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Sonication of Explanted Prosthesis Combined with Incubation in BD Bactec Bottles for Pathogen-Based Diagnosis of Prosthetic Joint Infection

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Prosthetic joint infection (PJI) is a rare but refractory complication of arthroplasty. Accurate identification of pathogens is a key step for successful treatment of PJI, which remains a challenge for clinicians and laboratory workers. We designed a combined culture method with sonication of implants and incubation in a BD Bactec system to improve the effectiveness of pathogen diagnosis in PJI. The aims of this study were to investigate the diagnostic accuracy of sonicate fluid cultures in the BD Bactec system and to compare the results with those of synovial fluid cultures in the BD Bactec system. The prosthetic components removed were sonicated in Ringer's solution, and then sonicate fluid was incubated in Bactec bottles for 5 days. Synovial fluid was incubated in Bactec bottles for 5 days as a control. Synovial fluid cultures with Bactec bottles and sonicate fluid cultures with Bactec bottles showed sensitivities of 64% and 88%, respectively ($P = 0.009$), with specificities of 98% and 87% ($P = 0.032$), respectively. Sonicate fluid cultures with Bactec bottles were more sensitive than synovial fluid cultures with Bactec bottles regardless of whether antimicrobial agents were used within 14 days before surgery (81% versus 52%; $P = 0.031$) or not (93% versus 72%; $P = 0.031$). Sonication of explanted prostheses followed by incubation of the resulting sonicate fluid in Bactec bottles detected many more pathogens than did synovial fluid cultures with Bactec bottles. This method is also effective in cases with antibiotic treatment before surgery.

Prosthetic joint infection (PJI) is a rare but refractory complication of arthroplasty that leads to severe consequences if unresolved, not only for patients but also for society, because of long hospital stays, expensive treatments, and multiple operations (1). The pathogenesis of PJI is related to the ability of microorganisms to grow in biofilms, which makes such infections difficult to diagnose and to eradicate (2, 3). Routine cultures of periprosthetic tissues and synovial fluid are the standard method for making a microbiological diagnosis of PJI, but they yield false-negative results in up to 30% of cases (3, 4). Several efforts have been made to improve pathogen diagnosis in PJI, and implant sonication and tissue fluid culture in the BD Bactec system have been shown to be effective methods (3, 5–9). Sonication was used to dislodge bacteria embedded in the biofilms from the removed implants, while blood culture systems such as the BD Bactec system were used to shorten the duration of incubation (10–12). We set up a combined culture method with sonication of explanted implants and culture in the BD Bactec system, to improve the effectiveness of pathogen diagnosis in PJI. The purposes of the current prospective study were to investigate the diagnostic accuracy of sonicate fluid culture in the BD Bactec system and to compare this method with synovial fluid culture in the BD Bactec system.

MATERIALS AND METHODS

Study population. This cohort study was conducted in Shanghai Jiao Tong University Affiliated Sixth People's Hospital, in which over 2,200 primary and revision arthroplasties are performed each year. The study protocol was reviewed and approved by the institutional review board. Patients undergoing removal of a total knee or hip prosthesis because of aseptic failure (AF) or presumed infection were enrolled between August 2011 and May 2014. PJI was suspected preoperatively on the basis of a persistently painful prosthesis, a sinus tract around the joint, positive laboratory markers, cultures of preoperative aspirate fluid, and positive results of technetium-methylene diphosphonate (MDP) bone scintigraphy. Patients were excluded if no synovial fluid was collected for culture with BD Bactec bottles, prosthetic components were not ultrasonicated after being removed, or obvious contamination of a removed component occurred in the operating room. Medical records, including demographic characteristics, clinical, laboratory, histopathological, and microbiological data, type of surgical management, and information about the primary arthroplasty, subsequent revisions, and antimicrobial therapy, were reviewed and analyzed.

PJI definition. Patients were classified as having definite prosthetic infection if at least one of the following was present (13): (i) gross purulence at the surgical site, (ii) presence of a sinus tract communicating with the prosthesis, (iii) acute inflammation detected during histopathological examination of periprosthetic tissue, or (iv) microbial growth in intraoperative periprosthetic samples or sonicate fluid samples from the removed implant. The diagnosis of PJI was finally determined by clinicians after evaluation of all of the available preoperative and intraoperative information (14). If only one intraoperative culture was pathogen positive, then histopathological examination results showing acute inflammation con-

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firmed the infection. If the histopathological examination did not show signs of inflammation and other evidence (such as a sinus tract or purulence) was not noted, then the single detected microorganism was considered to represent contamination. Aseptic failure (AF) was defined as failure of the prosthesis in the absence of any of these findings. Previous antimicrobial therapy was defined as receipt of antimicrobial agents within 2 weeks before removal of the prosthesis.

Sample collection. All patients with preoperative diagnoses of aseptic failure received standard perioperative prophylaxis with cefuroxime. Typically, the first dose was administered 30 min before incision. In cases in which infection was suspected, antimicrobials were not administered until culture samples were obtained. Synovial fluid samples were collected intraoperatively and sent for microbiological analyses with Gram's stain and culture. Periprosthetic soft tissue with inflammatory changes was collected for microbiological and histopathological assays. The prosthetic components (including polyethylene and polymethylmethacrylate components, if present) were placed in a sterile, wide-mouth, polypropylene jar for ultrasonication. All specimens were transported to the laboratory and processed within 6 h.

Synovial fluid culture. Synovial fluid was collected in sterile vials. One milliliter of sample was inoculated into a BD Bactec Anaerobic Lytic/F bottle, and 1 ml was inoculated into a BD Bactec Plus Aerobic/F bottle. Bactec bottles were monitored until the end of the 5-day incubation. If a bottle tested positive, then Gram's staining and Wright's staining were performed; 0.1 ml turbid broth from BD Bactec Plus Aerobic/F bottles was inoculated into sheep blood agar (SBA), MacConkey agar, and chocolate agar for 48 h at 35°C in 5% CO_2, and 0.1 ml turbid broth from BD Bactec Anaerobic Lytic/F bottles was inoculated into anaerobic sheep blood agar (ASBA) for 72 h at 35°C in an anaerobic atmosphere. If there was no growth on subculture and no microbiological morphology with Gram's staining or Wright's staining, then results were recorded as negative. Each unique colony of isolated microorganisms was identified on the basis of growth characteristics, biochemical profiles, and antimicrobial susceptibility with standard microbiological techniques.

Sonicate fluid culture. The prosthetic components removed were placed in a sterile, wide-mouth, airtight, polypropylene container and transported immediately to the microbiology laboratory. The containers had been previously autoclaved at 121°C for 15 min and double packed. Sonication was performed according to the technique described by Trampuz et al. (7), with some modification. Sterile Ringer's solution (ranging from 150 to 250 ml, depending on the size of the implant) was added to the container in a laminar airflow biosafety cabinet. The container was vortex-mixed for 30 s with a Vortex-Genie (Scientific Industries), subjected to sonication (frequency, 28 ± 2 kHz) for 10 min in a 10-liter ultrasound bath (CQ-200B-DST; Shanghai Yuejin Medical Equipment Co.), and then vortex-mixed again for 30 s. A total of 30 ml of sonicate fluid was centrifuged at 4,000 rpm for 10 min, and the sediment was resuspended with 2 ml sterile Ringer's solution. One milliliter of resuspended fluid was inoculated into a BD Bactec Anaerobic Lytic/F bottle, and 1 ml was inoculated into a Bactec Plus Aerobic/F bottle. Bactec bottles were monitored until the end of the 5-day incubation. If a bottle tested positive, then turbid broth was subcultured and incubated as described for synovial fluid cultures.

Statistical analysis. The baseline characteristics of the PJI group and the AF group were compared with the Wilcoxon rank-sum test, the chi-square test, or Student's *t* test, as appropriate. The sensitivities and specificities of the different culture methods were compared with McNemar's test of paired proportions. Comparisons between categorical variables were performed using McNemar's test or Fisher's exact test, as appropriate. *P* values of <0.05 (for two-sided tests) were considered to indicate statistical significance. Sensitivities, specificities, positive predictive values, and negative predictive values were calculated with two-by-two contingency tables. Confidence intervals (CIs) were calculated as exact binomial confidence intervals. All tests were performed using MedCalc for Windows (MedCalc v.12.3; MedCalc Software).

RESULTS

Demographic characteristics. A total of 132 cases were considered for inclusion in the study. Ten cases were excluded due to revision of a single part of the prosthetic components, and 8 cases were excluded because synovial fluid was not collected for culture in BD Bactec bottles or a prosthetic component was not ultrasonicated after being removed; 4 cases with obvious contamination of a removed component were also excluded. In the remaining 110 cases analyzed (Table 1), 60 patients had aseptic failure and 50 met the criteria for prosthetic joint infection. The mean age and gender ratio of the patients with PJI were similar to those of the patients with AF. There were proportionally more knee implants in the group with PJI than in the group with AF (40% and 3%, respectively; $P < 0.001$). In 5 cases of PJI (10%), debridement with implant retention was performed and only the mobile parts were exchanged and subjected to sonication. Among the remaining 45 PJI cases, a one-stage exchange was performed in 14 cases (28%) and a two-stage exchange was performed in the other 31 cases (62%). Fifty-nine patients with AF (98%) underwent a one-stage exchange. C-reactive protein (CRP) concentrations of >10 mg/liter and erythrocyte sedimentation rates (ESRs) of >30 mm/h were more prevalent in the group with PJI than in the group with AF (CRP concentrations of 34.3 and 4.17 mg/liter, respectively; $P < 0.001$; ESR values of 63 and 24 mm/h, respectively; $P < 0.001$).

Microbiological results. The microbiological findings in PJI are illustrated in Table 2. The most frequently isolated pathogens were coagulase-negative staphylococci (CoNS) and *Staphylococcus aureus*. Sonicate fluid cultures in Bactec bottles detected significantly more pathogens than did synovial fluid cultures in Bactec bottles (44 versus 32 cases; $P < 0.001$). Sonication obviously improved microbial detection, compared to synovial fluid cultures, which was particularly evident in CoNS recovery (21 and 14 cases, respectively; $P = 0.016$). Two cases of polymicrobial infections were detected only in sonicate fluid cultures in Bactec bottles. *S. aureus* in one case and *Acinetobacter baumannii* in another case were detected secondary to *Staphylococcus epidermidis*. Four cases of *Candida* were detected in both sonicate fluid cultures and synovial fluid cultures. No pathogens were detected in either synovial fluid cultures or sonicate fluid cultures in 6 cases of PJI, but two of those cases showed caseous necrosis and tuberculous nodules in the histopathological appearance of the periprosthetic tissue samples.

Among AF cases, 8 strains were detected in sonicate fluid cultures in Bactec bottles, i.e., 7 cases of CoNS and 1 case of *Burkholderia pickettii*. In one case, *S. epidermidis* was isolated from both sonicate fluid and synovial fluid cultures. Due to low colony counts, normal CRP levels, and an absence of any inflammatory histological evidence in this case, the strain was considered a contaminant.

Comparison of diagnostic techniques. Table 3 shows a comparison of diagnostic techniques in PJI and aseptic failure cases. Sonicate fluid cultures with Bactec bottles identified pathogens in cases of PJI (44 cases [88%]) more often than did synovial fluid cultures with Bactec bottles (32 cases [64%]; $P < 0.001$), while the former recovered contaminants in cases of AF (8 cases [13%]) more often than did the latter (1 case [2%]; $P = 0.016$). The sensitivity of sonicate fluid cultures with Bactec bottles (88% [95% confidence interval [CI], 76% to 95%]) was superior to that of synovial fluid cultures with Bactec bottles (64% [95% CI, 49% to

TABLE 1 Characteristics of 110 study patients with AF or PJI

Characteristic	Aseptic failure ($n = 60$)	Prosthetic joint infection ($n = 50$)	P
Age (median [range]) (yr)	65 (30–89)	64 (32–84)	0.860
Male (no. [%])	19 (32)	19 (38)	0.125
Type of prosthesis (no. [%])			
Knee ($n = 22$)	2 (3)	20 (40)	<0.001
Hip ($n = 88$)	58 (97)	30 (60)	<0.001
Type of revision surgery (no. [%])			
Debridement with prosthesis retention	0	5 (10)	<0.001
One-stage exchange	59 (98)	14 (28)	<0.001
Two-stage exchange	1 (2)	31 (62)	<0.001
Presence of clinical signs of infection (no. [%])			
Visible purulence	0	26 (52)	<0.001
Presence of sinus tract	0	12 (24)	<0.001
Preoperative laboratory findings			
Blood leukocyte count of >10×10^9 cells/liter (no. [%])	3 (5)	5 (10)	0.177
Blood leukocyte count (median [range]) ($\times 10^9$ cells/liter)	6.5 (3.5–12.3)	6.9 (3.6–15.1)	0.268
Erythrocyte sedimentation rate of >30 mm/h (no. [%])	11 (18)	41 (82)	<0.001
Erythrocyte sedimentation rate (median [range]) (mm/h)	24 (2–98)	63 (12–120)	<0.001
Serum C-reactive protein level of >10 mg/liter (no. [%])	4 (7)	32 (64)	<0.001
Serum C-reactive protein level (median [range]) (mg/liter)	4.17 (0.17–66.5)	34.3 (0.29–270)	<0.001
Inflammation in histopathology (no. [%])	0	43 (86)	<0.001
Received previous antibiotics (no. [%])	0	21 (42)	<0.001

77%]; $P = 0.009$). The specificity of sonicate fluid cultures with Bactec bottles (87% [95% CI, 75% to 94%]) was lower than that of synovial fluid cultures with Bactec bottles (98% [95% CI, 91% to 100%]; $P = 0.032$).

In the PJI group, 21 patients (42%) had received antimicrobial therapy within 14 days before sample collection (Table 4). Positive synovial fluid cultures with Bactec bottles were obtained in only 11 cases (52%), and positive sonicate fluid cultures with Bactec bottles were obtained in 17 cases (81%). Among cases with sonicate fluid cultures with Bactec bottles, pathogens were detected in 27 cases without previous antimicrobial therapy and in 17 cases with previous antibiotic usage (93% [27/29 cases] versus 81% [17/21 cases]; $P = 0.223$). Sonicate fluid cultures with Bactec bottles were more sensitive than synovial fluid cultures with Bactec bottles whether antimicrobial agents were used within 14 days before surgery (81% versus 52%; $P = 0.031$) or not (93% versus 72%; $P = 0.031$).

TABLE 2 Microbiological findings in 50 PJI cases according to type of diagnostic method

Finding	Synovial fluid	Sonicate fluid
Type of infection (no. [%])		
Monomicrobial	32 (64)	42 (84)
Polymicrobial	0 (0)	2[a] (4)
No. (%) of cases with no pathogen detected	18 (36)	6 (12)
No. (%) of detected pathogens	32	44
S. aureus	8 (25)	10 (23)
CoNS	14 (44)	21 (48)
Enterococci spp.	1 (3)	2 (5)
Escherichia coli	2 (6)	2 (5)
Enterobacter cloacae	1 (3)	1 (2)
Pseudomonas aeruginosa	1 (3)	1 (2)
Pseudomonas putida	1 (3)	1 (2)
Acinetobacter baumannii	0 (0)	1 (2)
Corynebacterium sp.	0 (0)	1 (2)
Candida spp.	4 (13)	4 (9)

[a] *S. aureus* plus *S. epidermidis* ($n = 1$) and *Acinetobacter baumannii* plus *S. epidermidis* ($n = 1$).

DISCUSSION

Accurate identification of pathogens is a key step for successful treatment of PJI, which remains a challenge for clinicians and laboratory workers. Routine cultures of periprosthetic tissue and synovial fluid are the most frequently utilized tests for PJI diagnosis (2, 13). Unfortunately, both methods suffer low sensitivity, likely due to biofilms forming on the prosthetic surfaces (15, 16). Tunney et al. first used bath sonication to dislodge adherent bacteria from explanted prosthetic hips (17); subsequently, Trampuz et al. investigated the effectiveness of this method for the diagnosis of hip and knee prosthetic joint infections (7). Compared to standard tissue culture, sonicate fluid culture improved the sensitivity from 61% to 79%. In the next decade, the feasibility and effectiveness of sonicate fluid culture were widely proved and each detailed step of the test was modified and improved, but the sensitivity of the method was never above 83%, which means that at least 17% of pathogens could not be isolated (18). Semiautomated methods such as the BD Bactec system have always been used for the incubation of specimens from various sterile sites (11). Recently, in-

TABLE 3 Comparison of diagnostic effectiveness for PJI between sonicate fluid cultures with Bactec bottles and synovial fluid cultures with Bactec bottles

Culture type	PJI (no. [%]) ($n = 50$)	AF (no. [%]) ($n = 60$)	Sensitivity (% [95% CI])[a]	Specificity (% [95% CI])	PPV (% [95% CI])	NPV (% [95% CI])
Sonicate fluid	44 (88)	8 (13)	88 (76–95)	87 (75–94)	85 (72–93)	90 (79–96)
Synovial fluid	32 (64)	1 (2)	64 (49–77)	98 (91–100)	97 (84–100)	77 (66–86)
P	<0.001	0.016	0.009	0.032	0.144	0.068

[a] CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value.

cubations of synovial fluid or periprosthetic tissue specimens in blood culture flasks showed higher sensitivity than routine cultures with blood agar plates (9, 19). To improve the sensitivity of sonicate fluid cultures, we devised a new method combining bath sonication of explanted prosthetic components and incubation in the BD Bactec system.

To set up a sonication protocol, it is important to control the frequency and duration of ultrasound treatment. Monsen et al. (10) recommended sonicating the explanted prosthetic components at a frequency of 40 kHz for 7 min, to balance eradiation of Gram-negative organisms and multiplication of Gram-positive organisms, which was followed by most other studies (3, 5, 20). In the present study, explanted prosthetic components were sonicated at a frequency of 28 kHz for 10 min. We considered this a reasonable protocol because there were no cases with positive synovial fluid culture results and negative results for sonicated samples, even for Gram-negative organisms. It is controversial whether prolonged incubations would increase bacteriological findings deemed clinically significant (3, 11, 21). In the majority of studies, the incubation periods were on the order of 5 days for aerobic cultures and 7 days for anaerobic cultures (6, 7, 22). Prolongation of periprosthetic tissue cultures identified an additional 26.4% of bacterial isolates, especially for aerobic, Gram-positive rods and small-colony variants of *Escherichia coli* (21, 23, 24). Recently, automated Bactec blood culture bottle methods have been used to identify the pathogens of PJI with periprosthetic tissue samples, and the majority of clinically significant organisms grow within 3 days even for *Propionibacterium* spp. Prolonged microbiological culture for 2 weeks is unnecessary when Bactec culture bottles are used (11). Therefore, the incubation period for Bactec blood culture bottles was 5 days after sonication of explanted prostheses in our protocol.

Previous antimicrobial treatment before surgery would increase culture-negative PJI cases. From the classic study by Trampuz et al., preoperative administration of antimicrobial agents can affect the sensitivity of sonicate fluid cultures, even with discontinuation of antimicrobial therapy 2 weeks before surgery (7). Portillo et al. (3) found that, despite the use of sonication, there remained 24% culture-negative PJI cases in the group with previous antibiotic treatment, which suggested that it is important, whenever possible, to avoid administering antibiotics before collecting samples. Therefore, Portillo et al. recommended that new diagnostic techniques should be investigated in order to reduce the incidence of culture-negative PJI (3). In the present study, pathogen recovery rates were higher in sonicate fluid cultures with Bactec bottles even in cases with antibiotic treatment before surgery (81%), and no statistical difference was detected for cases with versus without previous antimicrobial therapy. BD Bactec Plus Aerobic/F bottles have added antibiotic-inactivating resins to absorb most antibiotic in samples, for detection of bacterial pathogens in patients treated with antimicrobials (25). As a result, this may be a suitable culture method especially for PJI cases with antibiotic treatment before surgery.

TABLE 4 Effect of preoperative antimicrobial therapy on culture results for 50 patients with PJI

Culture type	No. (%) of cases with positive culture		
	Previous antibiotics ($n = 21$)	No previous antibiotics ($n = 29$)	P^b
Sonicate fluid	17 (81)	27 (93)	0.223
Synovial fluid	11 (52)	21 (72)	0.232
P^a	0.031	0.031	

[a] *P* value for comparison of positivity rates for the two culture methods between cases with previous antibiotics and cases without previous antibiotics.
[b] *P* value for comparison of positivity rates for the same culture method in cases with previous antibiotics and cases without previous antibiotics.

Direct inoculation of synovial fluid from PJI cases in blood culture vials was recommended by some authors (19, 26). In our study, however, the sensitivity of synovial fluid cultures with Bactec bottles was only 64%. In the study by Font-Vizcarra et al., cultures of synovial fluid samples in blood culture flasks exhibited greater sensitivity (90%) than cultures with swab samples (68%) or periprosthetic tissues (82%) (19). Possible reasons for the difference may be much more acute PJI in the cases reported by Font-Vizcarra et al. (19) and less previous antimicrobial treatment before surgery. In our cases, with much more chronic PJI and more previous antimicrobial treatment, cultures of sonicate fluid with Bactec bottles showed greater effectiveness than direct inoculation of synovial fluid samples in Bactec bottles.

To our knowledge, ours is the first study involving recovery of pathogens from PJI cases with sonicate fluid cultures in Bactec bottles. This has better sensitivity than synovial fluid cultures with Bactec bottles, but there are still several limitations to this study. First, although our method is useful for detection of fungal infections, it has no effectiveness for recovery of some organisms such as *Mycobacterium tuberculosis*, which need special culture media and are proved by histopathological evidence. Second, among patients with aseptic failure, 8 sonicate fluid cultures with Bactec bottles were positive and most of the isolates (7/8 cases) were CoNS. This indicated that additional use of Bactec bottles after sonication may increase the rates of isolation of CoNS from contamination or colonization, which is not involved in the pathogenesis of aseptic failure. Third, it might be better to perform some comparisons with the results of sonicate fluid cultures or conventional cultures of periprosthetic tissues, which could provide more information to evaluate the accuracy of such a culture method. Finally, the microbiological criteria retained as part of the PJI def-

inition when the sensitivity and specificity of this method were analyzed might have introduced a circularity.

In conclusion, sonication of explanted prostheses followed by incubation of the resulting sonicate fluid in Bactec bottles detected many more pathogens than did synovial fluid cultures with Bactec bottles. This may be a suitable culture method, especially for PJI cases with antibiotic treatment before surgery. Staphylococci (especially coagulase-negative staphylococci) were the predominant pathogens, but the significance of CoNS isolated from sonicate fluid cultures with Bactec bottles without other evidence of infection needs further evaluation, to classify the organisms properly as contaminants or pathogens.

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