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Sonication versus Tissue Sampling for Diagnosis of Prosthetic Joint and Other Orthopedic Device-Related Infections

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ABSTRACT Current guidelines recommend collection of multiple tissue samples for diagnosis of prosthetic joint infections (PJI). Sonication of explanted devices has been proposed as a potentially simpler alternative; however, reported microbiological yield varies. We evaluated sonication for diagnosis of PJI and other orthopedic device-related infections (DRI) at the Oxford Bone Infection Unit between October 2012 and August 2016. We compared the performance of paired tissue and sonication cultures against a "gold standard" of published clinical and composite clinical and microbiological definitions of infection. We analyzed explanted devices and a median of five tissue specimens from 505 procedures. Among clinically infected cases the sensitivity of tissue and sonication culture was 69% (95% confidence interval, 63 to 75) and 57% (50 to 63), respectively ($P < 0.0001$). Tissue culture was more sensitive than sonication for both PJI and other DRI, irrespective of the infection definition used. Tissue culture yield was higher for all subgroups except less virulent infections, among which tissue and sonication culture yield were similar. The combined sensitivity of tissue and sonication culture was 76% (70 to 81) and increased with the number of tissue specimens obtained. Tissue culture specificity was 97% (94 to 99), compared with 94% (90 to 97) for sonication ($P = 0.052$) and 93% (89 to 96) for the two methods combined. Tissue culture is more sensitive and may be more specific than sonication for diagnosis of orthopedic DRI in our setting. Variable methodology and case mix may explain reported differences between centers in the relative yield of tissue and sonication culture. Culture yield was highest for both methods combined.

KEYWORDS Prosthetic joint infection, accuracy, culture, diagnosis, orthopedic device-related infection, sensitivity, sonication, specificity

As more people benefit from arthroplasty surgery, the burden of orthopedic device-related infections (DRI) has grown. Chronic orthopedic infections significantly impact quality of life, and costs of orthopedic implant revision and infection management are projected to rise steeply. An estimated $1.6 billion will be spent in the United States alone over the next 5 years (1, 2).

Bacterial production of organized extracellular matrix (biofilm) presents particular challenges in the diagnosis and management of orthopedic device-related infections and associated osteomyelitis (3, 4). Successful management requires thorough excision of necrotic tissue with or without device removal or exchange, coupled with careful

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attention to soft tissue and bone reconstruction, accurate microbiological diagnosis, and targeted antimicrobial treatment (4, 5).

Collection of multiple intraoperative deep tissue samples has previously been shown to optimize diagnostic yield and enable assessment of whether cutaneous bacteria of low virulence are likely pathogens or contaminants in individual cases (6). However, a significant proportion of patients with clinical and histopathologic features of orthopedic DRI yield no positive bacterial cultures from operative specimens despite adequate tissue sampling and laboratory processing, and preoperative antibiotic use further decreases culture yield (5, 7).

Sonication of explanted devices may be used to separate adherent bacterial colonies in biofilm and might improve microbiological diagnostic yield (8). Some studies suggest sonication is more sensitive than tissue sample culture, but results vary between centers (see Table S1 in the supplemental material). Furthermore, although culture sensitivity increases with multiple tissue samples (6), few studies have routinely compared sonication with the 4 to 6 tissue samples recommended (6, 9) or used automated liquid culture methods that have been shown to optimize tissue culture sensitivity (10, 11).

We prospectively compared the performance of sonication and tissue sample culture for diagnosis of orthopedic DRI in a large cohort of patients managed at the Oxford Bone Infection Unit.

MATERIALS AND METHODS

Participants and setting. The Oxford Bone Infection Unit provides specialist multidisciplinary management of complicated bone and joint infections. It provides secondary level care locally and serves as a UK national referral center. Long established local protocols for the diagnosis of prosthetic joint and other orthopedic device-related infections include meticulous tissue sampling for microbiology and histology at the time of device removal (6).

In October 2012 we undertook a service improvement project to implement and locally evaluate sonication for diagnosis of orthopedic DRI. Surgeons were invited to submit explanted orthopedic devices for sonication. Results of both tissue and sonication culture were made available to the clinical team managing the patient, and clinical details were obtained for analysis from the patient notes. The project received institutional approval as a service improvement audit.

Sample processing. Specimen sampling and processing were performed as previously described (6, 11, 12). Antibiotics were withheld prior to surgery unless the risk of uncontrolled sepsis was considered high. Surgical antibiotic prophylaxis was delayed until after tissue sampling. Multiple tissue samples were obtained for culture and histology, using separate instruments for each sample and avoiding contact with the skin to minimize cross-contamination. Following removal, each device was placed immediately into a sterile, single-use, airtight container (13).

All samples were processed in a class 2 safety cabinet using aseptic technique. Each tissue sample was disrupted by vortexing with sterile glass beads in sterile saline, and equal aliquots of the resulting suspension were inoculated into Bactec Plus Aerobic/F and Bactec Lytic/10 Anaerobic/F bottles (BD Diagnostics, Sparks, MD). Bactec bottles were incubated at 37°C for 10 days or until they flagged positive. Sterile saline was added to the sonication container to cover at least 90% of the device. The container was vortexed vigorously for 30 s, sonicated in an ultrasound bath for 1 min, and vortexed again for 30 s. Aliquots (0.1 ml) of the sonication fluid were inoculated onto blood and chocolate agar; aerobic and anaerobic plates were incubated at 37°C for 5 and 10 days, respectively.

Gram stains were performed on all isolates. Positive Bactec bottles were subcultured onto agar, and isolates were identified by matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry (Bruker UK Ltd.). Drug susceptibility testing was performed using the BD Phoenix system (BD Diagnostics) or manual EUCAST methods. In keeping with existing guidelines, positive tissue sample culture was defined as isolation of indistinguishable organisms (with identical drug susceptibility profiles) from 2 or more independent tissue specimens (9, 13, 14) and positive sonication culture as $\geq$50 CFU per milliliter (CFU/ml) (15–23).

Statistical analysis. Data were analyzed by surgical procedure such that if a patient had more than one procedure during the evaluation period, each procedure would contribute to the analysis. In the absence of a single "gold standard," we used a range of published definitions of prosthetic joint infection (PJI) (Table S2), including the clinical definition used in previous studies of sonication (presence of a sinus OR visible purulence OR positive histology for infection) (12–14, 24) and combined clinical and microbiological definitions from the Infectious Diseases Society of America (IDSA) (14) and the International Consensus Meeting on Periprosthetic Joint Infection (consensus definition) (9). While the clinical definition excludes microbiological results from the definition, the IDSA and consensus definitions include both clinical features and microbiological parameters in the definition of infection. Since leukocyte counts are not performed routinely on joint fluid in our unit, for the purposes of our analyses we modified the consensus definition of PJI to replace elevated synovial leukocyte and neutrophil counts with visible purulence. Finally, to mitigate incorporation bias in favor of tissue culture in the IDSA and

TABLE 1 Explanted specimens received for sonication

Surgical procedure	Specimen type	No. (%)
Prosthetic joint revision surgery	Entire joint prosthesis (±fixation devices)	224 (44)
Debridement, antibiotics, and implant retention procedure for PJI	Prosthesis components (±fixation devices)	134 (27)
Metalwork removal for infected fracture	Orthopedic fixation devices	111 (22)
Surgical debridement for orthopedic device-related infections[a]	Cement	26 (5)
	Bone	10 (2)
Total		505

[a]Including repeat debridement of prosthetic joint infections and infected fractures.

consensus definitions, we compared the performance of both methods against a "composite definition" of PJI requiring either the clinical case definition (12) to be met or a positive culture from either tissue or sonication. We applied both the clinical and the composite case definitions to analyses of other (nonprosthetic-joint) orthopedic DRI.

We calculated the sensitivity and specificity of both culture methods using these definitions, and used McNemar's χ^2 test to compare proportions among paired samples from the same procedure. We calculated the combined sensitivity of sonication and tissue sample culture, first using the independent sonication and tissue sample culture results (independent analysis) and then in a combined analysis including as positive those with identical organisms isolated from both a single tissue specimen and from sonication (analogous to two independent tissue specimens).

To model the effect of the number of specimens taken on the sensitivity of tissue sample culture we used a computer algorithm to randomly sample *n* specimens from each procedure, excluding procedures from which fewer than *n* specimens were collected, and calculated the sensitivity based on this sample. For each value of *n* we repeated this process 100 times to estimate the mean sensitivity for *n* specimens.

We carried out a blinded case notes review to explore the significance of positive cultures in cases with discordant sonication and tissue sample cultures that did not meet the clinical definition of infection. Case notes were redacted for patient identifiers and the source (tissue or sonication) of isolates. An infectious diseases specialist and an orthopedic specialist in musculoskeletal infections who had not been involved in the patient's care then reviewed the case notes to judge whether the case was infected. If agreement could not be reached, a third musculoskeletal infection specialist adjudicated the case. We then recalculated the sensitivity and specificity of each culture method against a revised clinical definition of infection incorporating infection assignments of discordant cases from the case notes review.

To explore the effect of other factors on culture sensitivity, we compared the yield of sonication and tissue sample culture among subgroups defined by clinical features, time from device implantation to explantation, antibiotic exposure prior to explantation, isolation of "more virulent" organisms, and mixed infections. For this analysis we defined more virulent organisms *a priori* as Gram-negative bacilli, *Staphylococcus aureus*, *Staphylococcus lugdunensis*, enterococci, beta-hemolytic streptococci, milleri group streptococci, *Streptococcus pneumoniae*, and *Candida* species and less virulent organisms as other Gram-positive organisms, including coagulase negative staphylococci, viridans group streptococci, *Bacillus* species, and mycobacteria. Mixed infection was defined as isolation of more than one pathogen species by either method. Independent associations with a positive culture result from either method were explored using multivariable logistic regression.

Finally, we investigated the effect of lowering the thresholds of positive tissue sample and sonication culture on the performance of each method. We first explored the number of additional cases that would have been identified using the lower sonication threshold of 10 CFU/ml used in some studies (Table S1). Recognizing the polyclonal nature of many infections (25), and in keeping with common clinical practice, we also relaxed the stringent definition of identical tissue culture isolates used in the main analysis to allow up to two differences in drug susceptibility profiles between "indistinguishable isolates" and recalculated the sensitivity of tissue culture using this definition. Using these revised definitions we then carried out a further blinded case notes review of discordant cases that did not meet the clinical definition of infection and recalculated and compared the sensitivity and specificity of each method against the clinical definition of infection after incorporating final infection assignments of discordant cases from this case notes review.

RESULTS

Between 1 October 2012 and 12 August 2016, specimens for sonication were obtained from 528 procedures. We excluded 23 (4%) because <2 tissue specimens were received for culture, leaving 505 procedures on 463 patients in the final analysis (Table 1). Anatomical locations of explanted devices are summarized in Table S3 and Table S4. A median of 5 tissue specimens was obtained per procedure (interquartile range [IQR], 4 to 5).

The median age of patients was 68 years (IQR, 57 to 76). A total of 265 (52%) were male. Date of device implantation was available for 440 (87%), among whom the median time from device implantation to explantation was 28 months (IQR, 8 to 92). A total of 246/505

(49%) met the clinical definition of infection, including 169 PJI. Table 2 shows the number of cases meeting each of the other definitions of infection.

Diagnostic accuracy. Tissue sample culture was found to be more sensitive than sonication when analyzed independently, with an overall sensitivity against the clinical definition of 69% (63 to 75), compared with 57% (50 to 63) for sonication ($P < 0.0001$ [Table 2]). In modeling the effect of varying the numbers of tissue specimens obtained, tissue culture sensitivity increased as the number of specimens included in the analysis increased (Fig. 1).

Tissue sample culture was consistently more sensitive among PJI cases, irrespective of the definition of infection used. Despite smaller numbers of non-PJI cases, tissue culture still demonstrated greater sensitivity against the clinical definition of infection, with a trend toward superior sensitivity against the composite endpoint (Table 2).

The combined sensitivity of tissue and sonication culture was higher than for either method alone and increased in line with tissue sample culture as the number of tissue specimens increased (Fig. 1). Combining the independent tissue and sonication culture results, the overall sensitivities were 74% (68 to 79) and 77% (71 to 81) against the clinical and composite definitions of infection, respectively.

In 11 cases that were classed as culture negative by both sonication and tissue culture alone, the same organism was cultured from both sonication and a single tissue specimen, but below the 50-CFU/ml threshold for sonication. In 4 of these cases that grew coagulase-negative staphylococci, drug susceptibility testing was not performed on the sonication isolate so it was not possible to confirm that the tissue and sonication isolates were identical. Inclusion of the remaining 7 cases with identical isolates from tissue and sonication culture as culture positive gave overall sensitivities for tissue and sonication combined of 76% (70 to 81) and 78% (73 to 83) against the clinical and composite definitions of infection, respectively (Table 3).

Against the clinical definition of infection, the specificities of tissue and sonication culture were 95% (91 to 97) and 93% (89 to 96), respectively ($P = 0.394$).

Culture discordant specimens. Sonication and tissue sample culture results were discordant in 76 cases (Table 2). Of these, 54/76 (71%) met the clinical definition of infection: 27 with a sinus, 29 with visible purulence, and 48 with histological evidence of infection. Clinical and microbiological characteristics of the remaining 22 (29%) cases that did not meet the clinical definition of infection are summarized in Table 4. Following blinded case notes review, 6/9 tissue-positive/sonication-negative cases and 0/13 tissue-negative/sonication-positive cases were judged to be infected (Table 4). After assigning these 6 cases to the clinical infection category and excluding 3 cases classified as "uncertain," the sensitivities of tissue and sonication culture were 70% (64 to 75) and 56% (49 to 62), respectively ($P < 0.001$), and 75% (69 to 80) for the two methods combined. Specificities were 97% (94 to 99) for tissue, 94% (90 to 97) for sonication ($P = 0.052$), and 93% (89 to 96) for the two methods combined. Of 11 tissue-negative/sonication-positive cases not treated and followed up for a median of 3 years, 9 (82%) had a good outcome, 1 patient died of pneumonia, and 1 patient had persistent pain of uncertain etiology.

Subgroup analyses. Table 5 compares the sensitivities and specificities of tissue and sonication culture stratified by clinical and microbiological characteristics. While smaller numbers limit power in some subgroups, the point estimates suggest that tissue sample culture was more sensitive than sonication in most subgroups. However, no difference in culture yield was observed among cases caused by less virulent organisms. Organism virulence was the only factor independently associated with culture yield in multivariate logistic regression models of tissue and sonication culture (Table 5). The presence of more virulent organisms was strongly associated with positive sample tissue culture (odds ratio [OR], 8.5; 95% confidence interval [CI], 1.7 to 42.9; $P = 0.01$) but not with positive sonication culture (OR, 0.7; 95% CI, 0.2 to 2.4, $P = 0.587$).

TABLE 2 Sensitivity of tissue culture and sonication for diagnosis of prosthetic joint and other orthopedic device-related infections

Reference standard (definition of infection)	Total no. of infected cases	No. of cases positive by each method				Sensitivity, % (95% CI)			*P* value (tissue vs. sonication)
		Sonication positive		Sonication negative					
		Tissue positive[a]	Tissue negative	Tissue positive	Tissue negative	Tissue or sonication positive	Tissue	Sonication	
PJI									
Clinical	169	96	9	25	39	77 (70–83)	72 (64–78)	62 (54–69)	0.006
Consensus	150	99	6	30	15	90 (84–94)	86 (79–91)	70 (62–77)	<0.001
IDSA	177	100	8	33	36	80 (73–85)	75 (68–81)	61 (53–68)	<0.001
Composite	182	99	14	30	39	79 (72–84)	71 (64–77)	62 (55–69)	0.016
Other orthopedic device-related infection									
Clinical	77	32	3	17	25	68 (56–78)	64 (52–74)	45 (34–57)	0.002
Composite	91	34	11	21	25	73 (62–81)	60 (50–71)	49 (39–60)	0.077
All device-related infections (PJI and non-PJI)									
Clinical	246	128	12	42	64	74 (68–79)	69 (63–75)	57 (50–63)	<0.001
Composite	273	133	25	51	64	77 (71–81)	67 (61–73)	58 (52–64)	0.003

[a]Sonication positive, sonication culture positive (≥50 CFU/ml). Tissue positive, tissue culture positive (i.e., indistinguishable organisms isolated from at least two tissue specimens).

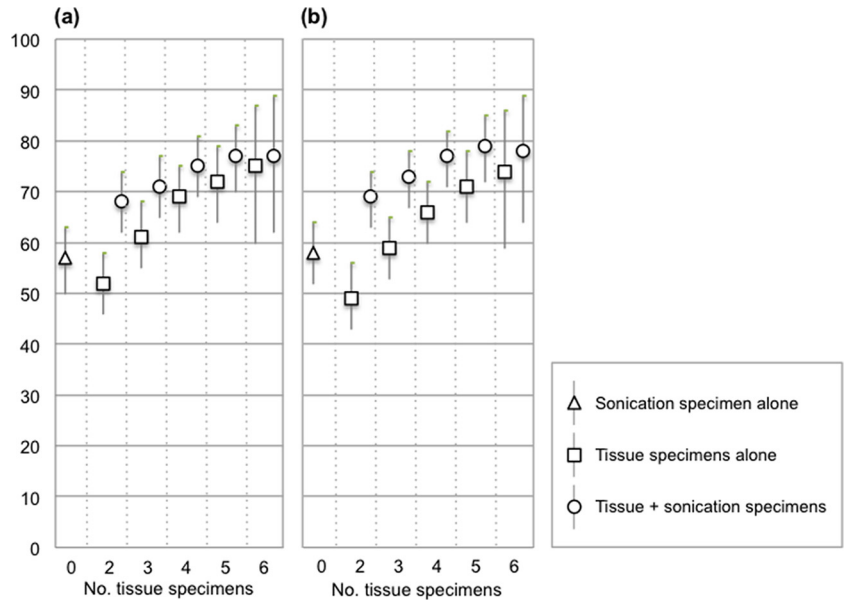

FIG 1 Sensitivity and 95% confidence intervals for sonication, increasing numbers of tissue samples, and sonication and tissue culture combined for clinical (a) and composite (b) definitions of infection. The effect of tissue sample number was modeled using a computer algorithm to randomly sample required number of specimens from the full set of specimens obtained in each case (see Materials and Methods).

Mixed infections. Tissue and/or sonication culture was positive for more than one bacterial species in 47/209 (22%) culture-positive cases. Of these mixed infections, 42 (89%) were positive by tissue sample culture and 18 (38%) by sonication ($P < 0.0001$ [Table S6]). The two methods shared at least one similar isolate in 35 (74%) cases of mixed infection, including 14 (30%) cases with identical isolates. Sonication identified mixed infection in 9 cases that were tissue culture negative; conversely, mixed infection was identified by tissue sample culture in 4 cases that were negative by sonication culture. In a further 3 cases of mixed infection by tissue sample culture, sonication yielded a completely different organism.

Sensitivity analyses. Reducing the sonication threshold to 10 CFU/ml would have classified an additional 78 cases as sonication culture positive, of which 41 (53%) met the clinical definition of infection. Of the 37 cases that did not meet the clinical definition of infection, 30 (81%) were not treated with antibiotics, had no evidence of infection during a median of 20 (IQR, 6 to 36) months of follow-up, and were judged

TABLE 3 Combined microbiological sensitivity of sonication and tissue culture

Reference standard definition of infection	Total no. of infected cases	Independent analysis[a]		Combined analysis[b]	
		No.	Sensitivity, % (95% CI)	No.	Sensitivity, % (95% CI)
PJI					
Clinical	169	130	77 (70–83)	133	79 (72–85)
Consensus	148	135	91 (85–95)	140	95 (90–98)
IDSA	179	138	77 (70–83)	143	80 (73–85)
Composite	184	143	78 (71–84)	148	80 (74–86)
Other orthopedic device-related infection					
Clinical	77	52	68 (56–78)	53	69 (57–79)
Composite	92	66	72 (61–81)	67	74 (64–83)
All device-related infections (PJI and non-PJI)					
Clinical	246	182	74 (68–79)	186	76 (70–81)
Composite	276	209	76 (70–81)	216	78 (73–83)

[a]Positive microbiology defined as positive sonication culture or identical isolates from ≥2 tissue specimens (positive tissue culture).
[b]Positive microbiology defined as positive sonication culture or identical isolates from ≥2 specimens of any type (tissue or sonication specimens).

TABLE 4 Clinical and microbiological characteristics of cases with discordant tissue and sonication culture results that did not meet the clinical definition of infection

Case no.	No. of mo since device implantation	Antibiotics in preceding 14 days	No. of tissue specimens	Sonication specimen	Sonication culture (CFU/ml)[e]	Tissue culture (no. specimens culture positive for each organism)[e]	Treated	Follow-up (mo)	Outcome	Diagnosis following blinded clinical review of case notes
Prosthetic joint infection (PJI) cases										
9	44	No	6	Knee components	*Propionibacterium acnes* (>100), CoNS[a] (>500)	*Bacillus* species (1)	Yes	85	Good	Not infected
70	5	Yes	5	Hip prosthesis		*Staphylococcus epidermidis* (4), *Enterococcus faecalis* (1)	Yes	2	Died of pneumonia	Infected (PJI)
77	37	No	4	Elbow components	CoNS (90)		No	25	Good	Not infected
171	52	No	4	Hip prosthesis	CoNS (>250)		No	33	Good	Not infected
241	13	No	5	Hip components	CoNS (>100)	*Moraxella osloensis* (1), *Micrococcus luteus* (1)	No	47	Good	Not infected (metallosis)
269	40	No	4	Knee prosthesis	*P. acnes* (>100)		No	44	Good[b]	Not infected (aseptic loosening)
334	0	No	5	Hip components		CoNS-1 (4), CoNS-2 (1)	Yes	6	Good	Probably not infected (loosening due to trauma)
397	124	No	4	Hip components		*S. epidermidis* (4)	Yes	19	Good	Probably not infected (aseptic loosening)
432	3	Yes	5	Hip prosthesis		*S. epidermidis* (3), *Bacillus* spp. (1)	Yes	18	Good	Infected (PJI)
491	193	Yes	4	Hip prosthesis		*S. aureus* (4)	Yes	5	Good	Infected (PJI)
Other orthopedic device-related (non-PJI) cases										
124	77	No	3	Bone knee	CoNS (>500)		No	49	Good	Not infected (mechanical failure)
179	248	No	3	Metalware spine	CoNS (<50)	CoNS (2), *S. epidermidis* (1)	No	23	Poor	Infected
197	8	No	5	Metalware tibia		*S. epidermidis* (2), *S. aureus* (1)	Yes	9	Good	Probably infected (infected nonunion)
210	17	No	5	Metalware tibia	CoNS (>100)		No	3	Died of pneumonia	Uncertain
262	4	No	4	Metalware	CoNS-1 (>100), CoNS-2 (>100)		No	45	Good	Not infected (aseptic nonunion)
339	7	No	4	Metalware femur	*P. acnes* (>250)		No	40	Poor (ongoing pain)	Uncertain
368	47	No	5	Metalware ankle/foot	CoNS[c] (<50)	CoNS (2), *S. epidermidis*-1 (2), *S. epidermidis*-2 (1)	Yes	24	Good	Probably not infected (aseptic nonunion)
376	1	No	4	Metalware ankle/foot	CoNS (>100)		No	36	Good	Not infected (aseptic nonunion)
429	3	No	3	Metalware femur	CoNS (>100), *Bacillus* spp. (>100)		No	5	Good	Not infected (mechanical failure; periprosthetic fracture)
489	9	No	4	Metalware ankle/foot	CoNS[d]		No	2	Good	Not infected
498	1	Yes	5	Metalware ankle/foot	CoNS (>250)		Yes	6	Good	Uncertain
535	22	No	5	Metalware ankle/foot		*S. epidermidis*-1 (4), *S. epidermidis*-2 (3), *S. epidermidis*-3 (1)	Yes	17	Good	Infected (infected nonunion and septic arthritis)

[a]CoNS, coagulase-negative staphylococci (not further identified).
[b]Later revision for metallosis, but no clinical or microbiological evidence of infection at repeat surgery.
[c]Sonication culture below the 50 CFU/ml threshold for positive culture.
[d]Number of CFU per milliliter not documented.
[e]Organism numerical suffixes denote different isolates of the same species; e.g., "CoNS-1" and "CoNS-2" denote two isolates of coagulase-negative staphylococci that are distinguished by their antimicrobial susceptibility profile.

TABLE 5 Effect of clinical and microbiological characteristics on tissue and sonication culture yield

Clinical definition of PJI and other orthopedic DRI clinical subgroup	No. of patients	No. sonication positive		No. sonication negative		Tissue sensitivity, % (95% CI)	Sonication sensitivity, % (95% CI)	*P* value
		Tissue positiveve	Tissue negative	Tissue positive	Tissue negative			
Time since device implantation								
<3 mo	41	18	2	9	12	66 (49–80)	49 (33–65)	0.035
3–24 mo	89	47	5	13	24	67 (57–77)	58 (47–69)	0.059
24 mo	106	59	4	19	24	74 (64–82)	59 (49–69)	0.002
Clinical features								
Sinus present	121	75	5	22	19	80 (72–87)	66 (57–74)	0.001
Visible purulence	139	91	6	23	19	82 (75–88)	70 (61–77)	0.002
No sinus or purulence	70	24	3	10	33	49 (36–61)	39 (27–51)	0.052
Antibiotic exposure prior to explantation[a]								
No recent antibiotics	104	56	3	18	27	71 (61–80)	57 (47–66)	0.001
Antibiotics within 14 days	109	50	8	19	32	63 (54–72)	53 (43–63)	0.034
Antibiotics within 7 days	77	32	5	13	27	58 (47–70)	48 (37–60)	0.059
Antibiotics within 3 days	61	27	4	11	19	62 (49–74)	51 (38–64)	0.071
Surgery while on antibiotics	49	23	4	9	13	65 (50–78)	55 (40–69)	0.166
Organism virulence								
More virulent organisms	132	93	4	35	0	97 (92–99)	73 (65–81)	<0.001
Less virulent organisms only	50	35	8	7	0	84 (71–93)	86 (73–94)	1.000

[a]Note categories of antibiotic exposure within 14 days are not mutually exclusive.

on blinded case notes review not to have been infected, 2 (5%) had other clinical evidence of infection, and the infection status of the remaining 5 (14%) who received antibiotic treatment was uncertain.

Relaxing the requirement for defining identical isolates to ≤2 differences in drug susceptibility profile would have classified an additional 21 cases as tissue sample culture positive, of which 18 (86%) met the clinical definition of infection. A further 2 (10%) cases were judged by blinded case notes review to be infected; the remaining case was not treated with antibiotics, had no evidence of infection during 21 months of follow-up and was judged not to have been infected.

Applying these lower thresholds, the sensitivities of tissue and sonication culture against the clinical definition of infection were 77% (71 to 82) and 72% (66 to 77), respectively ($P = 0.063$). The specificities were 96% (92 to 98) for tissue sample culture and 79% (74 to 84) for sonication ($P < 0.0001$).

DISCUSSION

This is the largest study to date comparing sonication with standard tissue sample culture for the diagnosis of orthopedic device-related infections. The results suggest that tissue sample culture is more sensitive than sonication for the microbiological diagnosis of both PJI and other orthopedic DRI in our setting.

There is wide variation between centers in the comparative yield of sonication and tissue samples (Table S1). Methodological differences between studies partly explain this heterogeneity; in particular, differences in the number of tissue specimens obtained for culture. Tissue sample culture yield depends critically on specimen number (6, 26), and current recommendations are to collect 4 to 6 specimens (6, 9). Most studies comparing sonication with tissue sample culture did not report the number of tissue specimens obtained (12, 18, 19, 22, 23, 27, 28), and many required a minimum of only 2 tissue specimens (12, 18, 29–31). Use of a suboptimal tissue sampling reference standard may therefore have overestimated the relative yield of sonication in some studies. Meticulous sampling to obtain a median of 5 tissue specimens per case in the current study allows a fairer comparison between tissue and sonication culture, and our analysis reinforces the importance of multiple tissue samples to maximize culture yield.

Differences in laboratory protocols may further affect culture yield. Automated liquid culture as used in this study improves tissue sample culture yield (10, 11) compared with that obtained with more traditional culture media. Variation was noted between studies in the quantitative threshold and culture duration used for sonication. We followed the sonication protocol and threshold for positivity most widely used in previous studies (15–23) but also explored the effect of lowering the sonication threshold (12).

Another reason for heterogeneity between published case series may be differences in the spectrum of cases included. PJI and other orthopedic DRI represent a spectrum of disease, from indolent infections with minimal soft tissue inflammation to more aggressive infections associated with marked soft tissue inflammation, purulence, and sinus formation (Fig. S2) (5, 12, 13).

We tested this hypothesis by comparing the performance of sonication among different clinical subgroups. While tissue sample culture yield was superior overall, sonication was equally sensitive among cases from which only less virulent organisms were isolated. This is consistent with our understanding of the pathophysiology of these more indolent infections, in which biofilm on the prosthesis predominates, with less soft tissue inflammation and lower bacterial density in tissue. At the other end of the spectrum, virulent organisms tend to cause more aggressive soft tissue inflammation with larger numbers of invading bacteria (Fig. S2). Interestingly, although other smaller studies have suggested that sonication may be superior to tissue culture in cases with recent antibiotic exposure and in mixed infections, this was not supported by our results (21, 23). One plausible explanation is that bone and soft tissue from which the tissue samples are taken is not sterilized due to the presence of biofilm and/or collections that persist despite antimicrobial therapy, just as biofilm persists on the surface of prostheses and other devices.

Strengths of this study include prospective inclusion of a large number and range of cases, suggesting that our findings should be generalizable to other settings. Rigorous collection of multiple specimens also ensured a robust tissue sample culture method for comparison. The stringent requirement for indistinguishable tissue culture isolates to have identical drug susceptibility profiles also maximized the specificity of tissue sample culture. We also explored the effect of relaxing this requirement in keeping with routine clinical practice and of reducing the sonication threshold.

In the absence of a perfect reference standard for PJI or other orthopedic DRI, we used a range of published reference definitions. Each of these definitions has limitations. Both the IDSA (13) and consensus (9) definitions, and the Musculoskeletal Infection Society definition (14) on which the later consensus definition is based, suffer from incorporation bias by including tissue culture in the definition. The clinical definition (12) circumvents this problem but does not capture the full spectrum of infection. To overcome these biases, we therefore also included a composite definition of PJI incorporating both tissue and sonication culture in addition to clinical features. While the effect size is slightly smaller using this composite reference standard, tissue culture still appears more sensitive than sonication.

The absence of a reliable clinical reference standard also makes the interpretation of culture specificity difficult, since the clinical significance of culture positive cases that do not meet the clinical definition is unclear. To address this, we conducted a detailed review of all cases with discordant culture results that did not meet the clinical definition of infection. Importantly, to avoid observer bias, reviewers were blinded to the source (tissue or sonication) of culture isolates. The results suggest that sonication may be less specific than tissue culture for clinically relevant infection, particularly when a lower sonication threshold is applied, and that in the absence of clinical or histological evidence of infection, a positive sonication culture may not indicate a need for antibiotic treatment if adequate tissue sampling has been performed and tissue sample cultures are negative. Whether some of these false-positive cases represent true infection that is effectively cured by device removal alone is unclear.

Our study has some limitations. In modeling the incremental yield of additional tissue cultures, we selected available specimens at random. This may not perfectly reflect surgical practice if surgeons are more likely to take samples from the highest yield sites first, based on their macroscopic appearance. This might therefore overestimate the incremental benefit of additional samples.

In the subgroup analyses, the definition of organism virulence is necessarily slightly arbitrary but nevertheless broadly correlates with clinical experience. Data on the time from device implantation also do not completely correlate with early versus late infection, since in many cases it includes a prolonged period from presentation with infection at another hospital, followed by referral and surgery at the Oxford Bone Infection Unit. This may explain why tissue sample culture is more sensitive even among the group $>$24 months from implantation, as this group does not only include late infections usually associated with less virulent organisms.

In summary, the results from this large prospective study suggest that tissue culture should remain the gold standard for microbiological diagnosis of PJI and other orthopedic DRI. The choice of method in a particular setting may, however, depend on existing infrastructure and available resources. If multiple tissue specimens cannot reliably be obtained, device sonication may provide a simpler though less sensitive alternative to tissue culture. Where rigorous tissue sampling can be established, culture methods should first be optimized (10, 11). Sonication may then have a complementary role in further optimizing microbiological yield.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/JCM.00688-18.

SUPPLEMENTAL FILE 1, PDF file, 0.8 MB.

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A.J.B., A.T., B.A.A., M.F., P.B., and R.N. designed and implemented the local evaluation of sonication to improve diagnosis of orthopedic device-related infection in Oxford. M.F., R.W., R.N., and S.O. were responsible for laboratory processing of the specimens. A.J.B., R.W., L.B., M.D., M.F., and M.W. collected and collated the data. A.J.B., A.T., B.A.A., B.K., D.S., M.A.M., M.S., and M.D. conducted the blinded review of discordant cases. A.J.B. performed the analyses and wrote the first draft of the manuscript with input from M.D. All authors contributed to preparation of the final manuscript. A.J.B. had full access to all the data in the study and final responsibility for the decision to submit for publication.

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