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ORIGINAL INVESTIGATION

Clinical utility of sonication for diagnosing infection and colonization of cardiovascular implantable electronic devices

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Abstract

Our study aimed to evaluate the sensitivity of the sonication tool for the microbiological diagnosis of cardiovascular implantable electronic device infections (CIEDIs). The extracted cardiac implants of 52 patients were assessed: 19 with CIEDI and 33 with elective generator replacement or revision without clinical infection. Sonication fluid culture of explanted CIEDs yielded higher numbers of microorganisms than pocket tissue or swab cultures. The sensitivity of sonication fluid culture was significantly higher than that of pocket swab and tissue culture for microbiological diagnosis of CIEDI. The microorganisms isolated most frequently via sonication of explanted CIEDs were Gram-positive cocci (70%), of which 50% was coagulase-negative *Staphylococcus*. Sonication fluid culture detected colonization in 36.4% of the non-infected patients. Sonication fluid culture represents a promising diagnostic strategy with increased sensitivity compared to conventional culture methods for microbiological diagnosis of cardiac devices associated with infection and colonization.

Keywords Sonication · Infection · Colonization · Cardiovascular implantable devices

Abbreviations

CRTDs	Cardiac resynchronization devices
CIEDIs	Cardiovascular implantable electronic device infections
CIEDs	Cardiovascular implantable electronic devices
CoNS	Coagulase-negative *Staphylococcus*
CFU	Colony-forming units
CIs	Confidence intervals
ICDs	Implantable cardiovascular defibrillators
LVEF	Left ventricular ejection fraction
MRSA	Methicillin-resistant *Staphylococcus aureus*
NICIEDs	Non-infected CIEDs
PPMs	Permanent pacemakers
PTC	Pocket tissue culture
SCF	Sonicate fluid culture
SWC	Swab culture

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Introduction

Over the past decades, cardiovascular implantable electronic devices (CIEDs), including permanent pacemakers (PPMs), cardiac resynchronization devices (CRTDs), and implantable cardiovascular defibrillators (ICDs), have been increasingly indicated worldwide for patients with ventricular arrhythmia, bradycardia, and heart failure, and for preventing sudden cardiac death [1, 2]. Such tools have reduced morbidity and mortality in cardiac patients and have proven cost-effective [3, 4]. In parallel, CIED infections (CIEDIs) have expanded at a rate that seems to have an augmented disproportionate pattern to the increased rate of the newly implanted devices [5]. CIEDIs are life-threatening conditions that increase morbidity and mortality risks and create a considerable cost burden on the health care system [6]. Device infection may be acquired due to generator or lead contamination at the time of implantation or replacement of the CIED system,

device erosion via intact skin, or hematogenous spread secondary to bloodstream infection [7]. CIEDIs are often challenging to treat, as intracardiac and extracardiac components can also become contaminated, necessitating complete system removal with the risk of death or serious complications [8]. Asymptomatic bacterial colonization of cardiac devices is prevalent and increases the threat of implant infection, which requires early diagnosis for implementing effective preventive measures and reducing infection [9]. Due to the high variability of CIEDI clinical findings, low accuracy of echocardiography, and poor sensitivity of blood and conventional peri-implantation tissue or fluid cultures, CIEDI diagnosis is challenging [10, 11]. False-negative microbiological outcomes ranging from 12 to 49% have been correlated with prior use of antibiotics and the existence of biofilm-mediated infection in which sessile bacteria are trapped in a polymeric matrix with altered morphology and gene expression on the implanted device surface, resulting in problems for the immune system [12]. Sonication, which uses ultrasound waves to mechanically disrupt biofilm on device surfaces, leading to the release of the fixed bacteria into the sonication fluid, has been indicated in the orthopedic setting for microbiological diagnosis of prosthetic joint infections and has higher sensitivity than conventional periprosthetic tissue culture [13]. The other advantages of sonication include the ability to perform molecular and immunological testing on the sonication fluid, quantifying the number of microorganisms, and detecting polymicrobial growth [14].

The present study was planned with the following objectives: (1) to evaluate the sensitivity of the sonication tool as compared to traditional culture methods for the microbiological diagnosis of CIEDIs; (2) to assess the utility of the sonication tool for detecting bacterial colonization of cardiac devices in patients who had undergone cardiac generator revision or replacement without signs of infection.

Subjects and methods

This was a cross-sectional descriptive study that involved 53 patients with PPMs and ICDs who had undergone surgical removal of a CIED because of infection or any other cause at the Cardiology Department of a tertiary university hospital between January 2018 and May 2019.

Infection was defined as either local infection of the generator pocket (acute inflammation with erythema, local warmth, tenderness, swelling, wound dehiscence, or purulent drainage with skin erosion) or definite CIED-associated infective endocarditis as defined by the Duke criteria [15, 16].

Generators (and leads, if removed) were aseptically extracted in the catheterization lab theater, collected in airtight containers, coated with 0.9% NaCl to prevent drying out, and submitted to culture after sonication. Implants were excluded if gross contamination occurred through extraction, transportation, or even during processing in the microbiology laboratory. Conventional and sonication fluid cultures were done in duplicate for each specimen. Five sterile cardiac systems were analyzed as negative controls and subjected to the same procedures.

Conventional microbiological methods

After the device had been removed, intraoperative generator deep-pocket samples were collected using a sterile fiber swab that had been moistened with sterile saline, then transported in Amies agar; in addition, a piece of the fibrous capsule was obtained for tissue culture. The tissue was homogenized for 1 min in 3 mL brain–heart infusion broth. The cotton swabs and homogenized tissue were inoculated to sheep blood agar, chocolate agar, and MacConkey agar, and incubated aerobically at 35–37 °C in 5–7% CO_2 for 48 h. An additional sheep blood agar plate was anaerobically incubated at 37 °C for 14 days. Microorganisms were identified by their growth characteristics, Gram-stained smears and standard biochemical workup and tested for their antibiotics susceptibility using Kirby–Bauer method.

CIED sonication

In the microbiology laboratory, sonication was performed as previously described [17]. Containers with the extracted implants were handled within 6 h of removal. The container was vortexed for 30 s, sonicated for 1 min at 40 kHz, and vortexed for 30 s at room temperature. A total of 0.1 mL of the resulting sonication fluid was inoculated onto aerobic and anaerobic sheep blood agar plates and checked daily for bacterial growth. Microorganisms were quantitated (i.e., number of colony-forming units [CFU]/mL sonication fluid), identified and tested for their antibiotic susceptibility using routine microbiological procedures. The growth of at least 20 CFU of the same bacteria from any plate was considered a positive sonication fluid culture.

Ethical approval

The Institutional Review Board approved the study protocol (IRB), Faculty of Medicine, code number: R/20.90.1003.

Statistical analysis

IBM SPSS Statistics for Windows (version 25.0) was used for analyzing study data. The Student t test and Mann–Whitney *U* test were used for comparing continuous variables. Categorical variables were compared using the chi-square test or Fisher's exact test. The sensitivity, specificity, positive

predictive value, and negative predictive value of the tissue, swab, and sonication fluid cultures were compared using the McNemar test of paired proportions. 95 percent confidence intervals (CIs) were calculated as exact binominal CIs. $P < 0.05$ (two-tailed) was considered significant for comparing differences.

Results

53 CIEDs were extracted from 53 patients due to insufficient battery charge of the device ($n = 25$), infection ($n = 19$), or device upgrading ($n = 9$). Due to apparent contamination detected during implant excision, one patient with insufficient battery charge was excluded from further study. In the total 52 implants collected, 48 (92.3%) were PPMs, and 4 (7.7%) were ICDs. 19 patients (36.5%) of the total patients included in the study met the CIED infection criteria, and 33 patients (36.5%) had non-infected CIEDs (NICIEDs). Generator pocket infection and device-related endocarditis were detected in 12 (63.2%) and 7 (36.8%) patients, respectively. The baseline characteristics of the 52 patients included in this study are presented in Table 1. Overall, sonicate fluid culture of the explanted CIEDs detected higher numbers of microorganisms than the pocket tissue and swab cultures (30 vs. 9 samples; $P < 0.001$).

In the CIEDI group, sonication fluid yielded positive culture for 18 patients (94.7%); device pocket traditional tissue culture was positive in 10 patients (52.6%), and only 6 patients (31.6%) had positive intraoperative pocket swab cultures. Conclusive microbial diagnosis was achieved for ten patients (52.6%) in the CIEDI group via a combination of the results of sonication of the extracted devices and pocket tissue/swab cultures. All positive cultures were concordant with each other, except for one swab sample that detected *Micrococcus* species as compared to no bacterial growth obtained by sonication fluid and tissue culture. In the NICIED group, all positive cultures (colonization) were concordant, and no pathogens were isolated among 11 (33.3%) patients. Colonization in the NICIED group was detected through SFC in 12 patients (36.4%), tissue culture in 5 patients (15.2%), and swab culture in 3 patients (9.1%), as illustrated in Table 2.

Regarding the duration of antimicrobial therapy, 18 of the 19 CIEDI patients (94.7%) had taken antibiotic therapy within 14 days before cardiac device excision. Of the 18 patients, sonication succeeded in detecting microorganisms in 94.4% of the patients (17/18), while tissue and swab cultures detected microorganisms in 55.6% (10/18) and 33.3% (6/18) of the patients, which was statistically highly significant ($P = 0.008$ and $P = 0.002$, respectively). Sonication also identified microorganisms in the only infected patient without previous antibiotic treatment, which was not the case for tissue and swab cultures. No NICIED patient had taken antibiotics before implant revision.

Table 3 shows that sonicate fluid culture had significantly higher sensitivity than swab and tissue cultures for microbiological diagnosis among 19 CIEDI patients (94.74% vs. 31.58% and 52.63%; $P < 0.001$ and $P = 0.008$, respectively). Conversely, swab and tissue cultures had significantly

Table 1 Baseline characteristics of all patients

	Subjects with CIEDI ($n = 19$)	Subjects with NICIED ($n = 33$)	*P* value
Demographic characteristics			
Age, years, median (range)	65 (35–74)	66 (38–77)	0.731
No. (%) male	15 (78.9)	17 (51.5)	0.050
No. (%) female	4 (21.1)	16 (48.5)	
Duration of CIED use, months (mean)	15.684	55.484	< 0.001
Clinical characteristics, *n* (%)			
Diabetes mellitus	17 (89.5)	17 (51.5)	0.006
Coronary diseases	8 (42.1)	13 (39.4)	0.848
Hypertension	15 (78.9)	23 (69.7)	0.469
Chronic renal failure	9 (47.4)	9 (27.3)	0.142
Heart failure	14 (73.9)	23 (69.7)	0.760
Smoking	11(57.9)	11(33.3)	0.084
Previous CIED infections	3 (15.8)	0 (0)	0.039
Left ventricular ejection fraction (LVEF) (mean)	53.5	53.9	0.844
No. (%) with the following indication for device implantation			
Atrioventricular block	11 (57.9)	27 (81.8)	0.163
Sick sinus syndrome	6 (31.6)	4 (12.1)	
Ventricular tachycardia	2 (10.5)	2 (6.1)	

Table 2 Microorganisms isolated from sonication fluid, pocket tissue, and swab cultures among patients with CIEDI and NICIED (colonized)

Subject group and culture results	No. of patients	Microorganisms (no. of isolates)
CIEDI subjects	19	
Positive SFC, PTC and SWC	5	CONS (2), MRSA (2), *Pseudomonas aeruginosa* (1)
Positive SFC and PTC	5	CONS (2), *Staphylococcus aureus* (1), *Klebsiella pneumoniae* (1), *Escherichia coli* (1)
Positive SFC only	8	CONS (4), *Klebsiella pneumoniae*(1), *Serratia marcescens* (1), *Proteus mirabilis* (1), polymicrobial {(*Proteus mirabilis* & *Klebsiella pneumoniae* (1[a])}
Positive SWC only	1	*Micrococcus* spp.
NICIDs subjects	33	
Positive SFC, PTC and SWC	3	CONS (2), *Staphylococcus aureus* (1)
Positive SFC and PTC	2	CONS (2)
Positive SFC only	7	CONS (3), *Staphylococcus aureus* (1), *Streptococcus* spp. (1), *Candida* spp. (1), *Klebsiella pneumoniae* (1)

Sonicate fluid culture (SCF), Pocket tissue culture (PTC), Swab culture (SWC), Coagulase-negative *Staphylococcus* (CONS)

[a]Only one CIEDI patient had a polymicrobial infection (*Proteus mirabilis* and *Klebsiella pneumoniae* on SFC)

Table 3 Compared sensitivities and specificities of tissue culture and swab culture relative to sonicate fluid culture

	Sensitivity (95% CI)	*P* value	Specificity (95% CI)	*P* value	PPV (95% CI)	NPV (95% CI)
Swab culture	31.58 (6/19) CI: 12.58–56.55	< 0.001	90.91 (30/33) CI: 75.67–98.08	0.004	66.67 CI: 36.06–87.64	69.77 CI: 62.53–76.14
Tissue culture	52.63 (10/19) CI: 28.86–75.55	0.008	84.85 (28/33) CI: 68.10–94.89	0.016	66.67 CI: 44.52–83.29	75.68 CI: 65.47–83.62
Sonicate fluid culture	94.74 (18/19) CI: 73.97–99.87		63.64 (21/33) CI: 45.12–79.60		60 CI: 48.55–70.46	95.45 CI: 75.39–99.31

PPV positive predictive value, *NPV* negative predictive value, *CI* confidence interval

The swab and tissue culture methods' specificities were compared relative to sonicate fluid culture via McNemar's paired proportions test

better specificity than sonicate fluid culture, i.e., 90.91% ($P = 0.004$) and 84.85% ($P = 0.016$), respectively.

Discussion

The correct clinical and microbiological diagnosis is critical for proper diagnosis and antibiotic management of pacemakers and cardiovascular implantable systems infections [18]. Even though tissue and swab cultures are usually performed clinically due to their simplicity, they are unable to identify the causative microbial agents in up to one-third of CIEDIs [19, 20]. Various studies have proven the superiority of sonication for identifying the causative pathogens of device-related orthopedic infections, particularly in patients with antibiotic treatment [21–23].

Mason et al. [17] showed that pacemaker and ICD generator ultrasonication improved the pocket infection diagnosis rate overdiagnosis by tissue and swab culture alone. In the present study, swab cultures identified pathogens in 6 patients (31.6%), and pocket tissue cultures yielded 10 positive results among 19 infected patients (52.6%). The low sensitivity of the swab and tissue cultures may be partially explained by the use of antibiotics during sample collection in 94.7% of the infected patients. Also, sonication isolated microorganisms in one infected patient who was not under antibiotic treatment, while swab and tissue cultures did not. Clearly, sonication increases diagnostic yield even with the use of antibiotics and with high statistical significance compared to tissue and swab cultures from infected patients ($P = 0.008$ and $P = 0.002$, respectively). This is in accordance with other studies that have hypothesized that CIEDIs are usually caused by low-virulence, biofilm-forming organisms that attach to PPM and ICD surfaces, making their detection easier through sonication [10, 22, 24]. Moreover, the higher sensitivity of sonication can be explained by the fact that all excised CIED systems (generator and leads) were obtained together in the same container, thus the sonication ultrasound dislodged great amounts of bacteria from the biofilm [11].

In line with other authors [12, 25], the most common pathogens isolated from the CIEDIs were gram-positive bacteria (both Coagulase-negative *Staphylococcus* and *S. aureus*). Here, *Staphylococcus* species were responsible for

most CIEDIs (11/18, 61.1%), encouraging the theory that wound contamination during implantation of the device is critical in creating the subsequent infection. Moreover, the biofilm formation is a cornerstone in the development of CIEDIs, demonstrating a survival instinct through which microorganisms can attach to foreign implants, resisting antibiotics and the host defense system 1000 times more than their planktonic forms. Also, Gram-negative bacteria were recorded as the causative agents of CIEDIs at a relatively higher rate, in agreement with other data [11, 26], which has a significant therapeutic indication. In our institution, empirical treatment usually does not cover Gram-negative bacilli.

Asymptomatic bacterial colonization has been associated with increased risk of developing future CIEDI, with rates varying from 21 to 27% when traditional cultures were used for isolating microorganisms [17, 27]. However, molecular methods detected microorganisms in 38.5–47.2% of uninfected CIEDs [28, 29]. In the present study, patients undergoing elective generator replacement or revision with no symptoms or clinical signs of infection had cultured microorganisms in 36.4% of CIEDs subjected to sonication, 15.2% of pocket tissue, and 9.1% of pocket swab cultures. Moreover, Oliva et al. [10] found that sonication of explanted systems was more sensitive than conventional culture for detecting bacteria in non-infected patients. The predominant microorganisms obtained in colonized patients were classified as belonging to the skin flora [28, 29]. We obtained similar results, where CoNS (58.3%) predominated over the other organisms.

The question of whether the isolation of microorganisms on devices undergoing elective revision indicates actual colonization, future clinical pocket infection, or possibly contamination is still being researched [11, 18]. We conducted all cultures in duplicate to eliminate the chance of contamination during laboratory processes. The biochemical and antibiotic susceptibility profiles of all bacteria found using sonication and conventional cultures were identical. In addition, five sterile cardiac systems were subjected to the same procedures as negative controls to exclude the laboratory contamination; no bacterial growth was identified among them.

Regarding the clinical characteristics of the infected patients, generator pocket infection was present in 63.2% of CIEDIs, while device-related endocarditis was detected in 36.8%. This was concordant with the results of Rohacek et al. [27], who documented that 66% of CIEDIs were related to pocket infection. Considering the infection time, most infected patients in our study presented early, i.e., within 12 months of implantation, which was in accordance with a prior study [30] but not with another that stated that two-thirds of patients with lead-associated endocarditis presented after 1 year [31].

Besides, 89.5% of CIEDI patients were diabetics versus 51.5% of NICIED patients ($P = 0.006$), so diabetes mellitus can be considered a risk factor for CIEDI, as previously documented [32].

The limitations of the present study are broadly present in the small sample size, besides the non-blinded involvement of infected and non-infected patients. Furthermore, it was challenging to assess the influence of bacterial colonization on the consequent generation of clinical infection due to the absence of prolonged follow-up. Moreover, we might not have detected highly fastidious organisms due to the absence of molecular methods.

In conclusion, we demonstrate that sonication has higher sensitivity compared to pocket tissue or swab cultures for both infected and non-infected cardiac devices, introducing it as a diagnostic tool for the microbiological diagnosis of CIEDIs, especially among patients taking systemic antibiotics. It is also helpful for detecting colonized CIEDs in patients intending elective device revision without clear infection signs.

Author contributions MSAH, AHE, KS conceptualized and designed the study protocol development, assessment, and writing the manuscript. MSAH, KS and AE designed the data collection instrument and coordinated and supervised data collection and statistical analysis. AHE performed laboratory analysis. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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Declarations

Conflict of interest There is no conflict of interest for this study.

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