JCM
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Diagnosis of Prosthetic Joint Infection by Use of PCR-Electrospray Ionization Mass Spectrometry

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We compared PCR-electrospray ionization mass spectrometry (PCR-ESI/MS) to culture using sonicate fluid from 431 subjects with explanted knee ($n = 270$) or hip ($n = 161$) prostheses. Of these, 152 and 279 subjects had prosthetic joint infection (PJI) and aseptic failure, respectively. The sensitivities for detecting PJI were 77.6% for PCR-ESI/MS and 69.7% for culture ($P = 0.0105$). The specificities were 93.5 and 99.3%, respectively ($P = 0.0002$).

PCR-electrospray ionization mass spectrometry (PCR-ESI/MS) was first described as a diagnostic strategy for infectious diseases in 2005 (1, 2) and advanced in 2008 (3). It has been used for broad-range bacterial and yeast detection in blood culture bottles and clinical samples (2, 4–7).

We previously showed that culture of fluid obtained by orthopedic implant vortexing/sonication is more sensitive than tissue culture for prosthetic joint infection (PJI) diagnosis (8). The fact that culture-negative cases remained suggests that a molecular approach may be helpful. Herein, we tested sonicate fluid using the BAC detection assay (Abbott Laboratories, Abbott Park, IL), which detects/identifies 3,400 bacteria, four antimicrobial resistance genes (*vanA*, *vanB*, bla_{KPC}, and *mecA*), and over 40 *Candida* species using multiplexed PCR followed by ESI/MS determination of the amplified product's base composition and software comparison to a commercial database.

(This study was presented in part at the 113th General Meeting of the American Society for Microbiology, 18 to 21 May 2013, Denver, CO.)

The study was approved by the Mayo Clinic Institutional Review Board. Mayo Clinic patients with hip ($n = 161$) or knee ($n = 270$) prostheses removed between April 2006 and May 2011 were studied. PJI was diagnosed using the Musculoskeletal Infection Society (MSIS) guidelines (9); for patients who did not have all data pieces required for classification by MSIS criteria, PJI diagnosis was made using Infectious Diseases Society of America (IDSA) guidelines (referred to herein as the "combined definition") (10). Prosthesis failure not meeting criteria for PJI was defined as aseptic failure. Of these subjects, 152 and 279 had PJI and aseptic failure, respectively, with the groups being similar in age, gender, reason for primary arthroplasty, and joint site (Table 1).

Removed prosthesis components were processed as previously described (8) and concentrated for culture (11–13); growth of ≥20 CFU/10 ml unconcentrated sonicate fluid was considered positive (13). A 1-ml aliquot of frozen unconcentrated sonicate fluid was thawed, and DNA was extracted and assayed using BAC Detection 2.0 assay plates and the PLEX-ID PCR-ESI/MS instrument, as previously described (14). A Q score (a rating between 0 and 1 which measures strength of the data for the identification of an organism) was generated, and results of ≥0.90 were reported. A level (a semiquantitative amount of amplicon detected as calculated using an internal calibrant) was also generated and reported in genome equivalents (GE)/well (1); failure of the calibrant to signal was interpreted as a failed report. Metzgar et al. have reported phylogeny covered by the assay and primers used, with limits of detection between 20 and 500 CFU/ml in blood (15). Results of culture and PCR-ESI/MS using three definitions of PJI are shown in Table 2. Using the combined definition, the sensitivities for detecting PJI were 77.6 and 69.7% for PCR-ESI/MS and culture, respectively ($P = 0.0105$), and the specificities were 93.5% and 99.3%, respectively ($P = 0.0002$), with analysis by the MSIS and the IDSA definitions of PJI yielding similar results (Table 2). Among the 35 PJI subjects who had received antimicrobials in the 14 days preceding surgery, 85.7% (30) and 65.7% (23) were positive by PCR-ESI/MS and culture, respectively ($P = 0.0196$). Among the 61 PJI subjects who had received antimicrobials in the 28 days preceding surgery, 85.7% (52) and 73.8% (45) were positive by PCR-ESI/MS and culture, respectively ($P = 0.0348$).

Organisms detected in PJI subjects by culture ($n = 106$) and PCR-ESI/MS ($n = 118$) are shown in Table S1 in the supplemental material. Polymicrobial sonicate fluid culture results included two cases with five or more species, while PCR-ESI/MS detected a maximum of three species.

Eighteen subjects with aseptic failure had positive PCR-ESI/MS results, and 14 of those had no microbial detection by any other method. Nine of these had water/soil bacteria detected, two had unidentifiable bacteria detected, and two and one had *Staphylococcus capitis* and *Lactococcus lactis* detected, respectively (Table 3). For the last three cases, infection was not suspected, no antimicrobials were given, and the subjects remained free from infection over 2 years of follow-up. Seven of 10 subjects with PCR-ESI/MS detection of water/soil organisms had samples collected

Received 15 November 2013 **Accepted** 18 November 2013
Published ahead of print 27 November 2013
Editor: W. M. Dunne, Jr.
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Supplemental material for this article may be found at http://dx.doi.org/10.1128/JCM.03217-13.

doi:10.1128/JCM.03217-13

TABLE 1 Characteristics of the 431 subjects with aseptic failure or PJI[a]

Characteristic	Subjects with: Aseptic failure ($n = 279$)	PJI ($n = 152$)	P
Demographics			
Age (yrs) [median (range)]	68 (24–91)	66 (31–92)	0.86
Females [no. (%)]	155 (55.6)	71 (46.7)	0.08
Reason for primary arthroplasty [no. (%)]			0.61
Osteoarthritis	208 (74.6)	116 (76.3)	
Bone fracture or trauma	35 (12.5)	18 (11.9)	
Inflammatory joint disorder[b]	19 (6.8)	9 (5.9)	
Avascular necrosis	12 (4.3)	3 (2.0)	
Congenital abnormalities	3 (1.1)	4 (2.6)	
Other[c]	2 (0.7)	2 (1.3)	
Site of arthroplasty [no. (%)]			0.56
Knee	172 (61.6)	98 (64.5)	
Hip	107 (38.4)	54 (35.5)	
Preoperative laboratory findings[d] [no./total (%)]			
Erythrocyte sedimentation rate > 30 mm/h[e]	42/255 (16.5)	84/143 (58.7)	<0.0001
Serum C-reactive protein > 10 mg/liter[e]	49/255 (19.2)	111/144 (77.1)	<0.0001
Synovial fluid leukocyte count > 1,700/μl[f]	30/135 (22.2)	66/78 (84.6)	<0.0001
Synovial fluid differential > 65% neutrophils[f]	12/130 (9.2)	65/77 (84.4)	<0.0001
Operative findings [no./total (%)]			
Sinus tract communicating with the prosthesis	0/279 (0)	38/152 (25.0)	
Histological acute inflammation	0/279 (0)	90/120 (75.0)	
Purulence in the affected joint space	0/279 (0)	111/152 (73.0)	

[a] Only the first prosthesis removal surgery was studied; subjects undergoing only polyethylene liner exchange were excluded. Patient characteristics were summarized as frequencies and percentages or median (minimum, maximum) and compared using the chi-square test or Wilcoxon rank sum test as appropriate (SAS version 9.2; SAS Inc., Cary, NC). All tests were two sided, and P values less than 0.05 were considered statistically significant.
[b] Includes rheumatoid arthritis, ankylosing spondylitis, and systemic lupus erythematosus.
[c] Includes bone neoplasia, arthrofibrosis, polymyalgia rheumatica, reflex sympathetic dystrophy, and acromegaly.
[d] ESR, CRP, and synovial fluid leukocyte count and differential within six preoperative months.
[e] The cutoff is taken from reference 21.
[f] The cutoff is taken from reference 22, a study which excluded subjects with underlying inflammatory joint diseases or connective-tissue diseases and evaluated only knee arthroplasties.

TABLE 2 Sensitivity and specificity of sonicate fluid culture and PCR-ESI/MS[a]

Definition of PJI	Parameter	% (no. positive/total) in: Sonicate fluid culture	Sonicate fluid PCR-ESI/MS	P
Combined	Sensitivity	69.7 (106/152)	77.6 (118/152)	0.0105
	Specificity	99.3 (277/279)	93.5 (261/279)	0.0002
IDSA	Sensitivity	73.1 (106/145)	81.4 (118/145)	0.0105
	Specificity	99.3 (284/286)	93.7 (268/286)	0.0002
MSIS	Sensitivity	76.7 (102/133)	82.7 (110/133)	0.0455
	Specificity	98.0 (292/298)	91.3 (272/298)	<0.0001

[a] Comparisons of sensitivities and specificities of PCR-ESI/MS and culture of sonicate fluid using three definitions of PJI with McNemar's test of paired portions (SAS version 9.2; SAS Inc., Cary, NC). All tests were two sided, and P values less than 0.05 were considered statistically significant.

within a one-month period and processed in succession (Table 3), suggesting either processing or reagent contamination. Three subjects (one each with *Streptococcus pseudopneumoniae*, *Klebsiella pneumoniae*, and group B *Streptococcus* sp.) had the same organism detected using other molecular methods (Table 3) and previous culture-positive infections with the organism detected by PCR-ESI/MS. Two subjects with aseptic failure had positive sonicate fluid cultures (>100 CFU/plate), one with viridans group streptococci and another with *Clostridium perfringens* and *Propionibacterium acnes* (Table 3).

There were no PJI cases caused by carbapenem-resistant *Enterobacteriaceae*. Three PJI cases yielded vancomycin-resistant *Enterococcus faecium*, with *vanA* being detected in all three. Of 42 cases with methicillin resistance detected in cultured staphylococci, 38 had *mecA* detected by PCR-ESI/MS, three did not have staphylococci (or *mecA*) detected by PCR-ESI/MS, and one had *Staphylococcus caprae* (but not *mecA*) detected by PCR-ESI/MS (with *S. caprae* detected by culture).

Of the 118 PJI cases that were positive by PCR-ESI/MS, 85 had microbiology concordant with culture, 17 were sonicate fluid culture negative (Table 4), 10 had an additional organism(s) detected in sonicate fluid culture (Table 5), four had an additional organism(s) detected by PCR-ESI/MS (Table 5), and two had discordant microbiology (Table 6). Of the 106 sonicate fluid culture-positive PJI cases, five were not identified by PCR-ESI/MS (Table 4). Ten PJI cases with no methicillin resistance detected in culture had *mecA* detected by PCR-ESI/MS (five had a level of ≤4, and three were culture-negative cases).

PCR-ESI/MS sensitivity (77.6%) for detecting PJI from sonicate fluid was equivalent to the sensitivity (77.1%) we recently reported with a genus-/group-specific rapid PCR panel assay targeting PJI bacteria (16) and was better than that of a 16S rRNA gene PCR assay, which had a sensitivity of 70.4% (17); both referenced studies used a set of samples similar to that studied herein (with the same subjects studied in some cases). PCR-ESI/MS was less affected than culture by prior antimicrobials; PCR-ESI/MS sensitivity (85.7%) among subjects receiving antimicrobial therapy within 14 or 28 days of surgery was equivalent to the sensitivity (87.9 and 86.2%, respectively) found with the PCR panel assay (16). However, PCR-ESI/MS specificity (93.5%) was less than the specificity of the PCR panel assay (97.9%) (16). Advantages of the PCR-ESI/MS assay include its multiplexed format, 0.04 ml sonicate fluid/PCR template DNA input (versus 2 ml using the previously described 16S rRNA [17] and panel [16] PCR assays), fast turnaround time (6.5 to 8.5 h versus 1 to 14 days for culture), and rapid detection of antimicrobial resistance markers. Other investigators have tested sonicate fluid using a commercial multiplex real-time PCR assay, Septifast (Roche Diagnostics, Basel, Switzer-

TABLE 3 Aseptic failure subjects with positive sonicate fluid microbiology[a]

Case type and no.	Antimicrobial treatment within 28 days of surgery, antibiotic (no. of days before surgery that treatment ended)	No. of positive tissues/ no. cultured and organism detected[b]	Synovial fluid culture result[c]	Sonicate fluid				
				16S rRNA PCR sequence-based identification[d]	PCR panel, assay positive[e]	Culture result (CFU/10 ml)	PLEX-ID result	Q score; level (GE/well)
Sonicate fluid culture negative and PCR-ESI/MS positive, with no microbial growth by any other method ($n = 14$)								
31	None	0/3	ND	ND	Neg	NG	*Staphylococcus capitis*	0.99; 205
54	None	0/3	NG	ND	Neg	NG	Bacteria detected, no identification	0.98; 10
62	None	0/3	NG	ND	Neg	NG	*Methylobacterium mesophilicum*	0.97; 6
68	None	0/2	ND	ND	Neg	NG	*Ralstonia solanacearum*	0.91; 24
							Burkholderia sp.	0.98; 175
71	None	0/2	NG	Neg	Neg	NG	*R. solanacearum*	0.96; 151
72	None	0/3	NG	Neg	Neg	NG	*R. solanacearum*	0.96; 127
75	None	0/3	NG	Neg	Neg	NG	*R. solanacearum*	0.94; 165
77	None	0/3	ND	Neg	Neg	NG	*R. solanacearum*	0.99; 246
							Burkholderia sp.	0.97; 33
							Pseudomonas mendocina	0.97; 20
190	None	0/3	ND	Neg	Neg	NG	*Stenotrophomonas maltophilia*	0.97; 69
191	None	0/3	NG	Neg	Neg	NG	Bacteria detected, no identification	0.95; 31
194	None	0/3	ND	Neg	Neg	NG	*Lactococcus lactis*	0.97; 10
228	None	0/3	NG	Neg	Neg	NG	*Kocuria rhizobhila*	0.98; 4
312	None	0/3	NG	Neg	Neg	NG	*K. rhizobhila*	0.91; 4
433	None	0/3	NG	Neg	Neg	NG	*S. capitis*	0.99; 205
Sonicate fluid culture negative and PCR-ESI/MS positive, with DNA detected by other methods ($n = 3$)								
299	Vancomycin (18)	0/3	NG	*Streptococcus* sp.	*Streptococcus* sp.	NG	*Streptococcus pseudopneumoniae*	0.99; 1509
321	None	0/3	NG	*Klebsiella pneumoniae*	ENT	NG	*K. pneumoniae*	0.98; 5
397	Penicillin, clindamycin (17)	0/5	ND	*Streptococcus* sp.	*Streptococcus agalactiae*	NG	Group B *Streptococcus* sp.	0.98; 4
Sonicate fluid culture positive ($n = 2$)								
73	None	0/3	ND	Neg	*Streptococcus* sp.	Viridans group *Streptococcus* sp. (>100)	*R. solanacearum*	0.97; 117
84	None	1/3 *Propionibacterium acnes*	ND	Neg	Neg	*Clostridium perfringens* (>100); *P. acnes* (>100)	Neg	NA

[a] ND, not done; Neg, negative; NG, no growth; ENT, *Enterobacteriaceae* assay (includes *K. pneumoniae*); NA, not applicable.

[b] Intraoperatively, tissues were collected for culture and histopathology and processed as previously described (16).

[c] Synovial fluids with volumes of >1 ml were inoculated into a Bactec Peds Plus/F bottle and incubated on a Bactec 9240 instrument (BD Diagnostic Systems, Sparks, MD) for 5 days (23). Synovial fluids with volumes of ≤1 ml were cultured as previously described (16). Any growth was classified and considered positive.

[d] Using primers which amplify the V3-V4 hypervariable region of the 16S rRNA gene with Roche LightCycler SYBR green detection followed by sequencing of amplified product, and using RipSeq software to analyze mixed sequences (17).

[e] A 10-assay panel of real-time FRET probe PCR assays (using Roche LightCycler) which detects multiple bacterial pathogens typically considered causes of PJI (staphylococci, streptococci, *Enterobacteriaceae*, anaerobic Gram-positive cocci, *Enterococcus*/*Granulicatella*/*Abiotrophia* sp., *Propionibacterium*/*Actinomyces* sp., *Pseudomonas aeruginosa*, *Corynebacterium* sp., and *Bacteroides fragilis* group) and *mecA* using a variety of gene targets (16, 24, 25).

TABLE 4 PJI subjects with discordant results between sonicate fluid PCR-ESI/MS and culture[a]

Case type and no.	Antimicrobial treatment within 28 days of surgery (no. of days before surgery that treatment ended)	No. positive/no. of tissues cultured and organism(s) detected[b]	Synovial fluid culture result[c]	Sonicate fluid				
				16S rRNA PCR sequence-based identification[d]	PCR panel, assay positive[e]	Culture, organism detected (CFU/10 ml)	PLEX-ID result	Q score; level (GE/well)
Sonicate fluid culture negative and PCR-ESI/MS positive ($n = 17$)								
53	None	0/3	ND	Neg	Neg	NG	*Rothia mucilaginosa*	0.95; 79
							Streptococcus sp.	0.93; 25
							Viridans/mitis group	0.97; 45
74	Vancomycin (0)	4/5 *Staphylococcus aureus*[f]	MS *S. aureus*	*S. aureus*	*S. aureus*	*S. aureus*[f] (<20)	*S. aureus*	0.97; 6
110	Oxacillin (0)	3/5 MR CNS	ND	*S. aureus*	*S. aureus*	CNS[f] (<20)	*S. aureus*	0.97; 6
							Staphylococcus lugdunensis	0.96; 3
181	Ciprofloxacin (0)	0/3	*Propionibacterium acnes*	Neg	Neg	NG	*P. acnes*	0.98; 22
195	None	0/5	ND	Neg	Neg	NG	*P. acnes*	0.98; 34
							Bacteria detected, no identification	0.96; 21
214	Ceftriaxone (0)	0/5	Group B *Streptococcus* sp.	*Streptococcus* sp.	*Streptococcus agalactiae*	Group B *Streptococcus* sp. (<20)	*S. agalactiae*	0.99; 139
217	None	2/9 *Escherichia coli*	NG	*E. coli/Shigella*	ENT	*E. coli* (<20)	*E. coli*	0.99; 152
							E. coli	0.87; 3
226	TMP/SFX, levofloxacin (3)	2/4 MR CNS	ND	CNS, *P. acnes*	CNS, *Prop.* sp.	NG	*P. acnes*	0.98; 44
250	Fusidic acid (2)	0/5	ND	CNS, *S. aureus*	CNS, *Prop.* sp.	CNS[f] (<20)	*Staphylococcus caprae* or *Staphylococcus epidermidis*	0.97; 62
							mecA	0.98; 190
280	None	1/7 CNS[f]	NG	*Streptococcus* sp., *Gemella* sp.	*Streptococcus* sp.	NG	Viridans/mitis group	0.97; 9
							S. lugdunensis	0.97; 3
352	Ampicillin/clavulanate (27)	2/3 MS *Staphylococcus warneri*	*S. lugdunensis*[f]	Neg	CNS	MS *S. lugdunensis* (<20)	*S. lugdunensis*	0.98; 9
369	Cefazolin (0)	2/3 MR *S. aureus*, *Corynebacterium* sp.	NG	Neg	Neg	NG	*S. aureus*	0.97; 3
							mecA	0.96; 3
379	Vancomycin (0)	0/7	NG	*Serratia marcescens*	ENT	NG	*S. marcescens*	0.99; 8
430	None	2/3 CNS[f]	MR CNS	*Enterococcus* sp.	CNS	NG	*S. epidermidis*	0.99; 37
							mecA	0.98; 45
431	None	0/3	NG	Neg	Neg	NG	*P. acnes*	0.99; 51
435	None	1/5 *P. acnes*	NG	Neg	Neg	NG	*S. marcescens*	0.98; 8
449	None	0/3	ND	Neg	Neg	NG	Viridans/mitis group	0.98; 19
Sonicate fluid culture positive and PCR-ESI/MS negative ($n = 5$)								
28	Clindamycin (12)	4/6 *Corynebacterium* sp.	*Corynebacterium* sp.	ND	*Coryne.* sp.	*Corynebacterium simulans* (>100)	Neg	NA
126	Levofloxacin (14)	2/5 MR CNS	MR CNS	Neg	CNS, *mecA*	*S. epidermidis*[f] (>100)	Neg	NA
289	None	2/2 CNS[f]	CNS[f]	Neg	CNS	MS CNS (>100)	Neg	NA
343	None	1/5 CNS[f]	ND	*P. acnes*	CNS	*P. acnes* (>100)	Neg	NA
412	None	1/4 CNS[f]	ND	Neg	CNS	MR CNS (>100)	Neg	NA

[a] ND, not done; Neg, negative; NG, no growth; MS, methicillin sensitive; MR, methicillin resistant; CNS, coagulase-negative staphylococci; ENT, *Enterobacteriaceae* assay (includes *E. coli* and *S. marcescens*); TMP/SFX, trimethoprim-sulfamethoxazole; *Prop.* sp., *Propionibacterium/Actinomyces* sp. assay (includes *P. acnes*); *Coryne.* sp., *Corynebacterium* sp. assay (includes *C. simulans*); NA, not applicable.

[b] Intraoperatively, tissues were collected for culture and histopathology and processed as previously described (16).

[c] Synovial fluid samples with volumes of >1 ml were inoculated into a Bactec Peds Plus/F bottle and incubated on a Bactec 9240 instrument (BD Diagnostic Systems, Sparks, MD) for 5 days (23). Synovial fluid samples with volumes of ≤1 ml were cultured as previously described (16). Any growth was classified and considered positive.

[d] PCR used primers which amplify the V3-V4 hypervariable region of the 16S rRNA gene with Roche LightCycler SYBR green detection followed by sequencing of amplified product, with RipSeq software to analyze mixed sequences (17).

[e] A 10-assay panel of real-time FRET probe PCR assays (using Roche LightCycler) which detects multiple bacterial pathogens typically considered causes of PJI (staphylococci, streptococci, *Enterobacteriaceae*, anaerobic Gram-positive cocci, *Enterococcus/Granulicatella/Abiotrophia* spp., *Propionibacterium/Actinomyces* spp., *Pseudomonas aeruginosa*, *Corynebacterium* spp., and *Bacteroides fragilis* group) and *mecA* using a variety of gene targets (16, 24, 25).

[f] Susceptibilities were not determined.

TABLE 5 PJI subjects with additional isolates found by either sonicate fluid PCR-ESI/MS or culture[a]

Case type and no.	Antimicrobial treatment within 28 days of surgery, antibiotic (no. of days before surgery that treatment ended)	No. positive/no. tissues cultured and organism detected[b]	Synovial fluid culture result[c]	Sonicate fluid				Q score; level (GE/well)
				16S rRNA PCR sequence-based identification[d]	Sonicate fluid PCR panel, assay positive[e]	Culture result and organism detected (CFU/10 ml)	PLEX-ID result	
Additional organisms detected by sonicate fluid culture ($n = 10$)								
192	None	4/6 MR CNS; 1/6 *Paecilomyces* sp.	NG	CNS	CNS	MR CNS (>100), *Corynebacterium* sp. (>100)	*Staphylococcus epidermidis* *mecA*	0.99; 132 0.98; 161
279	Vancomycin, piperacillin/tazobactam (14)	6/6 *Prevotella melanogenica*, *Actinomyces odontolyticus*, VS *Enterococcus* sp.; 5/6 viridans group *Streptococcus* sp.; 4/6 *Abiotrophia/Granulicatella* sp., anaerobic GPC, anaerobic GNC; 3/6 anaerobic GPB	ND	*P. melanogenica*	AGPC, *Actinomyces* sp., Ent/Abi/Gran sp., *Streptococcus* sp.	*P. melanogenica* (>100), *Finegoldia magna* (>100), *A. odontolyticus* (>100), VS *Enterococcus* sp. (>100), Viridans group *Streptococcus* sp. (>100), *Abiotrophia/Granulicatella* sp. (>100), *Veillonella* sp. (>100), *Pandoraea norimbergensis* (20–50)	*P. melanogenica* *F. magna*	0.98; 45 0.96; 7
287	None	4/4 MR *Staphylococcus aureus*, VS *Enterococcus* sp., *Actinomyces* sp.	VS *Enterococcus* sp., *Actinomyces* sp.	*S. aureus*	*S. aureus*, *Enterococcus* sp., *Actinomyces* sp., AGPC, *mecA*	*S. aureus*[f] (>100), *Enterococcus faecalis*[f] (>100), *Peptostrepto.* sp. (>100)	*S. aureus* *mecA*	0.99; 52 0.98; 159
300	Moxifloxacin, rifampin (0)	0/3	ND	Neg	CNS	MS CNS (20–50), MS *Staphylococcus lugdunensis* (20–50)	*S. lugdunensis*	0.98; 98
331	None	4/6 CNS[f], *F. magna*	MS CNS, MS *S. lugdunensis*	CNS, *F. magna*, *Corynebacterium* sp.	CNS, AGPC, *Coryne.* sp.	MS *S. lugdunensis* (51–100), *F. magna* (>100), MS *S. epidermidis* (51–100)	*S. lugdunensis*	0.97; 22
335	None	3/7 MS *S. aureus*, CNS[f]	ND	*S. aureus*, *Staphylococcus warneri*	*S. aureus*	MS *S. aureus* (>100), CNS[f] (51–100)	*F. magna* *S. aureus*	0.99; 77 0.99; 118
337	Levofloxacin (23)	3/3 MS *S. aureus*, *Pseudomonas aeruginosa*, *F. magna*, *Corynebacterium* sp., MS CNS	ND	*Pseudomonas* sp., *Peptoniphilis* sp.	*S. aureus*, *P. aeruginosa*, AGPC, *Coryne.* sp.	*S. aureus*[f] (>100), *P. aeruginosa* (>100), *F. magna* (20–50), *Peptostrepto.* sp. (20–50), *Actinomyces neuii* (20–50)	*S. aureus* *P. aeruginosa*	0.96; 4 0.99; 71
392	None	2/5 CNS[f]	CNS*	CNS	CNS, *mecA*	MR *S. epidermidis* (>100), *Propionibacterium acnes* (20–50)	*S. epidermidis* *mecA*	0.99; 132 0.98; 166

417	Cephalexin (0)	7/7 MR CNS, MS *S. aureus*, *Corynebacterium* sp.	ND	CNS	CNS, *mecA*	MR CNS (>100), MS *S. aureus* (51–100), *Corynebacterium* sp. (20–50)	*S. epidermidis* *mecA* *Corynebacterium striatum*	0.99; 62 0.96; 66 0.98; 16
428	None	1/4 CNS[f]	VS *Enterococcus* sp.	*Enterococcus* sp.	*Enterococcus* sp.	VS *Enterococcus* sp. (51–100), MR CNS (51–100)	*E. faecium*	0.99; 35
Additional organisms detected by sonicate fluid PCR-ESI/MS ($n = 4$)								
175	TMP/SMX (7)	5/5 MR *S. aureus*	*S. aureus*[f]	CNS, *F. magna*	*S. aureus*, *mecA*, AGPC, ENT	MR *S. aureus* (>100), *F. magna* (>100)	*S. aureus* *mecA* *F. magna* *E. cloacae complex*	0.99; 139 0.98; 176 0.97; 266 0.96; 12
238	Levofloxacin (0)	5/5 *Escherichia coli*, MR CNS; 1/5 *Corynebacterium* sp.	ND	*E. coli*/*Shigella* sp., *S. epidermidis*, *Corynebacterium* sp.	ENT, CNS, *mecA*, *Corynebacterium aurimucosum*	*E. coli* (>100), *S. epidermidis*[f] (>100), *Corynebacterium* sp. (51–100)	*E. coli* *S. epidermidis* *mecA* *Shigella boydii*	0.97; 119 0.97; 14 0.98; 27 0.96; 20
342	Vancomycin, rifampin (17)	0/3	CNS[f]	Neg	AGPC	MR CNS (51–100)	*S. epidermidis* *mecA* *F. magna*	0.99; 14 0.98; 9 0.98; 16
429	None	2/6 VR *Enterococcus faecium*, *Corynebacterium* sp.; 1/6 *E. faecalis*[f], MS *S. aureus*, MR CNS	VS *Enterococcus* sp.	*Enterococcus* sp.	*Enterococcus* sp.	*E. faecium*[f] (>100), *S. aureus* (<20)	*E. faecium* *vanA* *S. aureus*	0.99; 831 0.98; 28 0.98; 11

[a] MR, methicillin resistant; CNS, coagulase-negative staphylococci; NG, no growth; VS, vancomycin sensitive; GPC, Gram-positive cocci; GNC, Gram-negative cocci; GPB, Gram-positive bacilli; ND, not done; AGPC, anaerobic Gram-positive coccus assay (includes *F. magna*); *Actinomyces* sp., *Propionibacterium*/*Actinomyces* sp. (includes *A. odontolyticus*); Ent/Abi/Gran sp., *Enterococcus*/*Abiotrophia*/*Granulicatella* sp. assay; *Peptostrepto*., *Peptostreptococcus*; Neg, negative; MS, methicillin sensitive; *Coryne*. sp., *Corynebacterium* sp. assay; TMP/SFX, trimethoprim-sulfamethoxazole; ENT, *Enterobacteriaceae* assay (includes *E. cloacae* and *E. coli*); VR, vancomycin resistant.

[b] Intraoperatively, tissues were collected for culture and histopathology and processed as previously described (16).

[c] Synovial fluids with volumes of >1 ml were inoculated into a Bactec Peds Plus/F bottle and incubated on a Bactec 9240 instrument (BD Diagnostic Systems, Sparks, MD) for 5 days (23). Synovial fluids with volumes ≤1 ml were cultured as previously described (16). Any growth was classified and considered positive.

[d] PCR used primers which amplify the V3-V4 hypervariable region of the 16S rRNA gene with Roche LightCycler SYBR green detection followed by sequencing of the amplified product, with RipSeq software to analyze mixed sequences (17).

[e] A 10-assay panel of real-time FRET probe PCR assays (using Roche LightCycler) which detects multiple bacterial pathogens typically considered causes of PJI (staphylococci, streptococci, *Enterobacteriaceae*, anaerobic Gram-positive cocci, *Enterococcus*/*Granulicatella*/*Abiotrophia* spp., *Propionibacterium*/*Actinomyces* spp., *Pseudomonas aeruginosa*, *Corynebacterium* spp., and *Bacteroides fragilis* group) and *mecA* using a variety of gene targets (16, 24, 25).

[f] Susceptibilities were not determined.

TABLE 6 PJI subjects with discordant microbiology between sonicate fluid PCR-ESI/MS and culture

Case	Antimicrobial treatment within 28 days of surgery, antibiotic (no. of days before surgery that treatment ended)	No. positive/no. tissues cultured and organism detected[a]	Synovial fluid culture result[b]	Sonicate fluid				
				16S rRNA PCR, sequence-based identification[c]	PCR panel, assay positive[d]	Culture, organism detected (CFU/10 ml)	PLEX-ID result	Q score; level (GE/well)
153	Cefazolin (0)	1/2 *Propionibacterium acnes*	NG	*Streptococcus* sp.	*Streptococcus* sp.	*P. acnes* (20–50)	Group G *Streptococcus* sp.	0.98; 148
314	Cephalexin, levofloxacin (0)	2/3 group G *Streptococcus* sp.	NG	*Streptococcus* sp.	*Streptococcus* sp.	Group G *Streptococcus* sp. (>100)	*Streptococcus pyogenes*	0.99; 178

[a] Intraoperatively, tissues were collected for culture and histopathology and processed as previously described (16).
[b] Synovial fluids with volumes of >1 ml were inoculated into a Bactec Peds Plus/F bottle and incubated in a Bactec 9240 instrument (BD Diagnostic Systems, Sparks, MD) for 5 days (23). Synovial fluid samples with volumes of ≤1 ml were cultured as previously described (16). Any growth was classified and considered positive. NG, no growth.
[c] PCR used primers which amplify the V3-V4 hypervariable region of the 16S rRNA gene with Roche LightCycler SYBR green detection followed by sequencing of amplified product, with RipSeq software to analyze mixed sequences (17).
[d] A 10-assay panel of real-time FRET probe PCR assays (using Roche LightCycler) which detects multiple bacterial pathogens typically considered causes of PJI (staphylococci, streptococci, *Enterobacteriaceae*, anaerobic Gram-positive cocci, *Enterococcus*/*Granulicatella*/*Abiotrophia* sp., *Propionibacterium*/*Actinomyces* sp., *Pseudomonas aeruginosa*, *Corynebacterium* sp., and *Bacteroides fragilis* group) and *mecA* using a variety of gene targets (16, 24, 25).

land), which does not target anaerobes (18, 19); the PCR-ESI/MS assay studied detected nine anaerobic bacteria (see Table S1 in the supplemental material).

Despite the increased sensitivity of PCR-ESI/MS compared to culture, there remained 34 PCR-ESI/MS-negative PJI cases; 29 had negative sonicate fluid cultures. Using a similar PCR-ESI/MS technique on synovial fluid specimens, Jacovides et al. reported organism detection in four culture-negative PJI cases and in 88% of cases with presumed noninfectious failure (20). Differences between the study by Jacovides et al. and ours may be explained by differences in specimen type or software and hardware differences of the PCR/ESI-MS platform. The platform used by Jacovides et al. was an older version designed as a research tool, with no specific cutoff or reporting criteria defined within the software, whereas the system we evaluated was designed for clinical use, using a more stringent analysis.

In summary, PCR-ESI/MS is more sensitive but less specific than culture for PJI diagnosis when performed on material dislodged from the surfaces of explanted orthopedic prostheses and may be a useful tool for the rapid detection of PJI and/or as an adjunctive method for select cases of arthroplasty failure.

ACKNOWLEDGMENTS

Research grant support was provided by the National Institutes of Health (R01 AR056647 and R01 AI91594).

Robin Patel has research grant support from Pfizer, Pocared, Pradama, Astellas, 3M, nanoMR, Curetis, BioFire, and Tornier. She has patents on a pertussis PCR assay, an anti-biofilm substance, and a method/device for sonication.

We thank Robert T. Trousdale, David G. Lewallen, Mark W. Pagnano, Miguel E. Cabanela, Robert H. Cofield, Daniel J. Berry, Joaquin Sanchez-Sotelo, Michael J. Yaszemski, Michael J. Stuart, Franklin H. Sim, Bernard F. Morrey, Rafael J. Sierra, Tad M. Mabry, Diane L. Dahm, Stephen A. Sems, Joseph R. Cass, Bruce A. Levy, Michael E. Torchia, and Michael J. Taunton for submitting explanted prostheses for this study. We thank Melissa J. Karau and Suzannah M. Schmidt for the collection and storage of sonicate fluid specimens. Abbott Laboratories provided reagents and loaned a PLEX-ID PCR-ESI/MS instrument to Mayo Clinic for this study.

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