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ORIGINAL ARTICLE

Culturing periprosthetic tissue in blood culture bottles results in isolation of additional microorganisms

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Abstract

Despite low sensitivity, culture of periprosthetic tissue (PPT) specimens on agars and in broths has traditionally been used for the detection of causative microorganisms in patients suspected for prosthetic joint infection (PJI). The aim of this study was to evaluate the added diagnostic value of culturing PPT in blood culture bottles (BCB) over the conventional combination of standard agar and broth alone. This prospective cohort study was conducted over a 12-month period and included consecutive patients undergoing revision arthroplasty. Overall, 113 episodes from 90 subjects were studied; 45 subjects (50.0%) met the Infectious Diseases Society of America (IDSA) criteria for PJI, of whom the majority (75.6%) had an acute infection. Sensitivity and specificity of culture were assessed using IDSA criteria for PJI as gold standard. Although the increase in sensitivity from 84.44 (CI 70.54; 93.51) to 93.33% (81.73; 98.60) was not significant, added diagnostic value of culturing PPT in BCBs was demonstrated by the significantly higher number of detected pathogens in culture sets with BCBs compared to culture without BCBs (61 pathogens in conventional set versus 89 when BCBs were included for 57 PJI episodes, $P = < 0.0001$). In 17 (29.8%) episodes, microorganisms were cultured from BCBs only, and in 9 (52.9%) of these episodes, virulent pathogens were found. This study demonstrates that PPT culture in BCBs leads to isolation of additional microorganisms, both virulent and low-virulent, which were not cultured with use of agars and broths alone. Isolation of additional causative microorganisms has serious consequences for the treatment strategy in PJI.

Keywords Periprosthetic tissue · Blood culture · Prosthetic joint infection

Introduction

Culture of periprosthetic tissue (PPT) specimens on solid agars and in broths is routinely used in most medical microbiology laboratories to detect pathogens involved in prosthetic joint infections (PJI). Recent studies demonstrate that additional inoculation of PPT specimens in blood culture bottles (BCBs) may be more sensitive than conventional culture using agars and broth only, whilst maintaining specificity [1, 2]. Moreover, the use of BCBs may imply a reduced time to detection [1, 2].

Various microorganisms are acknowledged to play a role in PJI. Low-virulence microorganisms are known to cause low-grade chronic infections [3]. Traditionally, a relatively long incubation time in broths is assumed to be necessary to detect slowly growing, low-virulence pathogens such as *Cutibacterium acnes*. This microorganism that was formerly known as *Propionibacterium acnes* has shown to be particularly relevant in prosthetic joint infections of the shoulder [4, 5]. An extended incubation time does not necessarily result in additional PJI diagnoses, and in contrast, it may also increase the number of contaminants [6]. It is a common practice to subculture broths at the end of the incubation time or when visual growth appears. The former option means an

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unnecessary long time to pathogen detection whilst the latter option may implicate certain subjectivity since it is rather arbitrary whether or not to consider a broth cloudy. Blood culture systems on the other hand give a clear signal when growth is detected avoiding the subjective judgement of the microbiological analyst. Whether culture in BCBs ultimately improves patient care by an improved detection rate of pathogens is still subject of debate. To answer this question for our setting, a 1-year clinical validation study was conducted wherein the added diagnostic value of culturing PPTs in BCBs over conventional culture with agars and broths was evaluated. Further, the diagnostic value of an alternative set consisting of solid agars and BCBs, without broths, was investigated.

Materials and methods

Study population

The study population included consecutive patients undergoing revision arthroplasty surgery or debridement and implant retention (DAIR) for either presumed aseptic failure or suspected or proven septic failure of a knee or hip prosthesis. The study was conducted at the Elisabeth-TweeSteden Hospital, Tilburg, the Netherlands, from May 2017 until April 2018. The local Institutional Review Board ("Medisch Ethische Toetsingscommissie Brabant") approved the study protocol (NW2017-28).

Orthopaedic surgeons were instructed to take six PPTs during arthroplasty, as it is a common use based on the recommendation by Atkins et al. [7]. PPTs were excluded in case they consisted of bone instead of soft tissue because of limitations of the homogenisation machine. Additionally, cases were excluded if less than two specimens per arthroplasty were available for culture. In patients undergoing two-stage exchange or repetitive DAIR for management of PJI, samples from all procedures were tested with the new method (described in the "Microbiological methods" section). Results obtained with solid agars and broths were reported as usual. Until the first scheduled interim analysis, 6 months after the start of the study, results of BCBs were blinded; after superiority of BCBs over the conventional method was shown, further results were unblinded and used to determine optimal treatment strategies.

Microbiological methods

Periprosthetic fluid and tissue specimens were collected perioperatively at the judgement of the treating orthopaedic surgeon. Specimens were individually placed into sterile 30-mL screw-top vials for immediate transportation to the clinical microbiology laboratory. Subsequently, samples were homogenised using a Seward Stomacher 80 Biomaster (Seward brand, FermionX Ltd., Worthing, UK) operated on "high" in 5-mL brain heart infusion broth (BHI, Oxoid Ltd., Basingstoke, UK) for 1 min and subsequently inoculated on different media. First, 0.1 mL of homogenised substance was inoculated onto sheep blood, chocolate, MacConkey and Wilkins-Chalgren anaerobic agars (Oxoid Ltd., Basingstoke, UK) and incubated at 35 ± 1 °C for 4 days. Sheep blood, chocolate and MacConkey agars were incubated in 5% CO_2; Wilkins-Chalgren agar was incubated anaerobically. Second, 0.2 mL of the homogenised substance was inoculated into thioglycolate broth (Brewer, Oxoid Ltd., Basingstoke, UK), and 0.2 mL was inoculated into brain heart infusion broth (BHI, Oxoid Ltd., Basingstoke, UK). Both broths were incubated at 35 ± 1 °C for a maximum of 14 days. Broths (aliquots of 0.1 mL) were subcultured for 4 days at 35 ± 1 °C on Columbia colistin nalidixic acid (CNA) agar (5% CO_2), MacConkey agar and Wilkins-Chalgren agar (Oxoid Ltd., Basingstoke, UK) if visually cloudy or the end of the 14-day incubation period. When broths were subcultured before the end of the incubation period but subsequently no growth occurred, broths were again subcultured at the end of the 14-day incubation period. Third, 1 mL of the homogenised PPT substance was inoculated into a BCB containing BD Bactec^{tm} Plus Aerobic/F medium and a BCB containing Bactec Plus Anaerobic/F medium (BD Diagnostics, Sparks, USA). Two milliliters of fastidious organism supplement (FOS, BD Diagnostics, Sparks, USA) was added according to the instruction of the manufacturer. BCBs were placed in a Bactec FX-series instrument (BD Diagnostics, Sparks, USA). In keeping with prior published methods, BCBs were incubated for 7 days [1]. Bottles were subcultured only when the Bactec instrument flagged positive. These BCBs were subcultured on sheep blood, chocolate and Wilkins-Chalgren agars and incubated for 4 days as described above for broths. If synovial fluid specimens were obtained in the diagnostic flow before arthroplasty, an amount of 0.1 mL was inoculated on sheep blood, chocolate, MacConkey and Wilkins-Chalgren agars and incubated as described above for broths. Microorganisms were identified with the use of a Bruker Biotyper MALDI-TOF MS (Bruker, Bremen, Germany).

Definitions of PJI

In keeping with prior published methods, PJI diagnosis was made according to proposed definitions. We analysed sensitivity and specificity by using three different prevailing criteria sets: (1) Infectious Disease Society of America (IDSA), (2) Musculoskeletal Infection Society (MSIS) and (3) International consensus (IC) [8–10]. When applying the IDSA criteria, one supportive criterion was required for the definition PJI. Results for all media (agars, broths and BCBs) were included in the gold standard. Growth of low-virulent microorganisms was considered relevant (i.e. pathogenic) if

identical microorganisms with similar antibiograms were isolated from agar(s), broth(s) and/or BCB(s) from two or more PPTs. Growth of a virulent microorganism was considered relevant when isolated from agar(s), broth(s) and/or BCB(s) from one or more PPTs. The following cultured microorganisms were considered virulent: *Staphylococcus aureus*, *S. lugdunensis*, *S. schleiferi*, beta-haemolytic Streptococci, *Streptococcus anginosus* group, *S. bovis* group, *S. pneumoniae* and aerobic Gram-negative bacilli. Other cultured microorganisms such as other coagulase-negative staphylococci, enterococci and viridans streptococci were regarded as low-virulent. CRP, ESR and histology results were, if available, obtained from patient records pre-operatively from 3 weeks before until the day of surgery. Based on previous publications, an ESR of greater than 30 mm/h and a CRP of higher than 10 mg/L would represent elevated levels [9]. CRP levels below the cut-off of 10 mg/L were reported by the clinical chemistry laboratory as "< 10", a value of 10 was then used in our study. A synovial leukocyte count above 3000 cells/μL represented elevated levels, and for neutrophil percentages herein, a cut-off of 80% was applied [9].

Statistical analysis

Descriptive statistics were based on percentages and frequencies for categorical variables and means and standard deviations (SDs) or medians and interquartile ranges (IQRs) for continuous variables. For categorical data, proportions were compared using Fisher's exact test, and continuous variables were compared using the Mann-Whitney *U* test.

Three media sets were defined: (1) the conventional set consisting of agars and broths, (2) a complete set consisting of the conventional set plus BCBs and (3) an alternative set containing agars and BCBs.

First, culture results obtained with the three media sets were assessed for individual patients. These culture results were compared to the "gold standard reference" PJI or non-PJI according to IDSA, MSIS and IC criteria. Of note, culture results from all media were included in this reference. Then, sensitivity and specificity estimates for the three media sets were compared using McNemar's test for paired proportions. For this primary evaluation of the diagnostic performance, only samples from the first included arthroplasty per patient were analysed.

For the subsequent analyses evaluating microbiological culture results, all (repetitive) arthroplasty procedures, further referred to as episodes, were evaluated. Second, the number of pathogens cultured with the three media sets was analysed in the IDSA criteria–positive episodes. Numbers of pathogens isolated were compared using the Wilcoxon signed rank test. Third, positive episodes for which pathogens were found in broths and/or BCBs only were evaluated for IDSA criteria.

Results

Patient characteristics

During the study period, 120 revision arthroplasty or DAIR procedures were performed; of these, seven episodes were excluded from analysis because sample processing protocols were not followed correctly. Since 17 patients underwent one or more repetitive surgeries, the study cohort consisted of 113 arthroplasty episodes involving 90 unique patients. Table 1 shows the distribution of baseline characteristics stratified by meeting the IDSA criteria for PJI or not. A total of 45 patients (50.0%) met the criteria for PJI as defined by the IDSA. The majority (75.6%) of PJI patients presented with a duration of symptoms less than 6 weeks, implying acute infections. Of note, 16 PJI subjects (35.6%) had received antibiotics in 4 weeks prior to surgery, compared to one (2.2%) of subjects without PJI ($P = 0.001$). The median number of obtained PPTs was 6, and this was similar for the two groups.

Diagnostic performance

In Table 2, the performance of the study tests as expressed by sensitivity and specificity is shown. Culture results for 90 patients were included. When applying the IDSA criteria, 100% specificity was inferred since all patients from whom a single virulent microorganism was cultured were recognised as cases even when no other characteristics included in this gold standard were present. For the MSIS and IC on the other hand, these patients were regarded as having false-positive cultures.

With the assumption of the IDSA criteria as the composite gold standard, clinical sensitivity of culture was not significantly increased upon addition of BCBs. Sensitivity increased from 84.44 (CI 70.54; 93.51) to 93.33% (CI 81.73; 98.60), respectively, when BCBs were added to the conventional culture set consisting of solid agars and broths ($P = 0.125$). Additionally, sensitivity increased to 88.89% (CI 75.95; 96.29), when BCBs were added to the conventional culture set consisting of solid agars and broths were left out ($P = 0.688$). Results were comparable when the MSIS or IC criteria were used as gold standard (data not shown).

Microbiological culture results

The distribution of cultured pathogens in PJI cases according to the IDSA criteria is shown in Table 3. As can be expected from a cohort of patients in which the majority has an acute infection, a large part of isolated microorganisms consisted of so-called virulent microorganisms, such as *S. aureus* and Gram-negative bacilli. In 22 (38.6%) of all culture positive episodes, more than one pathogen was isolated.

Table 1 Distribution of characteristics among patients stratified according to whether or not IDSA criteria for PJI were met

	Meeting IDSA criteria		
	No ($n = 45$) (50.0%)	Yes ($n = 45$) (50.0%)	P value
Variable			
Median age, years (IQR)	69.0 (65.0–77.5)	74.0 (67.5–81.5)	0.047
Median implant age, days (IQR)	1779.0 (567.5–4964.5)	32.0 (14.5–833.5)	< 0.0001
Median days of complaints prior to surgery (IQR)[a]	205.0 (114.5–535.5)	12.0 (4.0–39.0)	< 0.0001
Acute infection (%)	N.A.	34 (75.6)	N.A.
Median number of periprosthetic tissue cultures per patient (range)	6 (2–7)	6 (3–8)	0.206
Prosthetic joint type in situ from which periprosthetic tissue was sampled, no (%)			
Hip	27 (60.0)	33 (73.3)	0.180
Knee	17 (37.8)	11 (24.4)	0.172
None (re-implantation in two-stage procedure)	1 (2.2)	1 (2.2)	1.000
Surgery performed, no (%)			
DAIR	2 (4.4)	30 (66.7)	< 0.0001
2-stage exchange	8 (17.8)	4 (8.9)	0.215
Girdlestone	3 (6.7)	3 (6.7)	1.000
Revision arthroplasty (including 1-stage exchange)	32 (71.1)	8 (17.8)	< 0.0001
Presenting complaints, no (%)			
None	4 (8.9)	0	0.117
Pain	26 (57.8)	13 (28.9)	0.010
Erythema along incision	0	8 (17.8)	0.003
Swelling	0	6 (13.3)	0.013
Drainage from the incision	0	24 (53.3)	< 0.0001
Sinus tract	0	7 (15.6)	0.006
Fever	0	3 (6.7)	0.121
Loosening	14 (31.1)	2 (4.4)	0.002
Fracture	1 (2.2)	1 (2.2)	1.000
Other	14 (31.1)	7 (15.6)	0.134
Patients who received antibiotics in 4 weeks prior to surgery, no (%)	1 (2.2)	16 (35.6)	< 0.0001
Median pre-operative CRP, mg/L (IQR)[b]	29.0 (10.0–96.0)	23.0 (10.0–100.25)	0.962
Median pre-operative ESR, mm/h (IQR)[c]	38.0 (13.25–93.75)	43.0 (30.5–69.0)	0.530
Pre-operative synovial fluid			
Patients from whom an aspirate was performed, no (%)	8 (17.8)	11 (24.4)	0.303
Of whom had a positive synovial fluid culture, no (%)	0	8 (72.7)	0.006
Median synovial fluid neutrophil percentage (IQR)[d]	2.0 (1.0–4.0)	73.0 (62.5–42,450.0)	0.003
Of which median synovial fluid neutrophil percentage (IQR)[d]	40.0 (34.0–56.0)	96.0 (84.0–96.5)	0.010
Histology, no (%)			
Patients from whom histology was performed, no (%)	16 (35.6)	19 (42.2)	0.333
Of whom had inflammation, no (%)	0	8 (42.1)	0.003

[a] Median days of complaints prior to surgery applicable to 39 patients not meeting IDSA criteria and to 45 cases meeting IDSA criteria. [b] CRP was determined in seven non-PJI and in 36 PJI patients; [c] ESR was determined in 16 non-PJI and in 33 PJI patients. [d] Available for seven non-PJI and five PJI patients

With the assumption of the IDSA criteria as the composite gold standard, the number of isolated pathogens was compared for the three different media sets for all 57 episodes in the IDSA criteria-positive patients. Despite the use of solid agars, broths and BCBs, PPTs gave culture-negative results for four episodes (7.0%). Table 4 illustrates the different numbers of cultured pathogens for each media set. Set 1 (agars and broths) resulted in a lower number of isolated pathogens than set 2 (agars, broths and BCBs; 61 versus 89 pathogens, respectively, $P = < 0.0001$). Set 3 (agars and BCBs) resulted in a higher number of pathogens isolated than set 1 (83 versus 61, respectively, $P = 0.001$). Furthermore, negative cultures were found more frequently in set 1 ($N = 12$) when compared to set 2 ($N = 4$, $P = 0.010$) or set 3 ($N = 8$, $P = 0.025$).

Table 2 Sensitivity and specificity of PPT culture for 45 PJI cases and 45 non-PJI cases according to the IDSA criteria

Culture set	Sensitivity (95% CI)	Specificity (95% CI)
Solid agars and broths	84.44 (70.54–93.51)	100.00 (92.13–100.00)
Solid agars and BCBs	88.89 (75.95–96.29)	100.00 (92.13–100.00)
Solid agars, broths and BCBs	93.33 (81.73–98.60)	100.00 (92.13–100.00)

For 17 episodes, pathogens were found with BCBs alone, and in nine of these occasions, virulent microorganisms were involved (Table 5). For six episodes, microorganisms considered as causative agent were found with broths only; in five of these episodes, only low-virulent bacteria were isolated (Table 6). Of note, from some episodes, multiple organisms were cultured. Isolation of pathogens with BCBs or broths only could not be explained by recent prior use of antibiotic therapy (data not shown). In addition, recent prior use of antibiotics was not associated with negative microbiological cultures (data not shown).

Discussion

In this clinical validation study, the value of BCBs in addition to solid agars plus broths was assessed. PPT culture in BCBs resulted in isolation of additional microorganisms, which were not cultured with the use of agars and broths alone. Less negative cultures were found for the IDSA criteria–positive patients with the use of BCBs in addition to conventional culture sets of agars and broths. These findings can imply important modifications in treatment strategy. Despite these beneficial findings, culture in BCBs did not significantly increase the number of PJI diagnoses according to the gold standard.

Table 3 Distribution of 89 pathogens isolated from 57 episodes (fulfilling IDSA criteria for PJI)

Microorganism	Percentage	Involved episodes (*N*)
Staphylococcus aureus	17.98	16
Staphylococcus lugdunensis	3.37	3
Other coagulase-negative staphylococci	24.72	22
Corynebacterium spp.	12.36	11
Dermabacter hominis	2.25	2
Streptococcus dysgalactiae	1.12	1
Streptococcus oralis	2.25	2
Enterococcus faecalis	8.99	8
Enterococcus faecium	1.12	1
Cutibacterium acnes	1.12	1
Escherichia coli	7.87	7
Enterobacter cloacae	3.37	3
Enterobacter aerogenes	1.12	1
Morganella morganii	1.12	1
Klebsiella pneumoniae	1.12	1
Pseudomonas aeruginosa	10.1	9

In contrast to the recent report by Peel et al., in our study, most patients had an acute periprosthetic infection [1]. We found that a large part of isolated microorganisms consisted of virulent microorganisms such as *S. aureus* and Gram-negative bacilli. The total percentage of Gram-negative bacilli (24.7%) is relatively high in comparison to earlier data [1, 4]. Our findings corroborate the study by Peel et al. from which an increase in sensitivity from 73 to 79% can be calculated when BCBs were added to the culture set. This calculation is based on the fact that PPTs gave culture-negative results for 24 PJI subjects, and PPT culture in BCBs resulted in eight additional microbiological diagnoses of PJI of 117 IDSA criteria–positive cases on 369 subjects in total [1].

The used cut-off to discriminate between acute and chronic infection was a duration of complaints of 6 weeks, since this is a common daily practice in our study centre. In literature, a cut-off duration of symptoms of less than 3 weeks up to 3 months, or an implant age of less than 30 days, has been suggested [5, 8]. In our hospital, DAIR is still considered when the duration of complaints is between 3 and 8 weeks. However, this neither influences the process of PPT sampling during surgery nor affects culture results.

The IDSA, MSIS and IC criteria are a set of clinical-pathological criteria and not diagnostic tests. Nevertheless, these definitions are often used as gold standard references. Although the definitions are rather similar, there are certain differences. The IDSA criteria do not include inflammatory markers and synovial fluid cell counts. However, the IDSA criteria provide other features that may support the diagnosis of PJI, such as growth of a virulent organism from a single culture or the finding of acute inflammation by PPT histopathology. Because in a significant proportion of all subjects there was no clinical suspicion for infection, CRP and ESR were available for only 43 (47.8%) and 49 (54.4%) out of 90 subjects, respectively. Additionally, histology and synovial fluid analysis were performed in only 35 (38.9%) and 19 (21.1%) subjects, respectively. Nevertheless, in general, we observed agreement in terms of classification of cases. Four cases with conflicting classifications either fulfilled the IDSA criteria (and not MSIS and IC) due to a virulent microorganism from one PPT ($N = 2$) or due to inflammation upon histology without the isolation of pathogens ($N = 2$).

Table 4 Number of pathogens obtained per culture set (57 episodes included)

	Number of different microorganisms cultured per episode						Total number of microorganisms by method:	P<0.0001	P=0.001	P=0.014
Culture set	**0**	**1**	**2**	**3**	**4**	**5**				
Agar + Broth	12	34	8	1	2	0	61	↕	↕	
Agar + Broth + BCB	4	32	10	8	2	1	89			↕
Agar + BCB	8	28	11	7	3	0	83			

In 17 of 57 PJI episodes, pathogens were found with BCBs only. In nine of those episodes, virulent microorganisms were cultured. Unexpectedly, these were microorganisms generally not considered difficult to culture: *S. aureus*, *S. lugdunensis*, *Escherichia coli* and *Pseudomonas aeruginosa*. We suppose that this can only partly be explained by the relative high inoculum in BCBs in comparison to broths and agars (five times and ten times higher, respectively); Peel and colleagues inoculated both broths and BCBs with 1 mL and still they observed a substantial increase in sensitivity in favour of BCBs [1]. Isolation of pathogens with BCBs only could not be explained by antibiotic therapy in the preceding 4 weeks before surgery. Recent prior use of antibiotics was not associated with negative microbiological cultures, neither for the conventional culture set (solid agars and broths) nor for the complete set including BCBs. Only four out of the 17 episodes would have been misclassified as non-PJI when BCBs were not part of the culture set, since in the other 13 episodes, either causative agents were cultured with agars and/or broths as well, or non-microbiological criteria had led to PJI-classification.

Inoculation of BCBs with homogenised tissue is considered off-label use. In four cases, the lipophilic *Corynebacterium tuberculostearicum* was the presumed pathogen isolated from solid agars and broths whilst in BCBs, no growth was present. One of the patients was already discharged from the hospital before culture results were complete. For two out of four patients, antibiotic therapy was not covering *Corynebacterium tuberculostearicum*, and until now, no signs of recurrent infection are observed. Still, *Corynebacterium* spp. may certainly be of relevance in PJI [11]. In one case, broth yielded growth of *E. coli* whilst agar plates and BCBs lacked growth. The clinical relevance of this finding remains unknown since the isolate was obtained from BHI only (and not thioglycolate) and only grew in broth that was subcultured after 14 days of incubation. Furthermore, no clinical signs of infection were observed.

It has been suggested that a 14-day incubation time may be necessary to detect *C. acnes* when using broths [6, 12]. In our study, involving only patients undergoing hip or knee arthroplasty, *C. acnes* was found in one case (with a hip prosthesis), whilst *S. aureus* was also cultured from the same episode. We cultured *C. acnes* from five out of six PPTs on solid Wilkins-Chalgren agar, broths and BCBs. In this case, broths were not of additional value in the detection of *C. acnes.* How this would relate to patients with shoulder PJI, and thus a higher a priori chance for infection with this microorganism, is unknown [13].

Results of this study should not be extrapolated to just any clinical setting since the performance of this new culture method is dependent on the characteristics of the study population. In the present setting, clinical presentation ranged from presumed aseptic failure to chronic infection to acute septic failure.

Table 5 Pathogens cultured with BCBs only (out of 57 episodes)

Low-virulent microorganisms[a]	Involved episodes (*N*)
Staphylococcus haemolyticus	3
Staphylococcus caprae	1
Staphylococcus epidermidis	7
Enterococcus faecalis	2
Corynebacterium pseudodiphtheriticum	1
Virulent microorganisms	
Staphylococcus lugdunensis	2
Staphylococcus aureus	4
Escherichia coli	2
Pseudomonas aeruginosa	2

[a] For low-virulent pathogens, identical microorganisms were isolated from two or more PPTs

Table 6 Pathogens cultured with broths only (out of 57 episodes)

Low-virulent microorganisms[a]	Involved episodes (*N*)
Corynebacterium amycolatum	1
Corynebacterium jeikeium	1
Corynebacterium tuberculostearicum	4
Virulent microorganisms	
Escherichia coli	1

[a] For low-virulent pathogens, identical microorganisms were isolated from two or more PPTs

Our study has several limitations. First, sampling of PPTs to be cultured depends on the judgement of the treating orthopaedic surgeon, which inevitably induces sample bias. Second and in line with previous publications, culture results were incorporated in the gold standard reference. This inevitably implies an incorporation bias and therefore overestimation of sensitivity and specificity [1, 6, 12, 14, 15]. An alternative approach may be the Bayesian latent class modelling. This was recently applied by Peel et al. [1] and assumes that no gold standard exists. In the current study, we chose to use the prevailing IDSA criteria as gold standard. In the daily routine practice in the multidisciplinary counselling between orthopaedic surgeon and clinical microbiologist, these gold standard criteria for PJI are used to provide support in decision making. It may for instance differentiate between "culture-positive PJI", "culture-negative PJI" and "no PJI". The third limitation is the lack of a power calculation before start of the study. This was not performed since the prevalence of PJI in our hospital was unknown. Partly due to dependency of the applied gold standard, sensitivities and specificities reported in literature vary considerably between culture methods. Thus, it was decided to perform a clinical validation with a duration of 1 year instead. In retrospect, reasoning from the obtained sensitivities and specificities, our study may have been underpowered for assessment of diagnostic performance [16].

In our laboratory, sonication of explanted prostheses (parts) and subsequent culturing of sonication fluid are not part of the routine procedure. Although some research groups encourage culture of sonication fluid at all times, this procedure is time consuming and may be at the detriment of specificity [17]. Large studies show that the sensitivity of sonication fluid culture is not per se higher than standard culture [18, 19]. A recent study by Yan et al. could not find a significant difference in sensitivity of tissue culture in BCBs compared to sonicate fluid culture [20].

This study demonstrates that PPT culture in BCBs leads to isolation of additional microorganisms, both virulent and low-virulent, which were not cultured with the use of agars and broths alone. These findings are of clinical importance since optimal treatment strategy, including surgical strategy and antibiotic treatment, must be established on all causative agents to optimise the chance of cure.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical approval The study has been performed in accordance with the institutional research committee ("Medisch Ethische Toetsingscommissie Brabant"; study protocol NW2017-28) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Study formal consent was not required since culturing blood culture bottles was already a method implemented by others as a standard procedure in their laboratories, and blood culture bottles were added to the conventional set of solid agars and broths.

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