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Comparison of PCR-Electrospray Ionization Mass Spectrometry with 16S rRNA PCR and Amplicon Sequencing for Detection of Bacteria in Excised Heart Valves

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Identification of the causative pathogen of infective endocarditis (IE) is crucial for adequate management and therapy. A broad-range PCR-electrospray ionization mass spectrometry (PCR-ESI-MS) technique was compared with broad-spectrum 16S rRNA PCR and amplicon sequencing (16S rRNA PCR) for the detection of bacterial pathogens in 40 heart valves obtained from 34 definite infective endocarditis patients according to the modified Duke criteria and six nonendocarditis patients. Concordance between the two molecular techniques was 98% for being positive or negative, 97% for concordant identification up to the genus level, and 77% for concordant identification up to the species level. Sensitivity for detecting the causative pathogen (up to the genus level) in excised heart valves was 88% for 16S rRNA PCR and 85% for PCR-ESI-MS; the specificity was 83% for both methods. The two molecular techniques were significantly more sensitive than valve culture (18%) and accurately identified bacteria in excised heart valves. In eight patients with culture-negative IE, the following results were obtained: concordant detection of *Coxiella burnetii* ($n = 2$), *Streptococcus gallolyticus* ($n = 1$), *Propionibacterium acnes* ($n = 1$), and viridans group streptococci ($n = 1$) by both molecular tests, detection of *P. acnes* by PCR-ESI-MS whereas the 16S rRNA PCR was negative ($n = 1$), and a false-negative result by both molecular techniques ($n = 2$). In one case of IE caused by viridans streptococci, PCR-ESI-MS was positive for *Enterococcus* spp. The advantages of PCR-ESI-MS compared to 16S rRNA PCR are its automated workflow and shorter turnaround times.

Diagnosis of infective endocarditis (IE) remains challenging. A multidisciplinary approach by microbiologists, infectiologists, surgeons, and cardiologists is needed for adequate management. Identification of the pathogen is crucial for selecting appropriate antimicrobial therapy (1–4). According to the modified Duke criteria, positive blood cultures (BCs) remain the cornerstone of the microbiological diagnosis of IE (4). Three sets of BCs, taken before starting antimicrobial therapy, detect 96% to 98% of bacteremia (5, 6). Unfortunately, BCs are negative in 2% to 31% of IE patients due to prior antimicrobial therapy or fastidious (*Brucella* spp., fungi) or intracellular (*Coxiella burnetii*, *Bartonella* spp., or *Tropheryma whipplei*) microorganisms, resulting in obscured diagnoses (7–9). During the last decade, molecular techniques performed directly on excised heart valves have emerged. Broad-range PCR, targeting the 16S rRNA gene, followed by subsequent sequencing of the amplicon proved to be superior to the culture of excised valves (VC) (8, 10–12). The sensitivity and specificity of 16S rRNA PCR for detecting the causative microorganism ranged from 61% to 90% and 97% to 100%, respectively, whereas the sensitivity and specificity of VC ranged from 23% to 31% and 67% to 87%, respectively (10–12). The use of different or no criteria for defining the definite microbiological cause of IE complicates the diagnostic comparison of different tests.

In 2005, an innovative technology, combining broad-range PCR with electrospray ionization-time of flight mass spectrometry (PCR-ESI-MS) amplicon analysis was introduced (13). After a broad-range PCR, ESI charges amplicons and moves them into a MS that sensitively and accurately measures the mass/charge (m/z) ratio of negatively charged oligonucleotide ions. The composition of masses, corresponding to different charge states, appears as a peak distribution or mass spectrum. After deconvolution of this spectrum, the mass of the unfragmented amplicon is calculated and software algorithmically predicts its base composition. These calculations rely on the masses of the four nucleic acids (adenine, guanine, cytosine, and thymine) and DNA strand complementarity. A joint least-squares algorithm correlates potential identifications from across multiple genetic regions with a database for the final identification of the microorganism. The regions amplified vary by organisms and assay type and are not disclosed by manufacturers.

The PCR-ESI-MS Plex-ID BAC detection assay (Ibis Biosciences, Abbott, Carlsbad, CA) was evaluated in two studies for detection and identification of microorganisms in excised heart

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valves (14, 15). In these studies, the sensitivity of PCR-ESI-MS ranged from 66% to 90% for detecting the causative microorganism. The suboptimal sensitivity reported by Brinkman et al. may be due to the fact that formalin-fixed paraffin-embedded valves were used in their evaluation (14). The other study included only 10 frozen valves from definite IE patients (15).

The new Iridica BAC-SFT assay (Ibis Biosciences, Abbott, Carlsbad, CA) was CE marked for in vitro diagnostics (IVD) in 2015. Compared to previous Ibis assays, the Iridica BAC-SFT has enhanced sensitivity due to optimized PCR conditions and human DNA-tolerant reagents with an improved downstream processing and analysis step (16–19). Until now, the Iridica system has not yet been evaluated for the detection of microorganisms in excised heart valves. The aim of this study was to compare the performance of the Iridica PCR-ESI-MS test with 16S rRNA PCR for the detection and identification of bacteria in excised heart valves.

(These findings were presented in part at the 26th European Congress of Clinical Microbiology and Infectious Diseases, Amsterdam, The Netherlands, 9 to 12 April 2016.)

MATERIALS AND METHODS

Case selection. Frozen excised heart valves of 34 definite IE patients according to modified Duke's criteria (4) and six nonendocarditis patients with degenerative heart failure were retrospectively selected for PCR-ESI-MS analysis. Cases were selected based on routine 16S rRNA PCR results to include a wide variety of pathogens. All valves were obtained during cardiac surgery in UZ Leuven between December 2013 and March 2015. Stored valves were retrospectively analyzed with PCR-ESI-MS from January until June 2015.

Valve processing. Valves were aseptically removed, sampled, and transported in sterile containers. At the time of surgery, portions of the valve that showed signs of infection (vegetation, thrombi) were divided into three parts, the first for molecular analysis, the second for culture analysis, and the third for histopathological analysis. Valves were processed under a biosafety cabinet class 2 for molecular testing. The freshly excised valve was crushed and homogenized after adding UMD Universal kit Trypticase soy broth (TSB) buffer (Molzym, Bremen, Germany) in sterile M-tubes on the gentleMACS dissociator (Miltenyi Biotec, Bergisch Gladbach, Germany). This homogeneous suspension was divided into two aliquots, one for immediate processing for 16S rRNA PCR and one for retrograde PCR-ESI-MS analysis after storage (−80°C).

Culture and serology. In all IE patients, at least three sets of BCs were taken prior to surgery. *Coxiella burnetii* serology was performed in two cases of culture-negative IE and proved to be positive (antiphase I IgG antibody titer, >1:800) in both cases. Valves were cultured in Wilkins-Chalgren broth for 7 days with subculturing if growth was observed. Matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) MS (Bruker Daltonics, Bremen, Germany) was used as the first-line identification method. If MALDI-TOF MS analysis could not resolve the identification of viridans streptococci, a Vitek 2 GP ID card was used (bioMérieux, Marcy l'Etoile, France). If Vitek 2 could not resolve identification, manual API-20 Strep (bioMérieux) identification was performed.

16S rRNA PCR analysis. DNA extraction, broad-spectrum 16S rRNA PCR, and sequence analysis were performed with the UMD Universal kit according to the recommendations of the manufacturer (Molzym, Bremen, Germany). Standard precautions were taken to avoid DNA contamination, and only high pure reagents were used. In brief, 800 μl, ±40% of the total crushed valve volume, was processed. Sample preparation included the lysis of human cells and the removal of human DNA prior to pathogen lysis and DNA purification using the patented Goffin-Meyvis method following the manufacturer's standard protocol. For each set of samples subjected to DNA extraction, a negative control was processed. A universal rRNA gene PCR assay of extracted bacterial DNA was performed in a GeneAmp PCR system 9700 (Thermo Fisher Scientific, Waltham, MA, USA). The UMD Universal kit provides paired primers that target conserved regions of the 16S rRNA genes of bacteria, 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. The detection of amplicons was performed by agarose gel electrophoresis. Amplicons from positive PCRs were purified with the QIAquick PCR purification kit (Qiagen, Hilden, Germany). Sequencing reactions were performed using BigDye Terminator cycle sequencing kit DNA (version 3.1; Thermo Fisher Scientific) according to the manufacturer's recommendations. The 16S rRNA PCR products were sequenced using sequencing primers (SeqGN16 and SeqGP16) that were supplied in the UMD Universal kit. The sequencing products were purified using the DyeEx 2.0 spin kit (Qiagen, Hilden, Germany) and analyzed with an ABI prism 3730 DNA analyzer (Thermo Fisher Scientific). Obtained sequences were compared with those in the GenBank database (http://www.ncbi.nlm.nih.gov/GenBank/) using the online BLAST software (http://blast.ncbi.nlm.nih.gov) and SepsiTest BLAST tool database (http://www.sepsitest-blast.net). Sequence similarity levels of ≥97% and ≥99% were used as cutoffs for genus and species identification, respectively (20).

PCR-ESI-MS analysis. Stored aliquots of crushed heart valves were thawed at room temperature. Testing was performed according to the manufacturer's recommendations using the Iridica BAC-SFT assay (Ibis Biosciences, Abbott, Carlsbad, CA). As described previously, the system performs all steps, including sample preparation, PCR, desalting, ESI-MS analysis, data processing, and reporting within a 6-h period (14). Broad-range microbial identification is provided using a signature database after mathematical matching of observed amplicon base compositions. Briefly, a frozen aliquot from the same crushed valve volume used for 16S rRNA PCR was thawed under a class 2 biosafety cabinet for molecular testing, and 300 μl (±35 mg of tissue) was chemically and mechanically lysed on the Iridica bead-beater and an extraction control was added to each sample. DNA extraction and PCR setup were automatically performed on the Iridica sample prep using prefilled individual disposable sample preparation cartridges and prefilled 16-well PCR strips, containing PCR amplification/inhibition controls. PCR was performed on the Iridica thermal cycler. The BAC strip consists of 16 wells, three of which contain primer pairs for the universal 16S rRNA gene and one of which contains primer pairs for the 23S rRNA gene. Other more specific conserved-site primer pairs target *Firmicutes*, *Staphylococcus* spp., *Enterobacteriaceae*, *Gammaproteobacteria*, and *Beta/Gammaproteobacteria*. Three wells contain specific primer pairs for *mecA*, *vanA*, *vanB*, and *Klebsiella pneumoniae* carbapenemase (KPC) antibiotic resistance genes. Four wells contain more discriminative primer pairs for *Candida* spp. detection and specification. The last well contains primers for a pumpkin DNA extraction control. PCR products were then desalted on the Iridica desalter and analyzed through ESI-MS on the Iridica mass spectrometer. The base compositions of the detected amplicons were deduced from the measured masses and compared with a reference database containing more than 6,000 bacterial species. Every result is accompanied by a Q score, which is a quality metric associated with the relative strength of the data supporting identification. The software only reported detections that had Q scores of ≥0.85.

Data analysis. The microbiological cause of IE was defined based on the criteria designed by Shrestha et al. (10) (Table 1). Discordant molecular results were resolved by 16S rRNA PCR analysis after one freeze-thaw cycle of the original stored crushed valve specimen, which is identical to the preanalytical sample conditions of PCR-ESI-MS analysis.

The result of any test modality was defined as true positive if the identification was identical up to the genus level to the microbiological cause defined according to the definitions in Table 1. Any pathogen detected by a test modality in the six nonendocarditis patients was considered to be a

TABLE 1 Criteria used to define the microbiological cause of infective endocarditis[a]

Result	Criterion
Considered causal for endocarditis	Same pathogen in blood culture, valve culture, and 16S rRNA PCR
	Same pathogen in at least one culture-based and 16S rRNA PCR, with a clinical presentation consistent with the pathogen identified
	A pathogen identified by any one modality—blood culture, valve culture, or 16S rRNA PCR—would only be considered if the same pathogen is identified in more than one specimen and if the clinical, operative, and histopathologic findings are consistent with endocarditis caused by that microorganism.
	A pathogen identified by any one modality—blood culture, valve culture, or 16S rRNA PCR —in only one specimen would only be considered if endocarditis caused by the pathogen has been well described and if the clinical, operative, and histopathologic findings are consistent with endocarditis caused by that microorganism.
Excluded as the cause of endocarditis	Single colony or rare growth of coagulase-negative staphylococci, viridans streptococci, *Propionibacterium acnes*, corynebacteria, or *Bacillus* spp. on a single valve culture, unless the same pathogen is also identified by a different modality
	Growth of coagulase-negative staphylococci, viridans streptococci, *Propionibacterium acnes*, corynebacteria, or Bacillus spp. on a single valve culture if there is another identified pathogen that could explain the endocarditis
	Positive 16S rRNA PCR results for coagulase-negative staphylococci, viridans streptococci, *Propionibacterium acnes*, corynebacteria, or *Bacillus* spp. on a single specimen if there is another identified pathogen that could explain the endocarditis
	Growth of any pathogen that is clearly inconsistent with endocarditis when interpreted in the context of clinical presentation, operative findings, histopathologic findings, and other microbiological findings

[a] Table 1 is adapted from Shrestha et al. (10).

false positive. Ninety-five percent confidence intervals (CI) were calculated with Analyse-it software (Analyse-it Software Ltd., Leeds, United Kingdom).

RESULTS

Overview of selected cases. The molecular- and culture-based microbiological results from 40 patients undergoing cardiac surgery with valve removal were compared and summarized in Table 2. IE cases were caused by viridans streptococci ($n = 14$), *Staphylococcus aureus* ($n = 5$), coagulase-negative staphylococci ($n = 4$), *Enterococcus faecalis* ($n = 3$), *Propionibacterium acnes* ($n = 2$), *C. burnetii* ($n = 2$), and *Granulicatella adiacens* ($n = 1$). In three IE cases, the causative microorganism was unknown, as 16S rRNA PCR and culture-based methods were negative. An overview of discordant molecular results (including resolution testing results) and double false-negative molecular results is given in Table 3.

BC and VC results. Twenty-four IE patients (71%) had positive BC results (in-house and/or in referring hospital) with the causative pathogen. In two of these patients (patients 7 and 8 in Table 3), there was a double negative molecular result (viridans streptococci [$n = 1$] and *Staphylococcus epidermidis* [$n = 1$]). Ten patients (29%) never had positive BC (in-house or in referring hospital), eight (24%) of which also had negative VC (culture-negative endocarditis) for the causative microorganism. Twelve endocarditis patients (35%) receiving adequate antimicrobial therapy before surgery, based on the susceptibility testing of the causative pathogen in referring hospitals, had sterile in-house BC and VC. However, PCR-ESI-MS and 16S rRNA PCR were able to detect bacterial DNA in 11 of these 12 patients.

Concordance molecular results. The positive/negative concordance between both molecular techniques was 98% in the 40 heart valves. In one valve, PCR-ESI-MS was positive while 16S rRNA PCR was negative (patient 3 in Table 3). In 30 heart valves with double positive molecular results, genus concordance was 97% and species concordance was 77%.

Molecular results in culture-negative IE. In two of the eight culture-negative IE cases, both molecular tests were negative. In one case, PCR-ESI-MS was the only method that detected and identified *P. acnes* (patient 3 in Table 3). In three cases, *Streptococcus gallolyticus*, viridans *Streptococcus* spp., and *P. acnes* were detected and identified by both molecular techniques as the causative pathogens (Table 2). Both molecular methods detected and identified *C. burnetii* in two cases of culture-negative IE.

Molecular detection of additional bacteria. PCR-ESI-MS additionally detected and identified *Staphylococcus saccharolyticus* and *Staphylococcus caprae* in two different IE cases caused by coagulase-negative staphylococci (patients 9 and 10 in Table 3). In another case of IE caused by viridans streptococci, PCR-ESI-MS additionally detected and identified *P. acnes* (patient 2 in Table 3). In all of these cases, BC, VC, or 16S rRNA PCR did not detect these microorganisms. However, 16S rRNA PCR additionally detected and identified *Parvimonas micra* in one case of IE caused by viridans streptococci; in this case, BC, VC, and PCR-ESI-MS could not detect this microorganism (patient 8 in Table 3).

Molecular misidentification and false-negative results. In one case of IE, PCR-ESI-MS detected and identified *Enterococcus* spp. (*Enterococcus durans*, *Enterococcus hirae*, or *Enterococcus mundtii*), whereas all other methods were concordantly positive for *Streptococcus mitis* (patient 1 in Table 3). The 16S rRNA PCR had no misidentifications. In one IE case caused by an unknown organism, PCR-ESI-MS solely detected and identified *P. acnes* (patient 3 in Table 3). In four cases of IE, there was a double negative molecular result (patients 4 to 7 in Table 3). These four patients received long-term effective antimicrobial therapy (4 to 10 weeks) and late cardiac valve surgery.

False-positive results. In 6 nonendocarditis valves, there was

TABLE 2 Molecular- and culture-based microbiological results from 40 patients undergoing cardiac surgery with valve removal

Method	Correct identification level of the causative pathogen	Causative pathogen in 34 infective endocarditis patients								Total no. (% of IE patients)
		S. aureus	Viridans streptococci	Coagulase-negative staphylococci	*E. faecalis*	*P. acnes*	*C. burnetii*	*G. adiacens*	Unknown	
16S rRNA PCR	Genus level	0	3	1	0	0	0	0	0	4 (12)
	Species level	5	10[a,b]	2	3	2[c]	2	1	0	25 (74)
	False negative	0	1	1	0	0	0	0	3	5 (14)
PCR-ESI-MS	Genus level	0	6[b]	0	0	0	0	0	0	6 (18)
	Species level	5	6[a]	3	3	2[c]	2	1	0	22 (65)
	Misidentification	0	1[d]	0	0	0	0	0	1[e]	2 (6)
	False negative	0	1	1	0	0	0	0	2	4 (11)
Blood culture	Growth and identification of causative pathogen	4	12	4	3	0	0	1	0	24 (71)
Valve culture	Growth and identification of causative pathogen	3	1	0	1	1	0	0	0	6 (18)
		False positive results in six nonendocarditis patients								Total no. (% of nonendocarditis patients)
16S rRNA PCR		0	0	1	0	0	0	0	0	1 (17)
PCR-ESI-MS		0	0	1	0	0	0	0	0	1 (17)
Blood culture		0	0	1	0	0	0	0	0	1 (17)
Valve culture		0	0	0	0	0	0	0	0	0 (0)

[a] Both molecular techniques detected *S. gallolyticus* in one case of culture-negative infective endocarditis.
[b] Both molecular techniques detected viridans *Streptococcus* spp. in one case of culture-negative infective endocarditis.
[c] Both molecular techniques detected *P. acnes* in one case of culture-negative infective endocarditis.
[d] This pathogen was identified as an *Enterococcus* spp.
[e] This pathogen was identified as *P. acnes* (it is unknown if this is a misidentification or true-positive result).

TABLE 3 Overview of discordant molecular results submitted to resolution testing, double false-negative molecular results, and molecular detection of additional microorganisms in 10 definite infective endocarditis patients

Patient	Valve culture result	Blood culture result	PCR-ESI-MS result	16S rRNA PCR result	Definite microbiological cause
1	*S. mitis*, *Staphylococcus hominis*,[a] and *Corynebacterium* spp.[a]	*S. mitis*	*Enterococcus* spp.	*S. mitis*[b]/*S. mitis*[e]	*S. mitis*
2	Negative	*S. epidermidis*[a] (two bottles out of 40 BC sets)	Viridans *Streptococcus* spp. and *P. acnes*[c]	Negative[b,d]/*S. mitis*[e]	viridans *Streptococcus* spp.
3	Negative	Negative	*P. acnes*	Negative[b,d]/Negative[d,e]	Unknown[f]
4	Negative	Negative	Negative[d]	Negative[d]	Unknown[f]
5	Negative	Negative	Negative[d]	Negative[d]	Unknown[f]
6	Negative	*S. epidermidis*	Negative[d]	Negative[d]	*S. epidermidis*
7	Negative	*S. mitis* and *Streptococcus parasanguinis*	Negative[d]	Negative[d]	Viridans *Streptococcus* spp.
8	Negative	*Streptococcus constellatus*	viridans or anginosus *Streptococcus* spp.	*S. constellatus* and *P. micra*[c]	*S. constellatus*
9	Negative	*S. epidermidis*	*S. epidermidis* and *S. caprae*[c]	*S. epidermidis*	*S. epidermidis*
10	Negative	*Staphylococcus capitis*	*S. capitis* and *S. saccharolyticus*[c]	*S. capitis*	*S. capitis*

[a] This result was considered to be contamination.
[b] This was a discordant molecular result.
[c] Detection of additional microorganisms by one molecular method occurred.
[d] This was considered a false negative molecular result.
[e] This was the result of resolution testing after one freeze-thaw cycle.
[f] Definite infective endocarditis was confirmed by histologic examination of the valve.

one case with double positive molecular results and another case with positive BC (2 out of 36 BC bottles), all with coagulase-negative staphylococci. These results were considered false positive (contamination). There were no false-positive valve culture results in these 6 patients (Table 2).

Detection of resistance markers. Detection of *mecA*, *vanA*, and *vanB* resistance genes by PCR-ESI-MS in staphylococci and enterococci (11 isolates) is summarized in Table 4. One case of endocarditis was caused by a methicillin-resistant *S. aureus* (MRSA) species, and another case was caused by methicillin-resistant *S. epidermidis*. PCR-ESI-MS correctly detected the *mecA* gene in these valves.

Sensitivity and specificity of different test modalities. The sensitivity for detecting and identifying the causative microorganism (up to the genus level) in 33 IE patients was 88% (95% CI, 71% to 96%) for 16S rRNA PCR, 85% (95% CI, 67% to 94%) for PCR-ESI-MS, 73% (95% CI, 54% to 86%) for BC, and 18% (95% CI, 8% to 36%) for VC. IE patient 3 from Table 3 was excluded from sensitivity calculations, as the causative microorganism is unknown in this case (according to the definitions of Table 1), but *P. acnes* was detected by PCR-ESI-MS. As such, it is impossible to classify this result as a true- or false-positive result. Specificity, calculated in 6 nonendocarditis patients, was 83% (95% CI, 36% to 99%) for PCR-ESI-MS, 16S rRNA PCR, and BC. Specificity of VC was 100% (95% CI, 52% to 100%).

TABLE 4 PCR-ESI-MS detection of potential antibiotic resistance genes in infective endocarditis patients caused by staphylococci and enterococci

Detected and identified by PCR-ESI-MS (no.)	*mecA* gene		*vanA* and *vanB* genes
	Detected	Not detected	Not detected
S. aureus (5)	1	4	NA[a]
Coagulase-negative staphylococci (3)	1	2	NA
E. faecalis (3)	NA	NA	3
Phenotype culture-based susceptibility testing	All methicillin resistant	All methicillin susceptible	All vancomycin susceptible

[a] NA, not applicable.

DISCUSSION

Overall, PCR-ESI-MS performed equally in identifying the causative pathogen at the species level (74% versus 65%, respectively) and had similar false-negative rates (14% versus 11%, respectively) compared to the 16S rRNA PCR.

In *S. aureus*, *E. faecalis*, and *G. adiacens* endocarditis, both molecular techniques performed equally in detecting and identifying the causative pathogen at the species level. The 16S rRNA PCR correctly identified viridans streptococci at the species level in more cases than PCR-ESI-MS (71% versus 43%, respectively). PCR-ESI-MS wrongly identified *Enterococcus* spp. in one case of viridans streptococci IE, whereas 16S rRNA PCR never suggested any incorrect identifications. However, we cannot exclude that the identified *Enterococcus* spp. was part of a mixed infection, as enterococci are a well-known cause of IE (4, 9). This may also explain cases in which the molecular tests detected additional bacterial species (*P. micra* by 16S rRNA PCR and *P. acnes*, *S. saccharolyticus*, and *S. caprae* by PCR-ESI-MS), all of which are a potential microbiological cause of IE (21–24).

There was no difference in sensitivity between the two molecular techniques or BC. Only for valve culture was a significantly lower sensitivity observed (18%), which is in accordance with many publications (8, 10–12). Specificity ranged from 83% to 100% for all test modalities, but the number of nonendocarditis samples is certainly too low in this study to evaluate specificity. Compared to the results of the Plex-ID system in the paper of Brinkman et al., we observed less false-negative (34% versus 11%, respectively) and therefore more true-positive (66% versus 85%, respectively) identification results using the Iridica system (14).

There were eight cases of culture-negative IE, of which six had positive PCR-ESI-MS and/or 16S rRNA PCR results. In one IE patient, PCR-ESI-MS was the sole method that detected and identified *P. acnes*. It remains uncertain whether *P. acnes* is the causative pathogen in this case of IE or not. Two cases of *C. burnetii* culture-negative IE were accurately detected and identified by both PCR-ESI-MS and 16S rRNA PCR.

PCR-ESI-MS and 16S rRNA PCR were negative in two definite IE cases, with positive BC for the causative pathogen. In one histopathologically proven case, only 1 out of 88 BC bottles grew *S. epidermidis*. This patient was treated successfully with vancomycin-rifampin. According to the criteria of Shrestha et al. (10), *S. epidermidis* is defined as the cause of IE in this case (a pathogen identified by any one modality in only one specimen fulfills the criterion of the causative pathogen if the pathogen is well described as a cause of endocarditis and if the clinical, operative, and histopathologic findings are consistent with endocarditis caused by that organism), but it is likely that the *S. epidermidis* isolate that was cultured is just a contaminant. In the other case, multiple BCs were positive for viridans streptococci in the referring hospital, but in-house BCs and VC were sterile after 10 weeks of adequate antimicrobial therapy.

Both molecular techniques detected bacterial DNA, although effective antimicrobial therapy was initiated before surgery. Resistance gene detection by PCR-ESI-MS (*mecA*, *vanA*, and *vanB*) was correct in all tested species compared to phenotypical, culture-based susceptibility testing, but only two *mecA*-harboring organisms were included. This detection of resistance genes is an advantage compared to 16S rRNA PCR, although expansion of the available molecular targets to other resistance mechanisms may be warranted.

A limitation of our study is the fact that PCR-ESI-MS was performed on frozen samples whereas 16S rRNA PCR was performed on fresh samples. A freeze-thaw cycle may negatively impact the sensitivity of the assays, although this was not the case for 16S rRNA PCR, which was repeated on selected discrepant samples for resolution testing. Evaluation of preanalytical variables is certainly needed to better understand their influence. Another limitation of the study is the selection (based on routine 16S rRNA PCR to include a wide variety of pathogens for comparison reasons) of our samples, which introduces a bias. Analysis of consecutive samples is needed in a next step to further evaluate and compare the clinical performance of these tests.

To conclude, the new Iridica PCR-ESI-MS system proved to be sensitive and accurately identified bacteria and resistance genes in excised heart valves. BCs remain essential for early (before surgery) diagnosis of IE and have the advantage of rendering an isolate that can be subjected to susceptibility testing. Conversely, PCR techniques do not only have diagnostic impact in culture-negative IE cases but certainly also by confirming (or contradicting) the identity of the involved pathogen in BC-positive cases. The latter is especially important in cases in which common skin contaminants are cultured from few BC bottles and VC remains negative. In such a context, uncertainty about the pathogen remains if no PCR is performed.

Iridica PCR-ESI-MS has the advantage of a nearly fully automated process, resulting in shorter turnaround times than 16S rRNA PCR. Iridica PCR-ESI-MS provides additional information about the presence of antimicrobial resistance genes. However, primers targeting antibiotic resistance genes may be included in a 16S rRNA PCR. Newer methods, like amplicon next-generation sequencing or whole-genome sequencing analysis, are beginning to replace 16S rRNA PCR and may easily be used to detect antibiotic resistance genes. The purchase of the Iridica PCR-ESI-MS platform comes with a great investment cost but can ameliorate the workflow and potentially increase the clinical impact of the molecular analysis of excised heart valves. Real-life performance and clinical impact of PCR-ESI-MS should be evaluated in future prospective studies, analyzing large numbers of consecutive samples.

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REFERENCES

1. **Habib G, Lancellotti P, Antunes MJ, Bongiorni MG, Casalta JP, Del Zotti F, Dulgheru R, El Khoury G, Erba PA, Iung B, Miro JM, Mulder BJ, Płońska-Gościniak E, Price S, Roos-Hesselink J, Snygg-Martin U, Thuny F, Mas PT, Vilacosta I, Zamorano JL.** 2015. ESC guidelines for the management of infective endocarditis. Eur Heart J **36:**3075–3123. http://dx.doi.org/10.1093/eurheartj/ehv319.
2. **Baddour LM, Wilson WR, Bayer AS, Fowler VG, Tleyjeh IM, Rybak MJ, Barsic B, Lockhart PB, Gewitz MH, Levison ME, Bolger AF, Steckelberg JM, Baltimore RS, Fink AM, O'Gara P, Taubert KA.** 2015. Infective endocarditis in adults: diagnosis, antimicrobial therapy, and management of complications. A scientific statement for healthcare professionals from the American Heart Association. Circulation **132:**1435–1486. http://dx.doi.org/10.1161/CIR.0000000000000296.
3. **Cahill TJ, Prendergast BD.** 2016. Infective endocarditis. Lancet **387:**882–893. http://dx.doi.org/10.1016/S0140-6736(15)00067-7.
4. **Li JS, Sexton DJ, Mick N, Nettles R, Fowler VG, Ryan T, Bashore T, Corey GR.** 2000. Proposed modifications to the Duke criteria for the diagnosis of infective endocarditis. Clin Infect Dis **30:**633–638. http://dx.doi.org/10.1086/313753.
5. **Lee A, Mirrett S, Reller LB, Weinstein MP.** 2007. Detection of bloodstream infections in adults: how many blood cultures are needed? J Clin Microbiol **45:**3546–3548. http://dx.doi.org/10.1128/JCM.01555-07.
6. **Cockerill FR, Wilson JW, Vetter EA, Goodman KM, Torgerson CA, Harmsen WS, Schleck CD, Ilstrup DM, Washington JA, Wilson WR.** 2004. Optimal testing parameters for blood cultures. Clin Infect Dis **38:**1724–1730. http://dx.doi.org/10.1086/421087.
7. **Houpikian P, Raoult D.** 2003. Diagnostic methods. Current best practices and guidelines for identification of difficult-to-culture pathogens in infective endocarditis. Cardiol Clin **21:**207–217.
8. **Houpikian P, Raoult D.** 2005. Blood culture-negative endocarditis in a reference center: etiologic diagnosis of 348 cases. Medicine (Baltimore) **84:**162–173. http://dx.doi.org/10.1097/01.md.0000165658.82869.17.
9. **Poesen K, Pottel H, Colaert J, De Niel C.** 2014. Epidemiology of infective endocarditis in a large Belgian non-referral hospital. Acta Clin Belg **69:**183–190. http://dx.doi.org/10.1179/0001551214Z.00000000046.
10. **Shrestha NK, Ledtke CS, Wang H, Fraser TG, Rehm SJ, Hussain ST, Pettersson GB, Blackstone EH, Gordon SM.** 2015. Heart valve culture and sequencing to identify the infective endocarditis pathogen in surgically treated patients. Ann Thorac Surg **99:**33–37. http://dx.doi.org/10.1016/j.athoracsur.2014.07.028.
11. **Marsch G, Orszag P, Mashaqi B, Kuehn C, Haverich A.** 2015. Antibiotic therapy following polymerase chain reaction diagnosis of infective endocarditis: a single centre experience. Interact Cardiovasc Thorac Surg **20:**589–593. http://dx.doi.org/10.1093/icvts/ivv006.
12. **Vondracek M, Sartipy U, Aufwerber E, Julander I, Lindblom D, Westling K.** 2011. 16S rDNA sequencing of valve tissue improves microbiological diagnosis in surgically treated patients with infective endocarditis. J Infect **62:**472–478. http://dx.doi.org/10.1016/j.jinf.2011.04.010.
13. **Wolk DM, Kaleta EJ, Wysocki VH.** 2012. PCR-electrospray ionization mass spectrometry: the potential to change infectious disease diagnostics in clinical and public health laboratories. J Mol Diagn **14:**295–304. http://dx.doi.org/10.1016/j.jmoldx.2012.02.005.
14. **Brinkman CL, Vergidis P, Uhl JR, Pritt BS, Cockerill FR, Steckelberg JM, Baddour LM, Maleszewski JJ, Edwards WD, Sampath R, Patel R.**

2013. PCR-electrospray ionization mass spectrometry for direct detection of pathogens and antimicrobial resistance from heart valves in patients with infective endocarditis. J Clin Microbiol **51:**2040–2046. http://dx.doi.org/10.1128/JCM.00304-13.

15. **Wallet F, Herwegh S, Decoene C, Courcol RJ.** 2013. PCR-electrospray ionization time-of-flight mass spectrometry: a new tool for the diagnosis of infective endocarditis from heart valves. Diagn Microbiol Infect Dis **76:**125–128. http://dx.doi.org/10.1016/j.diagmicrobio.2013.02.007.
16. **Bacconi A, Richmond GS, Baroldi MA, Laffler TG, Blyn LB, Carolan HE, Frinder MR, Toleno DM, Metzgar D, Gutierrez JR, Massire C, Rounds M, Kennel NJ, Rothman RE, Peterson S, Carroll KC, Wakefield T, Ecker DJ, Sampath R.** 2014. Improved sensitivity for molecular detection of bacterial and *Candida* infections in blood. J Clin Microbiol **52:**3164–3174. http://dx.doi.org/10.1128/JCM.00801-14.
17. **Jordana-Lluch E, Carolan HE, Giménez M, Sampath R, Ecker DJ, Quesada MD, Mòdol JM, Arméstar F, Blyn LB, Cummins LL, Ausina V, Martró E.** 2013. Rapid diagnosis of bloodstream infections with PCR followed by mass spectrometry. PLoS One **8:**e62108. http://dx.doi.org/10.1371/journal.pone.0062108.
18. **Jordana-Lluch E, Giménez M, Quesada MD, Ausina V, Martró E.** 2014. Improving the diagnosis of bloodstream infections: PCR coupled with mass spectrometry. Biomed Res Int **2014:**501214.
19. **Jordana-Lluch E, Giménez M, Quesada MD, Rivaya B, Marcó C, Domínguez MJ, Arméstar F, Martró E, Ausina V.** 2015. Evaluation of the broad-range PCR/ESI-MS technology in blood specimens for the molecular diagnosis of bloodstream infections. PLoS One **10:**e0140865. http://dx.doi.org/10.1371/journal.pone.0140865.
20. **Clinical and Laboratory Standards Institute.** 2008. Interpretive criteria for identification of bacteria and fungi by DNA target sequencing; approved standard. CLSI document MM18-A. Clinical and Laboratory Standards Institute, Wayne, PA.
21. **Gomez CA, Gerber DA, Zambrano E, Banaei N, Deresinski S, Blackburn BG.** 2015. First case of infectious endocarditis caused by *Parvimonas micra*. Anaerobe **36:**53–55. http://dx.doi.org/10.1016/j.anaerobe.2015.10.007.
22. **Vandenesch F, Eykyn SJ, Bes M, Meugnier H, Fleurette J, Etienne J.** 1995. Identification and ribotypes of *Staphylococcus caprae* isolates isolated as human pathogens and from goat milk. J Clin Microbiol **33:**888–892.
23. **Krishnan S, Haglund L, Ashfaq A, Leist P, Roat T.** 1996. Prosthetic valve endocarditis due to *Staphylococcus saccharolyticus*. Clin Infect Dis **22:**722–723. http://dx.doi.org/10.1093/clinids/22.4.722.
24. **Westblom TU, Gorse GJ, Milligan TW, Schindzielorz AH.** 1990. Anaerobic endocarditis caused by *Staphylococcus saccharolyticus*. J Clin Microbiol **28:**2818–2819.