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Molecular Approach to Diagnosis of Cardiovascular Implantable Electronic Device Infection

Zerelda Esquer Garrigos,[1] M. Rizwan Sohail,[1,2] Kerryl E. Greenwood-Quaintance,[1] Scott A. Cunningham,[1] Prakhar Vijayvargiya,[1] Madiha Fida,[1] Paul A. Friedman,[2] Jayawant Mandrekar,[3] Daniel C. DeSimone,[1,2] Larry M. Baddour,[1,2] and Robin Patel[1]

[1]Division of Infectious Diseases and Departments of [2]Cardiovascular Diseases and [3]Health Sciences Research, Mayo Clinic College of Medicine and Science, Rochester, Minnesota

Background. Sonicate fluid (SF), a solution derived from vortexing and sonication of explanted cardiovascular implantable electronic devices (CIEDs), is a higher-yield specimen compared with swabs or tissues for culture-based detection of microorganisms associated with CIED infection. Despite this, SF culture fails to identify a causative organism in ~50% of cases. We aimed to evaluate the diagnostic performance of 16S ribosomal RNA gene (rRNA) polymerase chain reaction (PCR)/sequencing of SF and compare it with that of SF culture.

Methods. We identified 322 SF specimens from extracted CIEDs and reviewed clinical data for each patient. Subjects were classified as having or not having CIED infection. Cases were subcategorized as culture negative if no significant growth was reported from SF cultures and as culture positive if an organism was detected above predefined thresholds. 16S rRNA PCR/sequencing was performed, with the organisms identified reported according to Clinical and Laboratory Standards Institute guidelines for sequence data interpretation.

Results. A total of 278 SF samples corresponded to infected cases, of which 160 were culture positive and 118 culture negative. The remaining 44 were from noninfected cases, of which 2 were culture positive. Compared with SF culture, the sensitivity of 16S rRNA PCR/sequencing was higher (64% vs 57.5%, $P = .003$). 16S rRNA PCR/sequencing detected a potential pathogen in 28 of 118 culture-negative cases, identifying staphylococci in the majority (18/28).

Conclusions. 16S rRNA PCR/sequencing has higher sensitivity to detect bacteria in SF from extracted CIEDs than does SF culture.

Keywords. 16S rRNA PCR/sequencing; cardiovascular implantable electronic device infection; laboratory diagnosis.

Cardiovascular implantable electronic device (CIED) infection is accompanied by significant morbidity, mortality, and cost [1]. Once infected, device removal and pathogen-directed therapy are essential for cure. Identifying a causative organism is therefore critical for diagnostic certainty and the selection of appropriate therapy.

Our group previously demonstrated that culture of sonicate fluid (SF), a solution derived from vortexing and sonication of extracted CIEDs, is a higher-yield methodology than swab or tissue cultures for the detection of microorganisms associated with CIED infection. Despite this, culture of SF fails to identify a pathogen in ~50% of presumed CIED infections [2, 3]. Possible explanations for the low sensitivity of culture include loss of viability during collection and/or processing, the fastidious nature of the causative organism(s), effects of prior antimicrobial therapy, and/or the presence of biofilms. When the etiologic agent is not identified, patients may be treated with empiric broad-spectrum antibiotics, leading to excess cost, treatment failure, selection of antibacterial resistance, dysbiosis, and/or drug toxicity [4].

To overcome limitations of traditional culture approaches, molecular methods have emerged. One of these is polymerase chain reaction (PCR) and sequencing targeting the 16S ribosomal RNA (rRNA) gene, which is universally present in bacteria [5]. This method has been incorporated into the diagnostic algorithm for infective endocarditis given its higher sensitivity compared with culture when performed on extracted valvular tissue [6]. Its utility has also been described for other specimen types in which an infection is suspected but cultures are negative [7–9]. 16S rRNA PCR/sequencing has advantages compared with culture, including potential identification of fastidious/nonculturable organisms and nondividing bacteria present due to the effect of host response or antibiotic therapy. Moreover, if rapidly performed, it may provide faster results than culture-based approaches, expediting antibiotic de-escalation and early discharge. Recent reports also suggest the potential utility of this method as an antimicrobial stewardship tool [10]. However, there are challenges with this, including cost, lack of standardized criteria for interpretation of results, risk of

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Correspondence: R. Patel, Mayo Clinic, 200 1st Street SW, Rochester, MN 55905 (patel.robin@mayo.edu).

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exogenous DNA contamination leading to false-positive results, and lack of provision of susceptibility data.

Despite the high percentage of culture-negative cases in CIED infection described by our group and others [2, 3], the utility of 16S rRNA PCR/sequencing for microbial detection and identification in these cases has not been previously reported. The current investigation evaluated the diagnostic performance of 16S rRNA PCR/sequencing on SF from extracted CIEDs and compared results with those of culture of the same specimen type, with a goal of developing an algorithm for utilization of this molecular method in the diagnosis of CIED infection.

METHODS

Study Group Classification and Definitions

We identified 323 SF specimens from extracted CIEDs collected and stored at −80°C in the Infectious Diseases Research Laboratory, Mayo Clinic, Minnesota, from January 2012 through July 2017. Fungal infections were excluded. The study was approved by the Mayo Clinic Institutional Review Board.

We reviewed clinical data for each patient and classified them as having or not having CIED infection using predefined criteria (Table 1) [11]. Infections were then subcategorized as definite, probable, or possible. Cases were independently evaluated by 3 infectious diseases physicians, who reached consensus (agreement between all 3 or 2 of 3) conclusions. Asymptomatic patients undergoing device extraction for indications other than infection were classified as noninfected. Clinical, laboratory, and imaging data were reviewed to ensure absence of findings of infection.

Significant microbial growth was defined as isolation of 20 or more colony-forming units (cfu)/10 mL of SF. Cases were classified as SF culture positive if significant microbial growth was reported or as culture negative if SF yielded no or insignificant growth. Device swabs, pocket tissue, or swab cultures obtained at the time of CIED removal, collectively and hereafter referred to as intraoperative cultures, were recorded and considered positive if colony growth was reported in 2 or more quadrants of the culture plate [2]. Blood cultures were considered positive if an organism(s) was identified from 2 or more sets. Delayed bacterial growth was defined as microbial identification after 48 hours of incubation. Culture positive infections were classified as monomicrobial if a single organism was isolated in culture and as polymicrobial if 2 or more organisms were isolated.

A 16S rRNA PCR-positive sample was defined as having a crossing point (Cp) of 32 or fewer cycles with a peak height fluorescence of greater than 0.3, whereas a negative result corresponded to a Cp of more than 32 cycles or peak height fluorescence of 0.3 or less. Following sequencing of 16S rRNA PCR–positive specimens, results were considered 16S rRNA PCR/sequencing positive if a bidirectional or unidirectional sequence was generated and percentage identities for the organism(s) identified were 98% or greater. The organism identified was reported according to Clinical and Laboratory Standards Institute guidelines [12]. Samples were deemed 16S rRNA gene PCR/sequencing negative in any of the following scenarios: PCR negative, PCR positive with uninterpretable mixed sequence, or PCR positive with the percentage of identities less than 98% to any organism. Details regarding sample processing, microbiological, and molecular testing are described in the Supplementary Methods.

Table 1. Cardiovascular Implantable Electronic Device Infection Case Definition and Classification Criteria

Pocket findings:
1. Physical examination: device erosion through skin, purulent drainage from generator pocket, fluctuance, sinus tract
2. Intraoperative findings: purulence within the generator pocket site[a]
Clinical findings:
Major
1. Two or more positive blood cultures for typical organisms for CIED infection, such as *Staphylococcus aureus*, CoNS, or enterococci with no alternative source
2. TEE findings consistent with vegetation on the device lead and/or right heart valve
3. Positron emission tomography/computed tomography imaging consistent with device infection
Minor
1. Prolonged (>72 h) bacteremia with microorganism other than listed in major criteria
2. TEE findings not meeting major criteria
3. Recent pocket manipulation (<3 mo of presentation)
4. Fever (38°C or higher)
5. Embolic phenomena (typically septic pulmonary emboli from lead vegetations or right-sided endocarditis)
6. Pocket erythema or tenderness
CIED infection classification criteria:
1. Definite CIED infection: combination of any 2 major clinical findings or 1 or more pocket findings
2. Probable CIED infection: 1 major clinical finding and 1 or more minor clinical finding
3. Possible CIED infection: suspected CIED infection case that does not meet "definite" or "probable" criteria

Table adapted from DeSimone and Sohail (2018) [11]. Abbreviations: CIED, cardiovascular implantable electronic device; CoNS, coagulase-negative *Staphylococcus* species; TEE, transesophageal echocardiogram.

[a]The following criterion was removed for the purpose of the study: positive cultures (significant microbial growth) from explanted CIED.

Statistical Methods

Descriptive summaries are reported as counts and percentages for categorical variables and as medians and interquartile ranges (IQRs) for continuous variables. Comparisons were performed using chi-square tests for categorical variables and the Wilcoxon rank sum test for continuous variables. Sensitivity and specificity between SF culture and 16S rRNA gene PCR/sequencing were compared using McNemar's test. All statistical tests were 2-tailed, with $P < .05$ considered statistically significant. Analyses were performed using SAS software version 9.4 (SAS Institute).

RESULTS

Overall, 322 SF samples were included, 278 from subjects with CIED infection, of which 118 (42.4%) were categorized as culture negative and 160 (57.5%) as culture positive, with the remaining 44 being from noninfected cases, of which 2 were culture positive (4.5%) and 42 culture negative (95.4%) (Figure 1). Clinical and device characteristics are summarized in Table 2.

Of the 278 CIED infections, 76.3% (212 of 278) were classified as definite, 16.5% (46 of 278) as probable, and 7.2% (20 of 278) as possible (Table 2). Other clinical, imaging, and microbiologic details of both groups are summarized in the Supplementary Table.

Performance of SF 16S rRNA PCR/Sequencing Compared With SF Culture

Among the infected cases, 178 (64.0%) were classified as 16S rRNA PCR/sequencing positive and 100 (35.8%) as negative (Table 3). In the noninfected cases, only 1 (2.7%) was 16S rRNA PCR/sequencing positive, with the remainder being negative (43, 97.7%).

Compared with SF culture, the sensitivity of 16S rRNA PCR/sequencing in definite cases of CIED infection was higher (76.4% vs 69.3%, $P = .001$). When analyzing all cases of CIED infection, 16S rRNA PCR/sequencing retained its higher sensitivity (64.0% vs 57.5%, $P = .003$). The specificity of the 2 methods in all CIED infection cases was not different (97.7% and 95.4%, $P = .563$) (Table 4).

SF Culture–positive, 16S rRNA Gene PCR/Sequencing–positive Infected Cases

Of the 160 culture-positive cases, a majority were monomicrobial (125, 78.1%) (Table 5). 16S rRNA PCR/sequencing was positive in 115 (92.0%) of these. The most common organism type identified with both methods was *Staphylococcus* species (sp.). Genus-level concordance between SF culture–positive and 16S rRNA gene PCR/sequencing–positive cases was 92.1% (106 of

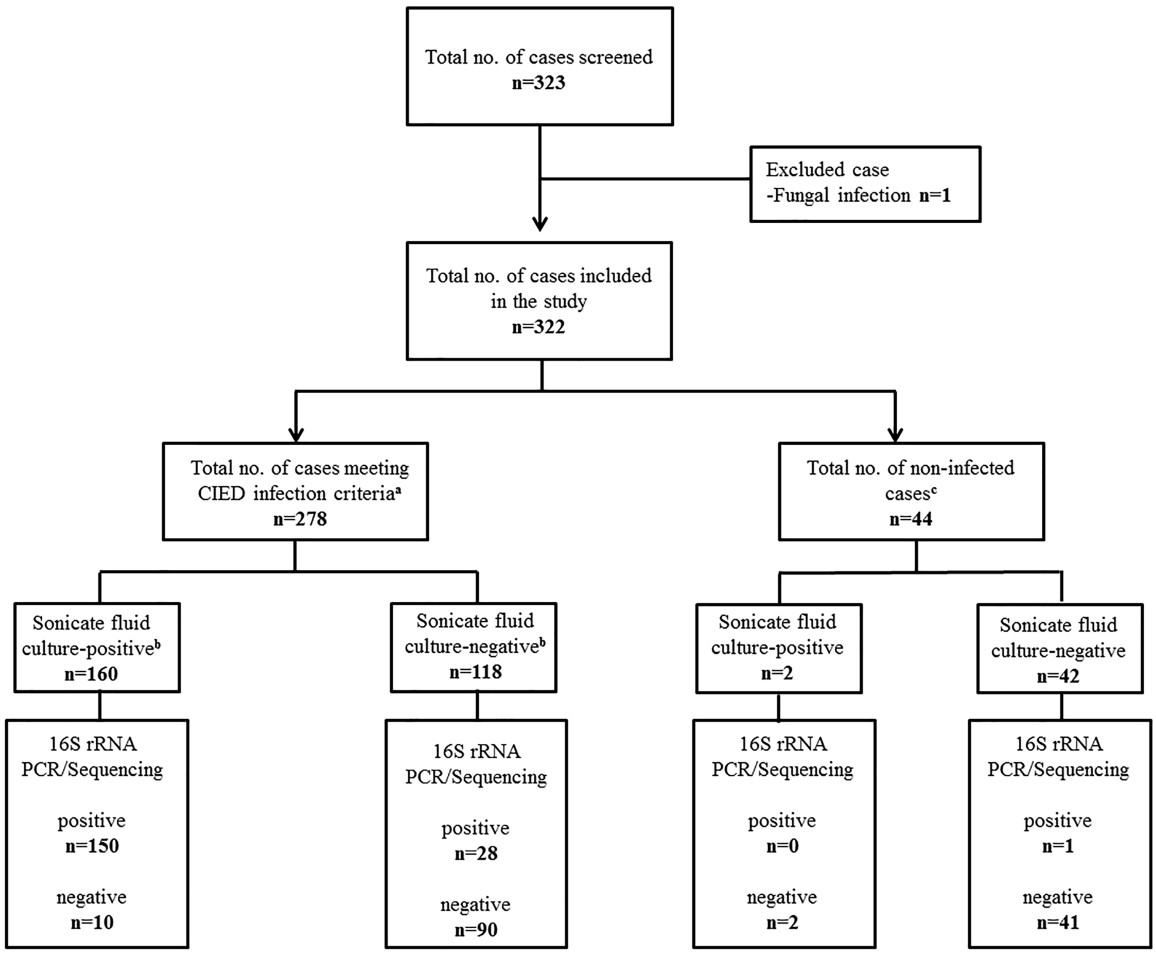

Figure 1. Study group classifications. Abbreviations: CIED, cardiovascular implantable electronic device; PCR, polymerase chain reaction; rRNA, ribosomal RNA. [a]See Table 1 for CIED infection criteria. [b]Cases were classified as culture positive if significant microbial growth was reported from sonicate fluid (20 or more colony-forming units from 10 mL) or culture negative if sonicate fluid yielded no or insignificant growth. [c]Noninfected cases included asymptomatic patients who underwent device extraction for indications other than infection.

Table 2. Demographic and Clinical Characteristics of Infected and Noninfected Patients

	Infected Group (n = 278)	Noninfected Group (n = 44)	*P* Value
Male, n (%)	208 (74.8)	25 (56.8)	.013
Caucasian, n (%)	268 (96.4)	43 (97.7)	.653
Age at diagnosis (median, IQR), y	70 (58.0–78.2)	65.5 (55.2–78.7)	.415
Type of device (PPM, ICD, CRT), n (%)	123 (44.2), 108 (38.8), 47 (16.9)	26 (59.0), 15 (34.0), 3 (6.8)	.104
Infected group case classification, n (%)		…	…
Definite	212 (76.3)		
Probable	46 (16.5)		
Possible	20 (7.2)		
Clinical presentation, n (%)			
Local infection			
Device pocket infection	101 (36.3)		
Generator or lead erosion	29 (10.4)		
BSI	15 (5.3)		
Lead endocarditis	8 (2.8)		
Valvular endocarditis	3 (1.0)		
Systemic infection			
CIED-BSI	33 (11.8)		
CIED-lead endocarditis	65 (23.5)		
CIED-valvular endocarditis	24 (8.6)		
Indication for CIED removal, n (%)			
End-of-life designation	…	40 (90.9)	
Lead revision	…	3 (6.8)	
Malfunction	…	1 (2.2)	

Abbreviations: BSI, bloodstream infection; CIED, cardiac implantable electronic device; CRT, cardiac resynchronization therapy; ICD, implantable cardiac device; IQR, interquartile range; PPM, permanent pacemaker.

115). Most genus-level discordant cases (5 of 9) were due to *Cutibacterium acnes* found by SF culture with *Staphylococcus* sp. detected by 16S rRNA PCR/sequencing. Among these discrepant detections, blood cultures identified the organism detected by 16S rRNA PCR/sequencing in 3 of 5 cases; in 1 case the organism detected by SF culture was also isolated from intraoperative cultures, whereas in the other, the organism identified by 16S rRNA PCR/sequencing was also isolated from intraoperative cultures (Table 5).

Table 3. Microbiologic Characteristics of the Infected Patients

	Infected Group (n = 278)
Sonicate fluid microbiology	
Culture positive	160 (57.5)
Polymicrobial	35/160 (21.8)
Monomicrobial	125/160 (78.1)
Microbial yield >100 cfu/10 mL	123 (76.8)
Culture negative	118 (42.4)
Gram-stain positive[a]	72/228 (31.5)
16S rRNA PCR/sequencing	
Positive, negative[b]	178 (64.0), 100 (35.8)
Sequence data	
Bidirectional sequence, forward sequence only	158 (88.7), 19 (10.6)
Reverse sequence only	1 (0.5)
Bacterial identification	
Genus level only, species level	118 (66.2), 60 (33.7)

Data are presented as no. (%).
Abbreviations: cfu, colony-forming units; PCR, polymerase chain reaction; rRNA, ribosomal RNA.
[a]Gram stain was only performed on 228 specimens.
[b]Negative results included 46 PCR-positive samples with uninterpretable mixed sequences, 4 PCR-positive samples with percentage identities <98% for the organism identified (cases 69, 155, 182, and 155), and 50 PCR-negative samples. Details are shown in the Supplementary Table. Negative 16S rRNA PCR/sequencing results in the noninfected group included 7 of 44 PCR-positive samples with uninterpretable mixed sequences, 2 of 44 PCR-positive samples with percentage identities <98% for the organism identified (cases 274 and 308), and 35 of 44 PCR-negative samples. Details are shown in the Supplementary Table.

SF Culture–positive, 16S rRNA Gene PCR/Sequencing–negative Infected Cases

16S rRNA PCR/sequencing did not detect an organism in 10 SF culture–positive cases, all of which had culture yields of 20–100 cfu/10 mL. Six were culture positive for *C. acnes*, 3 for *Staphylococcus* sp., and 1 for *Stenotrophomonas* sp. (cases 37, 59, 65, 87, 189, 213, 218, 221, 240, and 268). Two of the cases with *C. acnes* detected by SF culture had growth of *Klebsiella pneumoniae* or *Staphylococcus aureus* on blood cultures (cases 189 and 218, respectively).

SF Culture–positive Polymicrobial Infected Cases

As Sanger sequencing is only able to detect monotemplates, we separately analyzed the 35 infected cases from which more than 1 organism was detected by SF culture. A potential pathogen was identified by 16S rRNA PCR/sequencing in all 35 polymicrobial infections. With the exception of 2 cases, the organism identified by 16S rRNA PCR/sequencing was detected in culture at more than 100 cfu/10 mL. There was 1 case in which 16S rRNA PCR/sequencing detected an organism at

Table 4. Comparative Analysis of Sonicate Fluid Culture and 16S rRNA Polymerase Chain Reaction/Sequencing

	Sonicate Fluid Culture	16S rRNA PCR/ Sequencing	*P* Value
Definite cases of CIED infection			
Sensitivity	147 (69.3)	162 (76.4)	.001
Combined definite, probable, and possible cases of CIED infection			
Sensitivity	160 (57.5)	178 (64.0)	.003
Specificity	42 (95.4)	43 (97.7)	.563

Data are presented as no. (%). Refer to Table 1 for CIED infection classification. Abbreviations: CIED, cardiac implantable electronic device; PCR, polymerase chain reaction; rRNA, ribosomal RNA.

20–100 cfu/10 mL (*Staphylococcus* sp.) over that present at more than 100 cfu/10 mL (*C. acnes*), and another case in which SF culture yielded *Staphylococcus* and *Corynebacterium* sp. while *Finegoldia magna* was identified by 16S rRNA PCR/sequencing (Table 6).

SF Culture–negative, 16S rRNA Gene PCR/Sequencing–positive Infected Cases

Of the 118 SF culture–negative cases, 23.7% (28 of 118) had potential pathogens detected by 16S rRNA PCR/sequencing (Table 7). Most (23 of 28, 82.1%) were classified as definite and a majority were due to *Staphylococcus* sp.

Noninfected Cases

Two cases (2 of 44, 4.5%) were classified as culture positive with *Bacillus* sp. and *C. acnes* identified in one each, both at 20–50 cfu/10 mL. 16S rRNA gene PCR/sequencing was positive in 1 of 44 (2.2%) cases, detecting *Acinetobacter/Prolinobarus* sp. in a culture-negative case.

Other Culture Sources

One hundred thirty-eight CIED infection cases had positive blood cultures. Of those, 59 (42.7%) were SF culture positive and 73 (52.8%) were 16S rRNA PCR/sequencing positive. Concordance of 16S rRNA gene PCR/sequencing with blood culture results was higher than that of SF culture (n = 65 [89.0%] vs 47 [79.6%], $P = .002$). Of the 90 cases in which SF and 16S rRNA PCR/sequencing were negative, other intraoperative cultures yielded an organism in 5 cases (cases 1, 29, 150, 161, and 94). Details of blood and intraoperative culture results for both groups are summarized in the Supplementary Table.

Analysis of Antimicrobial Exposure

In cases where antibiotics were given prior to CIED extraction, SF culture was 3 times more likely to be negative when compared to 16S rRNA PCR/sequencing (odds ratios: 3.2 [1.6–6.3] vs 1.8 [0.9–3.5]; $P = .006$ and $P = .596$, respectively).

Analysis of Time to Detection

In SF culture–positive cases, time to microbial detection for aerobic or facultatively anaerobic bacteria ranged from 1 to 3 days, whereas for gram-positive anaerobes it was 7 to 14 days. There were 34 cases of delayed growth, of which 28 were identified by 16S rRNA PCR/sequencing.

DISCUSSION

Based on our analysis, 16S rRNA PCR/sequencing has higher sensitivity compared with SF culture and, therefore, could be considered in cases of suspected CIED infection, especially when no microbial growth is detected in intraoperative cultures after 48 hours of incubation. 16S rRNA PCR/sequencing generated a

Table 5. Organisms Detected in Sonicate Fluid by Method Detected in Infected Monomicrobial Cases

	Sonicate Fluid Culture (n = 125)	16S rRNA PCR/ Sequencing (n = 115/125)	Sonicate Fluid 16S rRNA PCR/ Sequencing	Blood Cultures	Intraoperative Cultures[a]
CoNS, n (%)	43 (34.6)	26 (22.6)	…	…	…
Staphylococcus sp., n (%)	…	23 (20.0)	…	…	…
Staphylococcus aureus, n (%)	40 (32.2)	32 (27.8)	…	…	…
Cutibacterium acnes, n (%)	32 (25.6)	19 (16.5)	…	…	…
GNB, n (%)	7 (5.6)	8 (6.9)	…	…	…
Other gram-positive bacteria, n (%)	3 (2.4)	7 (6.0)	…	…	…
Discordant detections (n = 9)					
(5)[b]	*C. acnes*	…	*Staphylococcus* sp.	*Staphylococcus* sp. (3)	*C. acnes* (1), *Staphylococcus epidermidis* (1)
(3)[c]	*C. acnes*	…	*Corynebacterium* sp.	(–)	*C. acnes* (1)
(1)[d]	*Staphylococcus gallinarum*	…	*Pseudomonas* sp.	(–)	(–)

Abbreviations: CIED, cardiovascular implantable electronic device; CoNS, coagulase-negative *Staphylococcus* species; GNB, gram-negative bacteria; PCR, polymerase chain reaction; rRNA, ribosomal RNA; –, negative; sp., species.

[a]Includes device swabs, pocket tissue, or swab cultures obtained at the time of CIED removal.

[b]Cases 16, 34, 96, 114, and 120. Details are shown in the Supplementary Table.

[c]Cases 25, 119, and 271. Details are shown in the Supplemental Table.

[d]Case 129. Details are shown in the Supplementary Table.

Table 6. Organisms Detected in Sonicate Fluid by Method Detected in Infected Polymicrobial Cases

Organisms Identified From Infected Polymicrobial Cases	Number of Cases (n = 35)	Organism Preferentially Detected With 16S rRNA PCR/sequencing	Number of Cases (n = 35)
CoNS >100 cfu/10 mL, *Cutibacterium acnes* 20–100 cfu/10 mL	8	*Staphylococcus* sp.	8
CoNS >100 cfu/10 mL, *C. acnes* >100 cfu/10 mL	4	*Staphylococcus* sp.	4
Corynebacterium sp. >100 cfu/10 ml, CoNS 20–100 cfu/10 mL	2	*Corynebacterium* sp.	2
C. acnes >100 cfu/10 mL, *Staphylococcus epidermidis* 20–50 cfu/10 mL	2	*Staphylococcus* sp.	1
		C. acnes	1
Staphylococcus sp. >100 cfu/10 mL, *Corynebacterium* sp. 20–100 cfu/10 mL	2	*Staphylococcus* sp.	2
Staphylococcus sp. 51–100 cfu/10 mL, *Corynebacterium* sp. 51–100 cfu/10 mL	2	*Finegoldia magna*[a]	1
		Staphylococcus aureus	1
CoNS >100 cfu/10 mL, *S. aureus* >100 cfu/10 mL	2	*S. epidermidis*	2
S. aureus >100 cfu/10 mL, *C. acnes* >100 cfu/10 mL	2	*Staphylococcus* sp.	2
S. aureus >100 cfu/10 mL, *C. acnes* >100 cfu/10 mL	2	*Staphylococcus* sp.	2
Pseudoclavibacter sp. >100 cfu/10 mL, *C. acnes* >100 cfu/10 mL	1	*Pseudoclavibacter* sp.	1
Staphylococcus lugdunensis >100 cfu/10 mL, *Staphylococcus capitis* 20–50 cfu/10 mL	1	*Staphylococcus* sp.	1
Staphylococcus schleiferi >100 cfu/10 mL, *S. capitis* >100 cfu/10 mL	1	*S. schleiferi*	1
Enterobacter cloacae complex >100 cfu/10 mL, *S. epidermidis* 20–50 cfu/10 mL	1	*Citrobacter/Enterobacter* sp.	1
Corynebacterium sp. >100 cfu/10 mL, *C. acnes* 20–50 cfu/10 mL	1	*Corynebacterium* sp.	1
C. acnes >100 cfu/10 ml, *Streptococcus mitis* group, not *Streptococcus pneumoniae* 51–100 cfu/10 mL	1	*C. acnes*	1
Enterobacter cloacae complex >100 cfu/10 mL, *Serratia marcescens* 20–100 cfu/10 mL	1	*E. cloacae/hormaechei*	1
S. aureus >100 cfu/10 mL, CoNS >20–50 cfu/10 mL	1	*Staphylococcus aureus*	1
Escherichia coli >100 cfu/10 mL, *Enterococcus faecalis* 20–100 cfu/10 mL	1	*Escherichia/Shigella* sp.	1
>4 organisms, all >100 cfu/10 mL	1	*Corynebacterium tuberculostearicum*	1
S. aureus >100 cfu/10 mL, *Corynebacterium amycolatum* >100 cfu/10 mL, *Moraxella nonliquefaciens* >20–50 cfu/10 mL	1	*S. aureus*	1

Abbreviations: cfu, colony-forming units; CoNS, coagulase-negative *Staphylococcus* species; PCR, polymerase chain reaction; rRNA, ribosomal RNA; –, negative; sp., species.

[a]Discordant sample: case 148. Details are shown in the Supplementary Table.

microbial diagnosis in 36.8% (56 of 152) of SF culture-negative and delayed-growth cases. Clinically, securing a microbiologic diagnosis in culture-negative infected CIED cases could help in the selection of targeted, narrow-spectrum antibiotic regimens, potentially leading to better treatment outcomes and fewer adverse events. When applied in cases of delayed culture positivity, molecular methods could expedite hospital dismissal planning. For patients who present with bloodstream infection (BSI) with no identifiable source and no evidence of generator pocket infection on physical examination, and who undergo device extraction for presumed CIED-associated BSI, 16S rRNA PCR/sequencing may be utilized when SF cultures are negative or discordant with blood culture results to determine whether the BSI may be related to the CIED, given the higher concordance of this method with positive blood culture compared with SF culture.

Table 7. Organisms Detected and Identified in Infected Culture-negative Cases Using 16S rRNA Polymerase Chain Reaction/Sequencing

	Number of Cases (%) (Total N = 28)
Staphylococcus sp.	8 (28.5)
Staphylococcus aureus	8 (28.5)
Corynebacterium sp. (not *Corynebacterium glutamicum*)	3 (10.7)
Cutibacterium acnes	2 (7.1)
Streptococcus sp.	2 (7.1)
Staphylococcus epidermidis	2 (7.1)
Enterococcus sp.	1 (3.5)
Serratia sp.	1 (3.5)
Mycobacterium iranicum	1 (3.5)

Abbreviations: rRNA, ribosomal RNA; sp., species.

For patients who undergo CIED extraction due to presumed infection (fever or other systemic signs of infection or abnormal echocardiogram) without physical examination findings suggesting pocket infection, 16S rRNA PCR/sequencing could be considered if SF cultures are negative. If blood, SF, intraoperative cultures, and 16S rRNA PCR/sequencing are all negative, an alternative explanation for the clinical presentation may be considered, with the decision to continue or stop antimicrobial therapy being based on clinical judgement on a case-by-case basis and not solely on the molecular result. A proposed algorithm for utilization of 16S rRNA PCR/sequencing in CIED infections is presented in Figure 2.

There are no publications regarding the utility of 16S rRNA PCR/sequencing method in the diagnosis of CIED infection. However, the usefulness of 16S rRNA PCR/sequencing

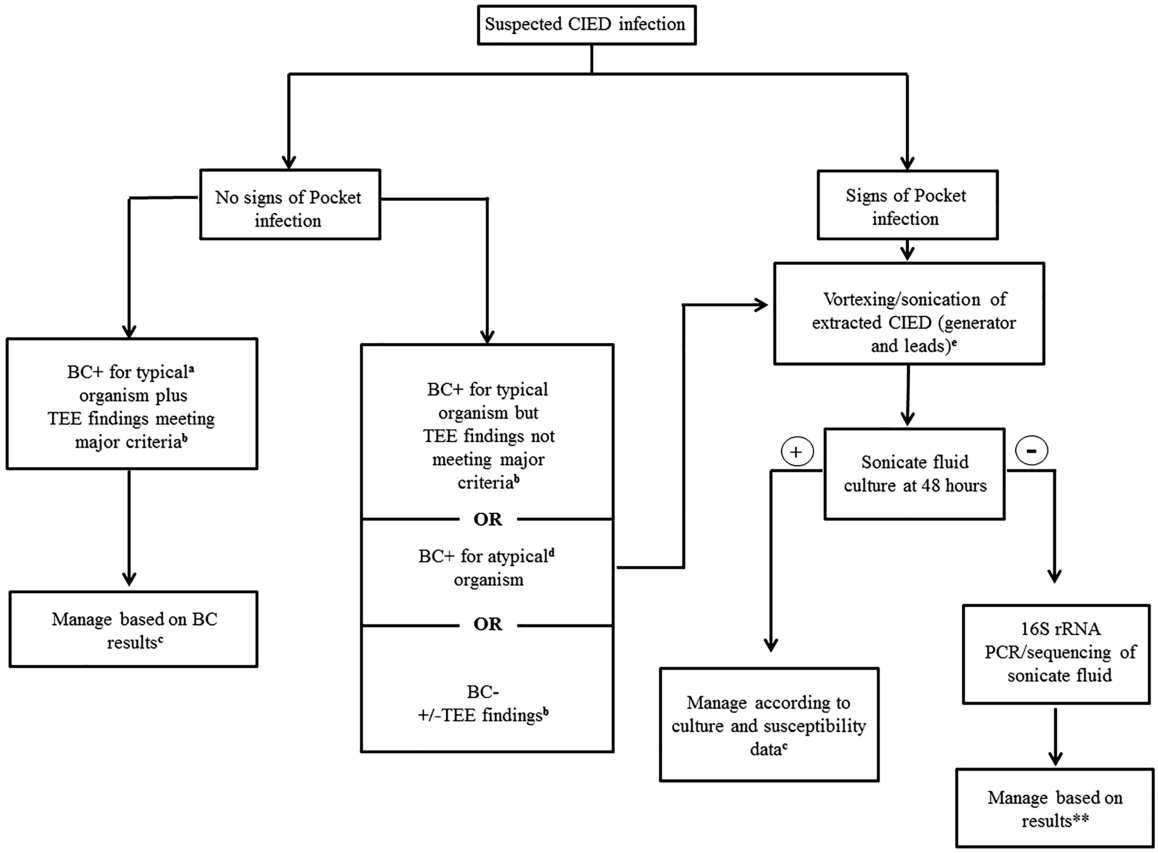

Figure 2. Proposed algorithm for utilization of 16S rRNA PCR/sequencing in the diagnosis of CIED infection in clinical practice. Abbreviations: BC, blood culture; CIED, cardiovascular implantable electronic device; PCR, polymerase chain reaction; rRNA, ribosomal RNA; TEE, transesophageal echocardiogram. [a]Typical organisms causing CIED infection: *Staphylococcus aureus*, coagulase negative *Staphylococcus* species, and *Enterococcus* species. [b]See Table 1 for TEE criteria. [c]Refer to American Heart Association CIED infection guidelines for further details on management. [d]Atypical organisms: other than *S. aureus*, coagulase negative staphylococci, or enterococci. [e]Vortexing/sonication: see Supplementary Methods. **If discrepant results between sonicate fluid culture, BC, and/or 16S rRNA PCR/sequencing are found, infectious diseases consultation is recommended for guidance.

performed on extracted heart valve tissues has been described, with sensitivities and specificities ranging from 61–96% and 77–100%, respectively [9, 13, 14]. The lower sensitivity of this molecular method in SF from extracted CIEDs compared with valve tissues likely relates to microbial load; in contrast to CIEDs where molecular tests are performed on dislodged biofilms, heart valve tissues generally have a higher burden of organisms circumscribed in a focal location.

Despite the risk of false-positive results due to exogenous DNA contamination, the specificity of 16S rRNA PCR/sequencing was similar to that of SF culture. Consistent with previously reported epidemiologic data on CIED infections, a majority, including SF culture-negative cases, were due to *Staphylococcus* sp. [15, 16]. A scant number of culture-negative cases were due to fastidious organisms (*C. acnes* [2 of 28], *Mycobacterium* sp. [1 of 28]).

Our observations support findings of reports that suggest that antibiotic exposure affects culture yield but not that of 16S rRNA PCR/sequencing [17]. We demonstrated that patients who received antibiotics prior to device extraction were more likely to have negative SF cultures compared with 16S rRNA PCR/sequencing results. Consequently, this method may be useful as an alternative to culture, particularly with antecedent antimicrobial therapy.

One limitation of the study is the retrospective nature of the design, with analysis of samples that had been stored for several years. SF culture was performed on a fresh specimen, whereas 16S rRNA PCR/sequencing was tested years later. Prospective studies are needed to evaluate the effect of specimen age on the results reported. Another limitation is the inability of the technology applied to decipher mixed sequences; this could be addressed using massive parallel sequencing in future studies.

Remarkably, of 10 SF culture–positive/16S rRNA gene PCR/sequencing–positive discordant cases, 5 had growth of *C. acnes* in SF culture while 16S rRNA PCR/sequencing yielded *Staphylococcus* sp. Three of these cases had positive blood cultures for *S. aureus*, suggesting that 16S rRNA PCR/sequencing detected a true pathogen missed by SF culture. Of note, culture-based methods may also select for certain bacteria, especially in cases of antecedent antibiotic therapy, due to differential effects

on one of the organisms. Interestingly, all 5 of these cases had received vancomycin therapy prior to device removal, with all *S. aureus* isolates showing susceptibility to this antibiotic, which could explain why this organism was not detected by SF culture. Another potential explanation for discrepant detection is difficulty in culturing the organisms present in biofilms. Last, contamination during sample processing or device extraction could also account for these results. We demonstrated that concordance of 16S rRNA PCR/sequencing with blood cultures is higher than that of SF culture (65 [89.0%] vs 47 [79.6%], $P = .002$). For cases in which the organism detected in blood differed from that identified by SF culture or 16S rRNA PCR/sequencing, a possible explanation is bacteremia from a non-CIED source, which is especially plausible in those with probable or possible CIED infection. Contamination of the device at removal and asymptomatic colonization are other potential explanations.

There were 10 cases in which 16S rRNA PCR/sequencing was negative but SF culture was positive. Six of these had *C. acnes* in SF cultures. Since this organism is ubiquitous in the environment, contamination during sample collection or processing is possible. DNA degradation and/or the presence of PCR inhibitors are potential reasons for negative 16S rRNA PCR/sequencing results. In 7 out of these 10 cases, 16S rRNA PCR was positive (Cp of ≤32) but sequencing was deemed negative because of uninterpretable results, which can occur with polymicrobial infections or with copy variants between individual 16S rRNA genes within the same bacterium (cases 37, 59, 65, 189, 218, 221, and 240).

SF culture– and 16S rRNA PCR/sequencing–positive cases in the noninfected group could be due to environmental contamination or asymptomatic colonization of the devices.

We had a large number of presumed CIED infection cases in which 16S rRNA PCR/sequencing and culture were negative. Although we utilized previously published criteria incorporating clinical, imaging, and operative data to define CIED infected cases, in the absence of a gold-standard test, comparing the performance of 2 methods for microbial diagnosis raises the question as to whether all cases classified as infected were infected. This is especially true for patients with systemic symptoms, no local signs of pocket infection, and no other alternative source of infection at the time of hospitalization. These cases were categorized as having CIED infection; however, there is a possibility that an unidentified alternative source of infection was present. It is also possible that cases who presented with nonspecific systemic symptoms, negative blood cultures (presumably due to prior antibiotic therapy), and "vegetations" on indwelling leads may have harbored bland clots, as current echocardiographic procedures cannot distinguish between the two [18]. In addition, patients presenting with BSI from non-CIED sources where the device was thought to be secondarily seeded were classified as having probable or possible CIED infection; however, given negative cultures and PCR, this may not always be the case. Without standardized criteria to define CIED infection, these challenges remain.

Another possibility is nonbacterial etiologies of CIED infection. Mycobacterial and fungal-specific tests were rarely performed on subjects reported herein, and the molecular technology employed only detects bacterial organisms (which do include mycobacteria). There may also be noninfectious causes of inflammation at the pocket site (eg, allergic reaction to device components) that could account for some microbiologically negative cases [19]. Finally, microbial concentrations below the lower limit of detection of 16S rRNA PCR/sequencing and uninterpretable mixed sequences could account for undetected cases (Table 3, footnote b).

Despite these limitations, results of our study suggest that 16S rRNA PCR/Sanger sequencing may have a role in the diagnosis of CIED infection, especially in culture-negative cases.

Conclusions

16S rRNA PCR/sequencing has a higher sensitivity to detect a potential pathogen in SF from extracted CIEDs compared with SF culture. Therefore, it may be reasonable to pursue 16S rRNA PCR/sequencing in cases in which cultures are negative. Moreover, given its higher concordance with blood culture results as compared with SF culture, 16S rRNA PCR/sequencing testing may be a potential adjunctive test to confirm CIED as a source of BSI.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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