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Diagnosis of prosthetic joint infections using UMD-Universal Kit and the automated multiplex-PCR Unyvero i60 ITI® cartridge system: a pilot study

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Abstract
Background Prosthetic joint infections (PJI) are associated with high morbidity and costs. Various efforts have been made to improve the diagnosis of PJI over the past years, but only few studies have assessed the diagnostic utility of nucleic acid amplification test (NAAT) techniques in this context. Here, we report our experience with a commercial 16S rRNA gene PCR and an automated multiplex-PCR cartridge system in identifying pathogens causing PJI.
Materials and methods A prospective single-centre study was performed including 54 patients with either septic or aseptic prosthetic joint replacement or surgical revision between February 2012 and April 2013. Conventional cultures of periprosthetic tissue samples were compared with the results of broad-range 16S rRNA gene real-time PCR (UMD-Universal Pathogen DNA Extraction and PCR Analysis, Molzym GmbH, Germany) and the multiplex-PCR Unyvero ITI® cartridge system (U-ITI; Curetis AG, Germany). Conventional culture and broad-range 16S rRNA gene real-time PCR were performed on all samples. U-ITI was used in a subgroup of 28 cases including all culture-positive cases. The agreement of the results from the methods was assessed.
Results Of 54 cases, seven were culture-positive. Broad-range 16S rRNA gene real-time PCR gave 6, U-ITI 3 concordant positive results. Of the 47 culture-negative samples, 46 were also negative by broad-range 16S rRNA gene real-time PCR resulting in a 96 % (52/54) agreement between 16S rRNA gene PCR and culture. Of the 21 culture-negative samples analysed with U-ITI, 20 gave negative results, including the single 16S rRNA gene PCR-positive/culture-negative specimen. The rate of agreement between U-ITI and culture results was 82 % (23/28).
Conclusion This pilot study gave no indication of superiority of the used NAATs over conventional culture methods for the microbiological diagnosis of PJI. Drawbacks are susceptibility to contamination in the case of 16S rRNA gene real-time PCR, labour-intensive DNA extraction and limited pathogen panel in the case of the multiplex cartridge PCR system. More prospective trials are needed to evaluate the diagnostic performance of NAATs and their impact on the clinical management of PJI.

Keywords Prosthetic joint infection · Orthopaedic surgical care · PCR · 16S rRNA gene real-time PCR

Abbreviations
CC Conventional culture

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NAAT Nucleic acid amplification test
PJI Prosthetic joint infection
U-ITI Automated multiplex-PCR Unyvero i60 ITI® cartridge system

Introduction

Over the past decade there has been a significant increase in the number of artificial joint replacements worldwide and especially in Germany. Prosthetic joint infections (PJI) represent a serious complication in the era of artificial body implants. PJI is a condition with high morbidity and costly economic side effects for endoprosthetic departments [1, 2]. The complex pathophysiology of PJI is closely related to the unique microbiological properties of biofilms, generated by microorganisms on artificial materials [3]. Biofilm-forming bacteria often remain in a dormant state complicating conventional diagnostic and therapeutic approaches [4]. Recently updated international guidelines on the management of PJI recommend the culture of 3–6 periprosthetic tissue samples as the diagnostic procedure of choice [5]. Various efforts have been made to improve the diagnostic tools for PJI over the past years. Culture samples obtained by sonication of removed artificial material were shown to be more sensitive than conventional tissue culture (78.5 vs. 60.8 %.), especially in patients who were exposed to anti-infective treatment 14 days before surgery (45 vs. 75 %)—the overall specificities were above 98 % [6, 7]. Only few studies have assessed the diagnostic utility of nucleic acid amplification test (NAAT) techniques in this context. The reported findings for different PJI-associated materials indicate a wide range of sensitivity (50–92 %) and specificity (65–94 %) [8–13]. Results from a recent study comparing 16S rRNA gene PCR and consecutive sequencing of sonication fluid with culture of synovial aspirates, tissue samples and sonication fluids reports that 16S rRNA gene PCR improved sensitivity [14]. An up-to-date large, prospective multicenter trial on 16S rRNA gene PCR applied to periprosthetic tissue samples showed a lack of sensitivity of this broad-spectrum molecular method (sensitivity 73.3 % and specificity 95.5 %) [8]. Almost no data are available on the role of multiplex-PCR assays for periprosthetic tissue samples. Using multiplex-PCR of sonication fluid, sensitivity (96 %) and specificity (100 %) for diagnosing PJI could be further improved and might help to differentiate between the clinical conditions of aseptic failure and PJI [15, 16]. However, the sophisticated infrastructure to obtain and process sonication fluids is not widely accessible, especially for endoprosthetic departments with external microbiological laboratories. To overcome these limitations, automated and easily operable multiplex-PCR systems are being developed, which provide rapid PCR results from periprosthetic tissue and bone samples regarding pathogen identification and genotypic resistance patterns. Here we report on our clinical experience comparing the automated multiplex-PCR Unyvero i60 ITI® cartridge (U-ITI) system with a 16S rRNA PCR assay and conventional culture in diagnosing PJI.

Materials and methods

Study design and ethical statement

This was a prospective study performed at Freiburg University Hospital, a large tertiary 1500-bed academic referral centre providing advanced specialized medical and surgical care including transplant centres and a level-1 trauma centre. The study protocol was approved by the ethics committee of the University of Freiburg (No. 60/12), and all patients gave written informed consent prior to any study-related procedures.

The recruited patients were either patients with septic or aseptic prosthetic joint exchanges or revisions admitted between February 2012 and April 2013. The protocol specified that at least three periprosthetic tissue samples (bone tissue and soft tissue) be taken during the surgical procedure for routine culture. Additional three periprosthetic tissue samples were removed simultaneously and stored at −80 °C in DNA-, RNA- and ATP free tubes (2 mL, biopur, Eppendorf AG, Hamburg)—without using any cryo-preservative agents. Synovial fluid was not processed. Frozen samples were thawed and split into portions for molecular assays. Clinical information was obtained from the medical records. A patient was regarded “clinically infected” based on the initial assessment (using medical history, clinical and radiological information) of the responsible orthopaedic surgeon prior to surgery.

Conventional culture microbiology

Tissue specimens were processed at the microbiology department on the basis of clinical routine protocols established within the QM-system of the laboratory (DIN EN ISO 15189 and DIN EN ISO 17025 certified) in accordance with “Microbiology Procedures Quality Standards (MiQ)” issued by the German Society for Hygiene and Microbiology. Briefly, tissue samples were minced and microscopically examined after Gram staining. Solid agar plates were inoculated for aerobic and anaerobic culture and brain heart infusion broth (Oxoid, Germany) as well as thioglycolate resazurin broth (Merck, Germany) for enrichment. All media were incubated for 14 days and inspected visually daily. Pathogen identification was done using MALDI-TOF MS (Bruker Daltonic GmbH, Germany). The diagnosis of

culture-positivity was based on the IDSA recommendations regarding the management of PJI [17]. In brief, two or more culture samples with the identical pathogen were considered as definitive positive. Growth of a highly pathogenic organism, e.g., *S. aureus* in a single sample was also reported as definitive positive. Growth of an organism known as common contaminant in a single tissue sample was evaluated as negative.

Broad-spectrum 16S rRNA gene real-time PCR

DNA extraction, broad-spectrum 16S rRNA gene real-time PCR and sequence analysis were performed with UMD Universal kit according to the instructions of the manufacturer (Molzym GmbH, Germany). Strict precautions were taken as advised by the manufacturer to avoid DNA contamination: only high-pure reagents were used. In brief a miliary portion of the tissue samples was processed. The manufacturer's protocol includes the lysis of human cells and the removal of human DNA prior to pathogen lysis and DNA purification. For each set of samples subjected to DNA extraction a negative control (DNA-free water) was processed. The broad-spectrum real-time PCR using SYBR Green was carried out in a Light Cycler 2.0 (Roche, Germany). The kit provides primer targeting conserved regions of the 16S rRNA genes of bacteria and fungi, mastermix, DNA-free water for negative controls, DNA for positive controls and internal control DNA. For each PCR run, negative and positive controls were included. Inhibition of the PCR was excluded by adding internal controls to each sample extract. A sample was considered PCR positive if the melting curve analysis showed a peak within the expected Tm range [17]. Amplicons from positive PCR reactions were purified with GFX PCR DNA Purification kit. Sequencing reactions were performed using the BigDye Terminator cycle sequencing kit DNA (version1.1; Applied Biosystem, Germany) according to the manufacturer's instructions. The 16S rDNA PCR products were sequenced using sequencing primers (SeqGN16, SeqGP16) supplied in the UMD Universal kit and analysed with a 3130 Genetic Analyzer (Applied Biosystem, Germany). Obtained sequences were compared with those in the GenBank database (http://www.ncbi.nlm.nih.gov/genbank/) using BLAST software (http://blast.ncbi.nlm.nih.gov). A sequence similarity level of ≥97 and ≥99 % was used as cutoff for genus and species identification, respectively. PCR-negative results were noted if melting curve analysis indicated the absence of bacterial DNA or sequencing analysis of poor mixed chromatograms did not generate sufficient sequence homology. The Ripseq mixed programme (www.ripseq.com, provided by Isentio, Bergen, Norway) was not used in this study. The pathogen identified by 16S rRNA gene PCR was assessed as reliable when at least two of the three samples investigated indicated the same pathogen-related sequence.

Multiplex PCR cartridge system

The Unyvero Implant and Tissue Infection cartridge application (U-ITI) is a semi-quantitative DNA test based on the parallel performance of eight multiplex PCR reactions and designed to detect up to 114 pathogen-associated nucleic acids and resistance markers in solid, fluid and highly viscous samples. Tissue samples were processed by Curetis AG with the i60 Unyvero assay according to the manufacturer's protocol. In brief, 27 mm^3 of the tissue was transferred into a Unyvero sample tube. Sample lysis comprised a 30-min protocol including mechanical, thermal, chemical and enzymatic sample treatment. After sample lysis, the sample was further processed in the Unyvero i60 cartridge. The cartridge is pre-loaded with reagents for DNA purification, PCR primers and probes for array hybridization. The U-ITI integrates and automates sample lysis, DNA purification, multiplex nucleic acid amplification by end-point-PCR using fluorescence-labelled primers in eight independent PCR chambers with individual detection array and qualitative amplicon detection by hybridization on a porous array membrane. PCR and array hybridization is performed with at least four probes per analyte. A series of images of the hybridization procedure over a specific temperature range is taken by a fluorescence camera. Results are derived from images processed by the Unyvero software, generating complete diagnostic information within 4.5 h. An internal control, a synthetic gene, without significant homology to known sequences, is co-processed in every PCR chamber to verify DNA purification, PCR and array hybridization. Analytical sensitivities regarding each microorganism and a complete list of all included pathogens and genetic resistance markers are disclosed in the manufacturer's manual. The result was reported as positive, when at least one of the analytes reached the threshold of positivity. The U-ITI was applied to 79 samples from 28 patient cases (23 cases with 3 samples, 5 cases with 2 samples).

Categorization of results

PCR-based results were compared to conventional culture (CC) results by defining five categories of agreement [18]. The results of molecular diagnostics were assessed as true positive if conventional culture was concordant positive (NAAT+/CC+). Culture-positivity and a negative molecular result were categorized as false negative (NAAT−/CC+). If the PCR-based method and culture did not yield a pathogen, the result was classified as concordant negative (NAAT−/CC−). Cases with culture-negative specimens but PCR-based positive results (NAAT+/CC−) were

Table 1 Descriptive information and overview

Decriptive information	
Total number of cases (*n*)	68
Cases inclusion (*n*)	54
Cases excluded (*n*)	14
Demographic information	
Male	23
Female	31
Average age in years (time of inclusion)	69.08
Clinically infected (PJI)	10
Hip prosthesis	21
Male	8
Female	13
Average age in years (time of inclusion)	68.29
Clinically infected (PJI)	3
Conventional culture positive	3
Conventional culture negative	0
Clinically no PJI	16
Conventional culture positive	1
Conventional culture negative	15
No clinical information on infection status at the time of inclusion	2
Conventional culture positive	0
Conventional culture negative	2
Knee prosthesis	33
Male	15
Female	18
Average age (time of inclusion)	69.76
Clinically infected (PJI)	7
Conventional culture positive	1
Conventional culture negative	6
Clinically no PJI	19
Conventional culture positive	1
Conventional culture negative	18
No clinical information on infection status at the time of inclusion	7
Conventional culture positive	1
Conventional culture negative	6

considered as conclusive positive or false positive based on an additional case by case review of the available clinical information by infectious disease physicians.

Results

Samples and clinical information were obtained from 68 patients of whom 14 were excluded because of insufficient clinical information or inappropriate pre-analytical tissue processing (Table 1). A total of 54 patients were included with either septic or aseptic prosthetic joint exchanges or revisions. Conventional culture detected the growth of possible pathogens in 16 patients. Considering the number of culture-positive samples per patient and the pathogenicity of the microorganism, seven patients were classified as culture positive (Table 2). The remainder of nine cases was interpreted as contamination of the conventional culture on the basis of the above mentioned criteria and finally assessed culture-negative (Table 1, 3). Four patients met the criteria for culture positivity regarding coagulase-negative staphylococci: two patients with *Staphylococcus lugdunensis* and one patient each with *Staphylococcus capitis* and *Staphylococcus epidermidis*, respectively. *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Enterococcus faecalis* were identified by culture and considered true positive in one patient each.

Broad-spectrum 16S rRNA gene real-time PCR was carried out for all samples of the 54 patients. Sequence analysis was successful with species identification in 30 cases (Table 2). Twenty-nine microorganisms identified by 16S rRNA gene sequencing in 23 cases were considered contaminants according to the above mentioned criteria and finally assessed 16S rRNA gene real-time PCR-negative (Table 3). Contaminating DNA originated from *Staphylococcus* spp. ($n = 13$), *Staphylococcus haemolyticus* ($n = 2$), *Staphylococcus epidermidis* ($n = 1$), *Staphylococcus hominis* ($n = 1$), *Pseudomonas pseudoalcaligenes* ($n = 6$), *Comamonas* spp. ($n = 5$) and *Pseudomonas* spp. ($n = 1$). Only seven cases met the criteria of the identical pathogen-related sequences in at least two samples. All internal and positive PCR controls yielded the expected results.

In 96 % of all cases (52/54) there was agreement between the results from 16S rRNA gene real-time PCR and conventional culture (Table 3). Six of the seven culture-positive cases gave concordant positive results in the 16S rRNA gene real-time PCR assay. Forty-six of 47 culture-negative samples were also estimated negative by 16S rRNA gene real-time PCR assay and categorized as concordant negative. The one 16S rRNA gene real-time PCR-positive/culture-negative sample (F-035) was interpreted as clinically true positive on the basis of prior culture results and antimicrobial treatment of the patient. In one culture-positive case with growth of *Staphylococcus capitis*, 16S rRNA gene real-time PCR could not identify the pathogen and was classified as false-negative.

The Unyvero i60 ITI cartridge (U-ITI) was applied to 79 samples of 28 patients (Table 3). In 23 cases three tissue samples and in five cases two samples were processed. In the five cases with only two tissue specimens investigated, the U-ITI result was concordant with culture findings. The overall degree of agreement between U-ITI and culture results in the 28 cases was 82 % (23/28). U-ITI identified 3 of 7 culture-positive cases revealing *S. aureus*, *P.*

Table 2 Disclosure of all included patient cases ($n = 54$)

Case number	Conventional culture result	16S-PCR result (positive: ≥2/3 samples positive for the same pathogen)	U-ITI result (positive: ≥1/3 samples positive for the same pathogen)	Contamination of 16S-PCR assay	Initially clinically infected (Y/N)	Exposure to antiinfective treatment within 14 days before sampling (Y/N)	Localisation	Gram staining
	Culture-positive cases							
F-003	*Pseudomonas aeruginosa*	*P. aeruginosa*	*P. aeruginosa*	–	y	y	Knee	Gram-Negative Rods
F-034	*Staphylococcus epidermidis*	*S. epidermidis*	Negative	–	y	n	Hip	Negative
F-041	*Staphylococcus capitis*	Negative	Negative	–	n	u	Hip	Negative
F-048	*Staphylococcus lugdunensis*	*S. lugdunensis*	Negative	–	u	n	Knee	Negative
F-051	*Enterococcus faecalis*	*E. faecalis*	*E. faecalis*	–	y	y	Hip	Negative
F-063	*Staphylococcus aureus*	*S. aureus*	*S. aureus*	–	y	u	Hip	Negative
F-065	*Staphylococcus lugdunensis*	*S. lugdunensis*	Negative	–	n	n	Knee	Negative
	Culture contamination							
F-019	*Propionibacterium* spp.	Negative	Negative	*Comamonas* spp.	n	n	Hip	Negative
F-039	*Staphylococcus hominis*	Negative	Negative	*Staphylococcus* spp.	n	n	Knee	Negative
F-061	*Staphylococcus epidermidis*	Negative	Negative	*P. pseudoalcaligenes Staphylococcus* spp.	n	n	Hip	Negative
F-068	*Micrococcus luteus*	Negative	Negative (*Micrococcus luteus* not within U-ITI panel)	*P. pseudoalcaligenes*	n	n	Hip	Negative
F-011	*Micrococcus luteus, Staphylococcus hominis, Staphylococcus epidermidis*	Negative	Negative	–	n	u	Hip	Negative
F-025	*Staphylococcus epidermidis, Streptococcus mitis*	Negative	Negative	–	n	n	Hip	Negative
F-028	*Streptococcus equorum*	Negative	Negative	–	u	n	Knee	Negative
F-047	*Streptococcus equorum, Propionibacterium acnes*	Negative	Negative (*Propionibacterium acnes* not included in U-ITI Panel)	–	n	n	Knee	Negative

Table 2 continued

Case number	Conventional culture result	16S-PCR result (positive: ≥2/3 samples positive for the same pathogen)	U-ITI result (positive: ≥1/3 samples positive for the same pathogen)	Contamination of 16S-PCR assay	Initially clinically infected (Y/N)	Exposure to antiinfective treatment within 14 days before sampling (Y/N)	Localisation	Gram staining
F-054	*Staphylococcus epidermidis, Staphylococcus pasteuri, Staphylococcus aureus* (Growth of each organism with only one colony forming unit in one tissue sample; the corresponding enrichment broth remained sterile)	Negative	Negative	–	y	y	Knee	Negative
	Culture negative cases							
F-007	Negative	Negative	nd	*S. epidermidis*	n	n	Knee	Negative
F-012	Negative	Negative	Negative	*Staphylococcus* spp.	n	y	Hip	Negative
F-015	Negative	Negative	nd	*Comamonas* spp.	u	n	Knee	Negative
F-021	Negative	Negative	nd	*Comamonas* spp. *S. hominis*	n	u	Knee	Negative
F-022	Negative	Negative	Negative	*Comamonas* spp. *S. haemolyticus*	u	y	Knee	Negative
F-024	Negative	Negative	nd	*Staphylococcus* spp.	n	n	Hip	Negative
F-026	Negative	Negative	Negative	*Staphylococcus* spp.	y	y	Knee	Negative
F-031	Negative	Negative	Negative	*Staphylococcus* spp.	n	y	Knee	Negative
F-032	Negative	Negative	nd	*Staphylococcus* spp.	n	u	Hip	Negative
F-037	Negative	Negative	Negative	*P. pseudoalcaligenes*	n	y	Hip	Negative
F-040	Negative	Negative	nd	*Staphylococcus* spp.		n	Hip	Negative
F-046	Negative	Negative	nd	*P. pseudoalcaligenes Comamonas* spp. *S. haemolyticus*	n	n	Knee	Negative
F-053	Negative	Negative	nd	*Staphylococcus* spp.	u	n	Knee	Negative
F-055	Negative	Negative	nd	*Staphylococcus* spp.	n	n	Hip	Negative
F-057	Negative	Negative	nd	*Staphylococcus* spp. *Pseudomonas* spp.	n	n	Knee	Negative
F-058	Negative	Negative	nd	*Staphylococcus* spp.	n	n	Knee	Negative
F-062	Negative	Negative	nd	*Staphylococcus* spp.	n	n	Knee	Negative
F-064	Negative	Negative	nd	*P. pseudoalcaligenes*	n	n	Knee	Negative
F-066	Negative	Negative	Negative	*P. pseudoalcaligenes*	y	n	Knee	Negative
F-005	Negative	Negative	Negative	–	n	n	Knee	Negative
F-006	Negative	Negative	nd	–	n	u	Knee	Negative

Table 2 continued

Case number	Conventional culture result	16S-PCR result (positive: $\geq$2/3 samples positive for the same pathogen)	U-ITI result (positive: $\geq$1/3 samples positive for the same pathogen)	Contamination of 16S-PCR assay	Initially clinically infected (Y/N)	Exposure to antiinfective treatment within 14 days before sampling (Y/N)	Localisation	Gram staining
F-008	Negative	Negative	Negative *Enterobacter cloacae* with 198 units at the threshold of positivity (250 units)	–	y	y	Knee	Negative
F-013	Negative	Negative	nd	–	n	n	Knee	Negative
F-014	Negative	Negative	nd	–	n	n	Hip	Negative
F-018	Negative	Negative	nd	–	n	n	Hip	Negative
F-030	Negative	Negative	nd	–	u	n	Knee	Negative
F-035	Negative	*S. aureus*	Negative	–	y	y	Knee	Negative
F-036	Negative	Negative	Negative	–	y	y	Knee	Negative
F-038	Negative	Negative	nd	–	u	n	Hip	Negative
F-042	Negative	Negative	nd	–	n	n	Knee	Negative
F-043	Negative	Negative	*Proteus* spp.	–	n	y	Hip	Negative
F-044	Negative	Negative	nd	–	n	n	Knee	Negative
F-045	Negative	Negative	nd	–	u	n	Knee	Negative
F-049	Negative	Negative	Negative	–	n	y	Knee	Negative
F-050	Negative	Negative	nd	–	n	n	Hip	Negative
F-052	Negative	Negative	nd	–	n	n	Knee	Negative
F-060	Negative	Negative	nd	–	u	n	Hip	Negative
F-067	Negative	Negative	nd	–	n	n	Knee	Negative

nd not done, *y* yes, *n* no, *u* uncertain

Table 3 Comparison of the different NAAT methods with conventional culture (CC)

	16S rRNA gene real-time PCR	U-ITI
Concordant positive (NAAT+/CC+)	6/7 (85.7 %)	3/7 (42.9 %)
Concordant negative (NAAT−/CC−)	46/47 (97.9 %)	20/21 (95.2 %)
Conclusive positive (NAAT+/CC−)	1/47	0/21
False positive (NAAT+/CC−)	0/47	1/21
False negative (NAAT−/CC+)	1/7	4/7
Overall agreement with CC	52/54 (96 %)	23/28 (82 %)
Total (*n*)	$n = 54$	$n = 28$

aeruginosa and *E. faecalis*. Results of the U-ITI molecular resistance typing function in comparison with conventional antimicrobial susceptibility testing are shown in Supplement Table 1. Of the 21 culture-negative samples analysed with U-ITI, 20 gave concordant negative results, including the single 16S rRNA gene real-time PCR-positive/culture-negative specimen (F-035). In this particular case *S. aureus* was detected by 16S rRNA gene real-time PCR. In one of 21 culture-negative samples U-ITI identified *Proteus spp*.-DNA (F-043). This finding was clinically assessed as false-positive, and furthermore it was not confirmed by broad-spectrum 16S rRNA gene real-time PCR.

Subgroup analysis

Patients were initially clinically categorized as “clinically infected” ($n = 10$), “no suspicion of infection” ($n = 35$) and “uncertain” ($n = 9$). Thirteen patients had been exposed to antimicrobial treatment within 14 days before sampling. Seven of these pretreated patients belonged to the subgroup labelled as “clinically infected”.

For “clinically infected” patients, culture confirmed the initial clinical suspicion only in 4 of 10 cases. 16S rRNA gene real-time PCR was concordant positive with culture findings in these 4 cases, and additionally identified *S. aureus* as likely causative agent in one culture-negative, antibiotic pretreated case (F-035). U-ITI was concordant positive in 3 of 4 culture-positive cases in this subgroup. U-ITI amplified *Enterobacter cloacae* DNA (198 units), just below the defined threshold of 250 units in one culture-negative, antibiotic pretreated patient in this subset. According to the manufacturer’s specifications this result was reported as negative. The clinical significance of this result remained unclear.

In three cases initially classified as “no suspicion of infection” ($n = 2$) or “uncertain” ($n = 1$) prosthetic joint infection was established by culture results. 16S rRNA gene real-time PCR confirmed the culture findings growing *S. lugdunensis* in two cases. U-ITI failed to verify these culture results because *S. lugdunensis* is not included in the detection panel. One case with positive cultures growing *S. capitis* could neither be verified by 16S rRNA gene real-time PCR nor by U-ITI.

Discussion

The current gold standard in diagnosing PJI is the conventional culture of periprosthetic tissue samples [19]. However, culture has limited sensitivity especially due to prior antimicrobial use, and its specificity is impaired by contaminating microorganisms of the skin which are often indistinguishable from true pathogens in this setting. Several technical advances have broadened the diagnostic options in recent years [6, 14–16]. Here, we report on a pilot study comparing conventional tissue culture with two PCR-based methods using prospectively collected tissue samples from patients with either septic or aseptic prosthetic joint replacements or revisions. Overall, we observed an agreement of the U-ITI cartridge results with culture results in about 82 % of all cases. 16S rRNA gene real-time PCR results were in line with the conventional culture results in about 96 % of the studied patient cases. These findings seem to be consistent with previously published data on NAAT techniques, indicating a relatively wide range of sensitivity and specificity [8–12]. NAATs may reasonably expand the diagnostic panel and contribute to a higher recovery rate especially after antimicrobial use.

However, each of the PCR-based methods has its specific drawbacks. Broad-range PCR is a very sensitive method and, thus, prone to false-positive results from contaminating DNA from different sources [20]. Attempts have been made to reduce the false-positive rates by introducing a detection threshold [21] but this may diminish the sensitivity of the test. In our study, 29 of 162 16S rRNA gene real-time PCR sequencing results, or 23 of 54 patient cases, were classified as contaminated especially by nonfermenters such as *Comamonas* spp. or *Pseudomonas* spp. and coagulase-negative staphylococci of the skin. As it is not advised by the manufacturer we did not define a detection limit for 16S rRNA gene real-time PCR. To overcome the contamination problem and as done in literature before [8], an organism identified by 16S rRNA gene real-time PCR was reported as true pathogen when at least two of the three samples investigated showed the same pathogen-related sequence. At this stage, the 16S rRNA gene real-time PCR assay is labour-intensive regarding its DNA

extraction procedure, which limits the introduction of this method into the clinical routine.

In contrast to the 16S rRNA gene real-time PCR assay, the U-ITI cartridge system is rapid and easy to operate. The test has an integrated thresholding algorithm. Amplification results reaching an arbitrary cut-off of 250 units are considered positive. Such a threshold can reduce false-positive results due to contamination and therefore might prevent unnecessary antimicrobial therapy. However, this approach poses the risk of false-negative results. In this pilot study we opted to report every single positive result as significant as recommended by the manufacturer. In one culture-negative, antibiotic pretreated and clinically infected case, the U-ITI cartridge amplified *Enterobacter cloacae* DNA at 198 units which is just below the defined threshold. The clinical significance of this result remained unclear. Compared to broad-range PCR assays, the U-ITI is limited to a fixed panel of pathogens. The U-ITI cartridge includes 94 primers (50 for bacterial or *Candida* species, and 42 resistance markers) and even though the test covers important gram-positive and gram-negative pathogens, relevant species such as *Staphylococcus lugdunensis* have not yet been included as a single primer in the latest version. In our study there were two culture-positive cases with *Staphylococcus lugdunensis*, a clinically important pathogen causing various infections including joint and prosthetic joint infection [22–24]. U-ITI might benefit from the inclusion of additional organisms into the panel.

Our pilot study has several limitations. First, the overall number and the number of culture-positive patients included is relatively low; therefore, the agreement rate of 82 % is mainly based on concordant negative results. Larger studies with higher number of cases are certainly desirable to determine sensitivity and specificity, but difficult to conduct and, therefore, even smaller sample size studies may give important insights. Second, our "first-experience" trial did not assess the influence of the NAAT results on the management of PJI. The only 4 U-ITI positive patient cases did not permit conclusions regarding the U-ITI molecular resistance typing function. We hypothesize that the automated U-ITI cartridge may facilitate the management of PJI, especially in countries with a high prevalence of multi-drug resistant organisms and may be appropriate to detect polymicrobial PJIs. UMD-Universal assay and the U-ITI cartridge are new complementary technologies in the diagnostic repertoire for PJI.

In conclusion, this pilot study gave no indication of superiority of the used NAAT over conventional culture methods for the diagnosis of PJI. Prospective and controlled trials will be necessary to evaluate the usefulness and the impact of NAATs on the clinical management of PJI.

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Conflict of interests JPB received research funding from Curetis AG, Holzgerlingen. AS recieved technical and financial support from Molzym GmbH, Bremen.

Ethics statement The study protocol was approved by the ethics committee of the University of Freiburg (No. 60/12), and all patients gave written informed consent prior to any study-related procedures.

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References

1. Sculco TP. The economic impact of infected total joint arthroplasty. Instr Course Lect. 1993;42:349–51.
2. Zimmerli W, Trampuz A, Ochsner PE. Prosthetic-joint infections. N Engl J Med. 2004;351:1645–54.
3. Li Y-H, Tian X. Quorum sensing and bacterial social interactions in biofilms. Sensors (Basel). 2012;12:2519–38.
4. Rodrigues LR, Bacterial Adhesion. Linke D, Goldman A, Editors. Dordrecht: Springer Netherlands; 2011;715:351–67.
5. Osmon DR, Berbari EF, Berendt AR, Lew D, Zimmerli W, Steckelberg JM, et al. Executive summary: diagnosis and management of prosthetic joint infection: clinical practice guidelines by the Infectious Diseases Society of America. Clin Infect Dis. 2013;56:1–10.
6. Trampuz A, Piper KE, Jacobson MJ, Hanssen AD, Unni KK, Osmon DR, et al. Sonication of removed hip and knee prostheses for diagnosis of infection. N Engl J Med. 2007;357:654–63.
7. Portillo ME, Salvadó M, Alier A, Martínez S, Sorli L, Horcajada JP, et al. Advantages of sonication fluid culture for the diagnosis of prosthetic joint infection. J Infect. 2014;69:35–41.
8. Bémer P, Plouzeau C, Tande D, Léger J, Giraudeau B, Valentin AS, et al. Evaluation of 16S rRNA gene PCR sensitivity and specificity for diagnosis of prosthetic joint infection: a prospective multicenter cross-sectional study. J Clin Microbiol. 2014;52:3583–9.
9. Vandercam B, Jeumont S, Cornu O, Yombi J-C, Lecouvet F, Lefèvre P, et al. Amplification-based DNA analysis in the diagnosis of prosthetic joint infection. J. Mol. Diagn. 2008;10:537–43.
10. Panousis K, Grigoris P, Butcher I, Rana B, Reilly JH, Hamblen DL. Poor predictive value of broad-range PCR for the detection of arthroplasty infection in 92 cases. Acta Orthop. 2005.
11. Lévy P-Y, Fenollar F. The role of molecular diagnostics in implant-associated bone and joint infection. Clin Microbiol Infect. 2012;18:1168–75.
12. Fenollar F, Roux V, Stein A, Drancourt M, Raoult D. Analysis of 525 samples to determine the usefulness of PCR amplification and sequencing of the 16S rRNA gene for diagnosis of bone and joint infections. J Clin Microbiol. 2006;44:1018–28.
13. Rice LB, Eckstein EC, DeVente J, Shlaes DM. Ceftazidime-resistant *Klebsiella pneumoniae* isolates recovered at the

Cleveland Department of Veterans Affairs Medical Center. Dis: Clin Infect; 1996.

14. Gomez E, Cazanave C, Cunningham SA, Greenwood-Quaintance KE, Steckelberg JM, Uhl JR, et al. Prosthetic joint infection diagnosis using broad-range PCR of biofilms dislodged from knee and hip arthroplasty surfaces using sonication. J Clin Microbiol. 2012;50:3501–8.
15. Portillo ME, Salvadó M, Sorli L, Alier A, Martínez S, Trampuz A, et al. Multiplex PCR of sonication fluid accurately differentiates between prosthetic joint infection and aseptic failure. J Infect. 2012;65:541–8.
16. Achermann Y, Vogt M, Leunig M, Wüst J, Trampuz A. Improved diagnosis of periprosthetic joint infection by multiplex PCR of sonication fluid from removed implants. J Clin Microbiol. 2010;48:1208–14.
17. Mühl H, Kochem A-J, Disqué C, Sakka SG. Activity and DNA contamination of commercial polymerase chain reaction reagents for the universal 16S rDNA real-time polymerase chain reaction detection of bacterial pathogens in blood. Diagn Microbiol Infect Dis. 2010;66:41–9.
18. Haag H, Locher F, Nolte O. Molecular diagnosis of microbial aetiologies using SepsiTest™ in the daily routine of a diagnostic laboratory. Diagn Microbiol Infect Dis. 2013;76:413–8.
19. Osmon DR, Berbari EF, Berendt AR, Lew D, Zimmerli W, Steckelberg JM, et al. Diagnosis and management of prosthetic joint infection: clinical practice guidelines by the Infectious Diseases Society of America. Clin Infect Dis. 2012;56:e1–25.
20. Clarke MT, Roberts CP, Lee PTH, Gray J, Keene GS, Rushton N. Polymerase chain reaction can detect bacterial DNA in aseptically loose total hip arthroplasties. Clin Orthop Relat Res. 2004;132–7
21. Tunney MM, Patrick S, Curran MD, Ramage G, Hanna D, Nixon JR, et al. Detection of prosthetic hip infection at revision arthroplasty by immunofluorescence microscopy and PCR amplification of the bacterial 16S rRNA gene. J Clin Microbiol. 1999;37:3281–90.
22. Bhumbra S, Mahboubi M, Blackwood RA. *Staphylococcus lugdunensis*: novel Organism causing Cochlear Implant Infection. Infect. Dis. Rep. 2014;6:5406.
23. Rose AM, Barnett J, Morris-Jones S, Marks DJB. *Staphylococcus lugdunensis* septic arthritis and epidural abscess in a patient with rheumatoid arthritis receiving anti-tumour necrosis factor therapy. Rheumatology (Oxford). 2014.
24. Inada N, Harada N, Nakashima M, Shoji J. Severe *Staphylococcus lugdunensis* keratitis. Infection. 2014.