R (5′-TTACCGCGGCTGCTGGC AC-3′) (2) in all the amplicons obtained using the BigDye Terminator method and detected in an ABI Prism 3130x1 genetic analyzer (Applied Biosystems Inc.). The sequences generated were compared with those stored in GenBank using BIBI software (http://pbil.univ-lyon1.fr/bibi). Sequence similarity was interpreted following reported criteria (12).

Estimation of analytical sensitivity. The sensitivity of the PCR assay was assessed by analyzing negative biopsy specimens spiked with 10-fold dilutions of *Staphylococcus aureus* processed in the same way as the clinical samples.

Statistical methods. The statistical study was performed as described by Atkins et al. (1), although we considered our defined criteria to be the gold standard for proven cases. All statistical analyses were performed using SPSS software (version 15.0).

We performed 3 statistical studies. (i) We compared the results obtained by culture and 16SPCR for each individual sample to calculate sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratio (LR), accuracy, and positive posttest probability. (ii) We studied the same parameters for 1, 2, or 3 positive cultures or 16SPCR, taking into consideration any number of samples sent for study. (iii) We analyzed the effect of the number of samples sent for study on the results obtained in order to establish the ideal number of samples that would permit the best combination of results.

The Ethics Committee of our institution approved the study.

RESULTS

Patients and PJI. A total of 122 patients (497 samples) were included. Forty patients fulfilled our criteria for infection (149 periprosthetic biopsy specimens and 27 synovial fluid samples); PJI was ruled out in the remaining 82 patients (292 periprosthetic biopsy specimens and 29 synovial fluid samples).

The main characteristics of the patients, microorganisms, and samples are shown in Table 1.

After 1 year of follow-up, no criteria for PJI were observed in the patients without PJI who had culture-positive or 16SPCR-positive results. These patients were considered definitely noninfected.

Microbiological cultures. Microorganisms were isolated by culture in 186 out of 497 clinical samples: 137 were from patients with PJI, and 49 were from patients without PJI.

Overall, 36 patients (137 samples) with PJI had 1 or more culture-positive samples. Table 2 shows the microorganisms isolated in samples from patients with PJI. In 4 patients (Table 2, patients 2, 22, 31, and 34), all the cultures were negative (14 samples). For 7 patients, causative bacteria were recovered only in enrichment medium (22 samples; Table 2, patients 1, 4, 13, 27, 30, 36, and 39). Contaminant microorganisms were isolated from 16 infected patients (19 samples, 11 samples with microorganisms grown exclusively in enrichment medium). Only 1 pathogen was considered causative in 33 patients. Nineteen patients with PJI had polymicrobial cultures, although the infections were considered to be polymicrobial in only 3 patients (Table 2, patients 13, 29, and 40).

Of the 82 patients without PJI, 1 or more microorganisms were isolated from 31 patients (49 surgical samples). All the isolates were considered to be contaminants according to our gold standard. For 26 patients (35 samples), microorganisms grew only in enrichment medium.

16S rRNA PCR. The analytical sensitivity of 16SPCR was 100 CFU of *S. aureus*/PCR.

16SPCR was positive for 123 samples out of 497: 119 from patients with PJI and only 4 from patients without PJI.

Table 1 compares the results obtained by both conventional culture and 16SPCR.

The results of 16SPCR were diagnostic for 3 patients (Table 2;

TABLE 1 Characteristics of patients with or without PJI and results obtained in sample analysis by culture and 16S PCR

Characteristic	Patients with PJI	Patients without PJI
No. of patients	40	82
Median (range) age (yr)	73 (49–96)	71 (33–92)
No. (%) of patients by:		
Sex		
Male	11 (27.5)	25 (30.5)
Female	29 (72.5)	57 (69.5)
Prosthesis type		
Knee	21 (52.5)	49 (59.75)
Hip	17 (42.5)	33 (40.25)
Shoulder	1 (2.5)	
Elbow	1 (2.5)	
No. of patients under antimicrobial treatment at time of surgery (range of no. of days of treatment)	8 (1–90)	
No. of samples analyzed	176	321
Biopsy samples	149	292
Synovial fluid samples	27	29
No. (%) of culture-positive samples	137 (77.84)	49 (15.26)
True positives	123 (69.86)	
False positives	14 (7.95)	49 (15.26)
True negatives		272 (84.73)
False negatives	39 (22.15)	
No. (%) of PCR-positive samples	119 (67.61)	4 (1.24)
True positives	116 (65.90)	
False positives	2 (1.7)	4 (1.24)
True negatives		317 (98.75)
False negatives	57 (32.86)	
No. of microorganisms isolated by culture		
S. aureus	29	5
CoNS[a]/*Staphylococcus epidermidis*	51	23
Enterococcus faecalis	17	6
Streptococcus agalactiae	3	0
Streptococcus viridans	8	4
Aerobic Gram-positive bacilli	3	8
Escherichia coli	9	0
Proteus mirabilis	7	0
Pseudomonas aeruginosa	4	0
Other aerobic Gram-negative bacilli	8	2
Propionibacterium acnes	5	2
Other anaerobes	5	0

[a] CoNS, coagulase-negative staphylococcus.

patients 2, 11, and 34), 2 of whom were infected by anaerobic bacteria and confirmed the culture results for 32 patients (Table 2). For patient 11, 16SPCR and sequencing identified the causative microorganism in 24 h, while the culture grew after 6 days of incubation.

Comparison between conventional microbiology and PCR. The results of the analysis performed according to the 3 different approaches were as follows.

(i) Per sample analysis and results for culture and 16SPCR according to our gold standard. Microbiological culture and 16SPCR results obtained by considering each sample individually were used to calculate the sensitivity, specificity, positive and negative predictive values, accuracy, and LR for a positive result to diagnose PJI, according to whether samples were obtained from patients with or without PJI (Table 3).

The results obtained demonstrate that 16SPCR is more specific than culture, although culture is more sensitive (Table 3). One 16SPCR-positive result was highly predictive of PJI (positive predictive values for PCR and culture, 94.3% and 66.1%, respectively; LRs for a positive result of PCR and culture, 31.04% and 4.05%, respectively; Table 3).

(ii) Per patient analysis to determine value of different number of culture- or 16SPCR-positive samples. To calculate the ideal number of positive samples optimally correlated with the presence or absence of infection, we repeated the model described by Atkins et al. (1). We calculated different statistical parameters for 1, 2, or 3 or more positive results (Table 4).

The prevalence of infection among the study population was 32.8%. The proportion of infected and control patients in our population at the beginning of this study enabled us to calculate an estimated pretest probability of infection of 17%, from which we calculated posttest probabilities for culture and 16SPCR results (Table 4).

Our results show that isolation of bacteria in 1 sample has poor specificity and cannot confirm PJI. The best combination of results for culture to diagnose PJI was obtained when the same microorganism grew in 2 or 3 specimens, irrespective of the number of samples studied per patient. In general, culture has good sensitivity but poor specificity. The specificity of culture for the diagnosis of PJI increases with the number of positive samples analyzed.

In contrast, 16SPCR is more specific than culture. One positive 16SPCR result has sufficiently good specificity, positive predictive value, and LR to suggest PJI (96.3%, 91.7%, and 22, respectively) and increase the positive posttest probability of PJI from 17% (pretest) to 81.8%. The best combination of results is obtained with only 1 positive result (Table 4). A positive 16SPCR result for 2 or more samples has high specificity but insufficient sensitivity. Two or more positive 16SPCR results have better parameters than culture.

(iii) Evaluation of ideal number of samples required for culture and 16SPCR to diagnose PJI. The number of samples received in the microbiology laboratory ranged from 1 to 7 (mean, 4 samples). A previously described mathematical model based on a binomial expansion (1) was applied to determine the predictive capacity of all possible test results obtained for culture and 16SPCR for 1 to 7 samples (Table 5).

Isolation of the same microorganism in 3 or more cultures out of 5 samples shows the best combination of results (sensitivity, 80%; specificity, 96.8%; and LR, 25.03). As for culture, the best combination of results is obtained when 5 samples are analyzed and 16SPCR is positive in 2 of them (sensitivity, 94%; specificity, 100%; and LR, 69.62). One 16SPCR-positive result is very suggestive of PJI, even when few samples are analyzed (Table 5).

DISCUSSION

16SPCR substantially improves the specificity of the microbiological diagnosis of PJI and requires fewer samples to diagnose PJI than conventional culture. Our study confirms the limitations of single-sample culture to establish the etiology of PJI. Concordance between positive cultures from several samples significantly improves diagnostic yield but still has low specificity.

Conventional culture is the traditional method used for the

TABLE 2 Characteristics of patients with PJI and results of microbiological analysis of their samples[a]

Patient no.	Age (yr)/ sex	Affected joint	Histology	Length of antimicrobial treatment at time of surgery (days)	Preoperative samples		Microbiology of operative samples					Comments
								Culture		Universal PCR		
					No. of samples studied	Culture results	No. of samples studied	No. of positive samples	Conventional identification	No. of positive samples	Molecular identification	
1	70/F	Knee	ND	1			5 (1 JF)	5	5 *S. aureus*	3	3 *S. aureus*	
2	73/M	Knee	+	0	3 joint fluid	3 *Gemella morbillorum*	5 (1 JF)	0		3	3 *G. morbillorum*	False-negative culture
3	54/F	Hip	+	0			5 (1 JF)	5	5 *S. aureus*	5	5 *S. aureus*	
4	71/F	Elbow	+	0	2 joint fluid	1 *S. epidermidis*	6 (3 JF)	1	1 *S. epidermidis*	1	1 *S. epidermidis*	
5	96/F	Hip	ND	17	1	*Providencia stuartii*	2	2	2 *P. stuartii*, 1 *P. acnes* FP	1	1 *P. stuartii*	
6	78/F	Knee	+	1			8	7	7 CoNS	2	2 CoNS	
7	74/M	Knee	ND	0	1 joint fluid	*S. agalactiae*	5 (1 JF)	4	3 *S. agalactiae*, 1 *S. viridans* FP, 2 *Enterobacter cloacae* FP, 1 *Bacillus* sp. FP	3	3 *S. agalactiae*	
8	79/F	Knee	ND	0	1 joint fluid	CoNS	5	5	3 *Morganella morganii*, 1 *S. epidermidis* FP	4	4 *M. morganii*	
9	64/M	Knee	+	0	1 joint fluid	CoNS	7 (2 JF)	4	3 *S. epidermidis*, 1 *P. acnes* FP	4	4 *S. epidermidis*	
10	71/M	Hip	ND	0	2 joint fluid	2 *Fascioloides magna*	3	3	1 *F. magna*, 1 *S. viridans* FP, 1 *P. acnes* FP	2	Mixed sequence	False-positive PCR
11	69/F	Hip	−	0			4	4	3 *G. morbillorum*, 1 *S. epidermidis* FP	4	4 *G. morbillorum*	Advance diagnosis by PCR
12	82/F	Hip	+	0	1 joint fluid	*E. coli*	3	1	1 *E. coli*	0		False-negative PCR
13	82/F	Hip	−	0			5 (1 JF)	5	3 *S. aureus*, 5 *E. coli*, 1 *Corynebacterium* sp. FP	4	3 *E. coli*, 1 *S. aureus*	Polymicrobial infection
14	79/F	Knee	ND	0	1 joint fluid	*S. aureus*	3 (1 JF)	3	3 *S. aureus*	3	3 *S. aureus*	
15	78/F	Hip	ND	0	1 joint fluid	*S. viridans*	1	1	1 *S. viridans*	1	1 *Streptococcus constellatus*	
16	82/F	Hip	ND	0			4	3	3 *P. aeruginosa*, 2 *P. mirabilis* FP, 1 *S. viridans* FP	2	2 *P. aeruginosa*	
17	73/M	Hip	ND	3	1 joint fluid	*S. epidermidis*	2	2	2 *S. epidermidis*, 1 *P. acnes* FP	2	2*S. epidermidis*	
18	69/M	Knee	+	0	1 joint fluid	*Staphylococcus lugdunensis*	3 (1 JF)	3	3 *S. lugdunensis*, 1 *S. epidermidis* FP	3	3 *S. lugdunensis*	
19	69/M	Knee	−	0			6 (3 JF)	3	3 *E. faecalis*	3	3 *E. faecalis*	
20	69/M	Knee	−	0			5	5	5 *E. faecalis*	5	5 *E. faecalis*	
21	82/F	Knee	+	10	1 joint fluid	*E. coli*	6	3	3 *E. coli*, 1 *Corynebacterium* sp. FP	3	3 *E. coli*	
22	61/F	Knee	+	30	2 joint fluid	CoNS	1	0		0		False-negative culture and PCR
23	83/F	Knee	ND	0			7	5	4 *S. lugdunensis*, 1 *S. epidermidis* FP	4	4 *S. lugdunensis*	
24	65/F	Knee	+	0			5	3	3 *S. viridans*	4	4 *S. anginosus*	
25	56/F	Hip	ND	90			5 (1 JF)	5	5 *E. faecalis*, 1 *P. acnes* FP	2	2 *E. faecalis*	
26	71/M	Knee	ND	0	2 joint fluid	*S. epidermidis*	4	4	3 *S. epidermidis*, 1 *E. faecalis* FP	2	2*S. epidermidis*	
27	70/F	Hip	ND	0			4	1	1 *E. faecalis*	4	4 *E. faecalis*	
28	73/F	Hip	ND	0			2	2	2 *S. aureus*, 1 *P. aeruginosa* FP	2	2 *S. aureus*	
29	86/F	Knee	ND	0	1 joint fluid	*S. epidermidis*, *E. faecalis*	3 (2 JF)	3	2 *S. epidermidis*, 2 *E. faecalis*	3	2 *S. epidermidis*, 1 *E. faecalis*	Polymicrobial infection
30	76/F	Hip	ND	0			7 (1 JF)	7	7 *S. epidermidis*	6	6 *S. epidermidis*	
31	73/F	Knee		0			2 (1 JF)	0		0		False-negative culture and PCR
32	56/F	Hip		0			6	6	6 *S. epidermidis*	3	3 *S. epidermidis*	
33	74/F	Hip	ND	0			2	2	2 *S. aureus*	2	2 *S. aureus*	
34	76/M	Knee	+	0	2 joint fluid, 3 blood culture	β-Hemolytic group G *Streptococcus*	6 (1 JF)	0		3	3 *Streptococcus dysgalactiae*	False-negative culture
35	73/F	Knee	+	0			3 (2 JF)	3	3 *S. aureus*	3	3 *S. aureus*	
36	77/F	Knee	ND	0			7 (2 JF)	5	3 *S. aureus*	7	7 *S. aureus*	
37	92/F	Hip	ND	0			4	4	4 *S. epidermidis*	0		False-negative PCR
38	93/F	Hip	ND	0			6	6	4 *S. epidermidis*, 1 *S. viridans* FP, 1 CoNS FP, 1 *Peptostreptococcus* sp. FP	6	6 *S. epidermidis*	
39	49/M	Shoulder	ND	0	1 joint fluid	*P. mirabilis*	3 (1 JF)	3	2 *P. mirabilis*, 1 *Enterobacter agglomerans* FP	1	1 *P. mirabilis*	
40	80/F	Hip	ND	0			6	6	3 *S. aureus*, 3 *P. mirabilis*	6	4 *P. mirabilis*, 2 *S. aureus*	Polymicrobial infection

[a] F, female; M, male; ND, not determined; JF, joint fluid; FP, considered false positive; CoNS, coagulase-negative staphylococcus. The number of joint fluid samples analyzed is shown in parentheses.

TABLE 3 Value of culture and 16SPCR for diagnosis of PJI considering the results obtained for each sample independently

Test	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Accuracy (%)	LR for a positive result (%)
Culture	75.9 (68.8–81.9)[a]	81.2 (76.7–85.0)	66.1 (59.1–72.5)	87.5 (83.3–90.7)	79.5 (75.7–82.8)	4.05 (3.18–5.13)
PCR	67.1 (59.7–73.6)	97.8 (95.6–98.9)	94.3 (88.7–97.2)	84.8 (80.8–88)	87.1 (83.9–89.8)	31.04 (14.8–65.06)

[a] Values in parentheses are 95% confidence intervals.

microbial diagnosis of PJI. It establishes etiology and determines antimicrobial susceptibility so that suitable antibiotic treatment can be administered. However, culture results do not have optimal specificity and are sometimes difficult to interpret, especially when few samples are analyzed. Our study and other studies highlight the poor specificity of culture, which is due to isolation of contaminants in samples from controls and infected patients, mainly when enriched media are used (1, 20). Such is the case of PJI, a low-burden infection in which enriched media are recommended.

Bacteriological culture of single samples obtained during clean surgical procedures has a high rate of false-positive and false-negative results (6). In our study, single-sample culture results revealed poor sensitivity and specificity for the diagnosis of PJI (75.9% and 81.2%, respectively).

Atkins et al. (1) demonstrated that the predictive value of cultures of samples obtained during surgery increased when the same microorganisms were isolated in more than 2 samples obtained during revision arthroplasty. This recommendation has been adopted elsewhere to interpret culture results (4, 9). However, similar studies to evaluate the utility of PCR-based methods in the diagnosis of PJI have not been conducted.

Given the limitations of culture, we evaluated the utility of 16SPCR in the diagnosis of PJI. We compared its results with those of conventional culture of synovial fluid and periprosthetic biopsy specimens taken during revision arthroplasty. This comparison was performed using a mathematical model previously validated for culture only (1).

Our results demonstrate that 16SPCR is significantly more specific than culture and has a better positive predictive value than culture for the diagnosis of PJI, considering both any number of samples analyzed and any number of positive results obtained. Identification of bacteria in only 1 periprosthetic sample by sequencing can confirm PJI, while confirmation by culture requires the isolation of the same microorganisms in 2 to 3 samples. However, culture is more sensitive than 16SPCR, and its specificity increases when the same microorganism is isolated from 2 or more samples. The best combination of sensitivity and specificity results is obtained when at least 5 samples are analyzed by culture or by PCR (1). In our study, 16SPCR established the etiology of PJI in only 3 patients. In the remaining patients, it proved useful mainly for the confirmation of culture results, although in most cases, 16SPCR was more rapid than culture. In addition, analysis based on 16SPCR can help clarify confusing culture results in selected samples.

In recent years, new microbiological methods for the diagnosis of PJI have been investigated, although previous reports reveal the following limitations: poorly formulated gold standard for comparison (17, 25); analysis of preoperative samples, postoperative samples, or implants in the same study (25); difficult interpretation of false-positive results when post-PCR sequencing is not used (16, 17, 19); and use of a low number of samples from noninfected patients (14). In this context, we were not able to draw clear conclusions about the utility of 16SPCR in the diagnosis of PJI.

Our work has some advantages over previous studies. First, we prospectively analyzed a high number of samples in the daily clinical and laboratory routine of a multidisciplinary team managing patients with PJI. Second, culture and PCR were performed in the routine work of a tertiary hospital clinical microbiology laboratory and not in an investigational context. Third, our study used a clinical-histopathological gold standard evaluated by an infec-

TABLE 4 Value of culture and 16SPCR for the diagnosis of PJI considering the number of positive samples

Test and no. of positive samples	No. of patients with positive specimens		Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	LR for a positive result (%)	Posttest probability of infection (%)
	PJI positive	PJI negative						
Culture								
≥1	36	27	87.8 (74.5–94.7)[a]	67.1 (56.3–76.3)	57.1 (44.9–68.6)	91.7 (81.9–96.4)	2.67 (1.92–3.71)	35.3 (24.7–44.7)
≥2[b]	25	5	61.0 (45.7–74.3)	93.9 (86.5–97.4)	83.3 (66.4–92.7)	82.8 (73.9–89.1)	10 (4.13–24.21)	67.2 (49.3–81.2)
≥3[b]	19	1	46.3 (32.1–61.3)	98.8 (93.4–99.8)	95.0 (76.4–99.1)	78.6 (69.8–85.5)	38 (5.27–274.03)	88.6 (68.2–96.6)
PCR results								
≥1	33	3	80.5 (66.0–89.8)	96.3 (89.8–98.7)	91.7 (78.2–97.1)	90.8 (82.9–95.3)	22 (7.17–67.49)	81.8 (66.4–91.1)
≥2[c]	24	1	58.5 (43.4–72.2)	98.8 (93.4–99.8)	96.0 (80.5–99.3)	82.7 (74.0–88.9)	48 (6.73–342.47)	90.8 (73.5–97.2)
≥3[c]	17	0	41.5 (27.8–56.6)	100 (95.5–100)	100 (81.6–100)	77.4 (68.5–84.3)	—[d]	—

[a] Values in parentheses are 95% confidence intervals.
[b] Same identification considering biotype and antibiotype.
[c] Same identification obtained by sequencing.
[d] —, not possible to calculate with 100% specificity.

TABLE 5 Probability of PJI considering results of culture or 16SPCR for 1 to 7 samples analyzed

Sensitivity								Specificity								LR							
Test and no. of positive samples	Probability for the following no. of samples analyzed:							Test and no. of positive samples	Probability for the following no. of samples analyzed:							Test and no. of samples	Probability for the following no. of samples analyzed:						
	1	2	3	4	5	6	7		1	2	3	4	5	6	7		1	2	3	4	5	6	7
Culture								Culture								Culture							
≥1	0.67	0.89	0.96	0.99	1	1	1	≥1	0.84	0.7056	0.5927	0.4979	0.4182	0.3513	0.2951	All negative	0.39	0.15	0.06	0.02	0.01	0	0
≥2		0.45	0.75	0.89	0.96	0.98	0.99	≥2		0.97	0.93	0.88	0.82	0.75	0.69	1 positive	4.19	1.65	0.65	0.25	0.1	0.04	0.02
≥3			0.3	0.6	0.8	0.9	0.96	≥3				0.986	0.968	0.944	0.913	2 positive		17.54	6.89	2.71	1.06	0.42	0.16
																≥3 positive			73.43	41.51	25.03	16.12	11.04
16S PCR								16S PCR								16S PCR							
≥1	0.64	0.87	0.95	0.98	0.99	1	1	≥1	0.98	0.97	0.95	0.93	0.92	0.9	0.89	All negative	0.37	0.13	0.05	0.02	0.01	0	0
≥2		0.41	0.7	0.86	0.94	0.97	0.99	≥2		1	1	1	1	1	0.99	1 positive	37.65	13.79	5.05	1.85	0.68	0.25	0.09
≥3			0.26	0.55	0.75	0.87	0.94	≥3			1	1	1	0.9999	0.9998	2 positive		1417.3	519.05	190.09	69.62	25.5	9.34
																≥3 positive			53,357.2	28,104.1	15,643.6	9,216.14	5,738.49

tious diseases consultant to classify patients infected or uninfected. Finally, we applied a validated mathematical model to evaluate the role of culture and 16SPCR in the diagnosis of PJI (1).

The main limitation of our study is the lack of implants. In recent years, implant sonication has been shown to play an important role in the diagnosis of PJI, since it allows direct study of the site of infection (11, 21).

16SPCR has important advantages over other molecular approaches, particularly the fact that only 1 PCR method is used to detect and identify the bacteria causing infection (22). However, its 3 main limitations are the inability of bacterial DNA to determine the viability of bacteria, low analytical sensitivity due to the presence of bacterial DNA in PCR reagents (8), and the difficulty in detecting mixed infections.

In our study, we used several measures to diminish the detection of bacterial contaminant DNA, such as purified *Taq* DNA polymerase, a reduced number of PCR cycles, and the amplification of 1,500 bp of the 16S rRNA gene. However, these approaches showed that 16SPCR was not very sensitive and produced false-negative results, especially when we consider that the burden of bacteria causing PJI is sometimes low. Development of reagents and polymerases that are completely free of residual DNA could enable us to overcome this important limitation in the near future.

In conclusion, our results demonstrate that 16SPCR is more specific and has a better positive predictive value than culture. It requires a lower number of samples and can provide results in 24 h. In our opinion, this technique should be used as a complement to culture. It could be included as part of the microbiology laboratory routine to improve the diagnosis of PJI, at least in reference laboratories and in selected cases when few samples are available for analysis, when culture is negative after 24 h of incubation for patients with suspected PJI, when evaluating unexpected culture results, and in the diagnosis of patients receiving antimicrobial treatment.

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