ESC European Society of Cardiology

Europace (2023) **25**, 578–585
https://doi.org/10.1093/europace/euac228

CLINICAL RESEARCH

Fluorescence *in situ* hybridization and polymerase chain reaction to detect infections of cardiac implantable electronic devices

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Received 22 July 2022; accepted after revision 23 October 2022; online publish-ahead-of-print 8 December 2022

Aims

In patients with infections of cardiac implantable electronic devices (CIEDs), the identification of causative pathogens is complicated by biofilm formations and previous antibiotic therapy. In this work, the impact of an additional fluorescence *in situ* hybridization (FISH), in combination with polymerase chain reaction and sequencing (FISHseq) was investigated.

Methods and results

In 36 patients with CIED infections, FISHseq of explanted devices was performed and compared with standard microbiological cultivation of preoperative and intraoperative samples. The mean age was 61.9 (±16.2) years; 25 (69.4%) were males. Most patients (62.9%) had heart failure with reduced ejection fraction. Infections occurred as endoplastits ($n = 26$), isolated local generator pocket infection ($n = 8$), or both ($n = 2$); CIED included cardiac resynchronization therapy defibrillator ($n = 17$), implantable cardioverter defibrillator ($n = 11$), and pacemaker ($n = 8$) devices. The overall positive FISHseq detection rate was 97%. Intraoperatively, pathogens were isolated in 42 vs. 53% in standard cultivation vs. FISHseq, respectively. In 16 of 17 FISHseq-negative patients, the nucleic acid strain DAPI (4′,6-diamidino-2-phenylindole) indicated inactive microorganisms, which were partially organized in biofilms ($n = 4$) or microcolonies ($n = 2$). In 13 patients in whom no pathogen was identified preoperatively, standard cultivation and FISHseq identified pathogens in 3 (23%) vs. 8 (62%), respectively. For the confirmation of preoperatively known bacteria, a combined approach was most efficient.

Conclusion

Fluorescence *in situ* hybridization sequencing is a valuable tool to detect causative microorganisms in CIED infections. The combination of FISHseq with preoperative cultivation showed the highest efficacy in detecting pathogens. Additional cultivation of intraoperative tissue samples or swabs yielded more confirmation of pathogens known from preoperative culture.

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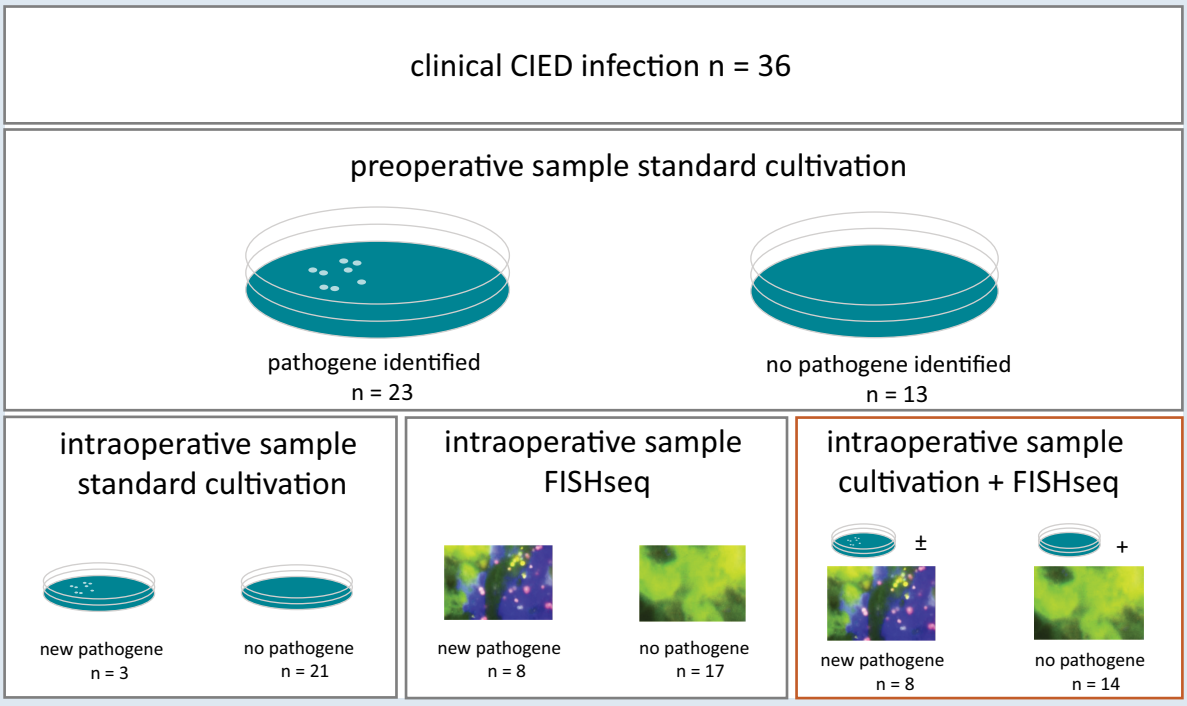
Graphical Abstract



Keywords FISHseq • Fluorescence *in situ* hybridization • CIED infection • Endoplastitis • Device infection

What's new?

- Fluorescence *in situ* hybridization sequencing (FISHseq) is a valuable diagnostic tool to detect pathogens in cardiac implantable electronic device infections.
- The overall positive FISHseq detection rate is 97%.
- Fluorescence *in situ* hybridization sequencing is especially effective in patients in whom preoperative standard cultivation fails to identify pathogens.
- A combined approach of FISHseq and standard cultivation shows the highest efficacy in detecting or confirming causative microorganisms.

Introduction

Cardiac implantable electronic devices (CIEDs), including pacemakers (PMs), cardiac resynchronization therapy (CRT), and implantable cardioverter defibrillators (ICDs), are potentially life-saving treatments for a continuously expanding field of cardiac diseases.[1]

Despite recommended preventive strategies, infection rates are increasing disproportionately compared with implantation rates.[2] The risk of infection following primary ICD implantation is 1.7% in the first 6 months and even higher for CRT implantations. In replacement or revision procedures, the risk increases two- to four-fold.[3,4] Cardiac implantable electronic device infections are associated with a negative impact on patient outcome and represent a financial healthcare burden.[5,6] Local infections cause an in-hospital mortality of 2–5%, which increases up to 15% in case of systemic infection.[7–9]

Initiation of effective antibiotic therapy and complete device explantation are key to successful treatment. Detection of the causative pathogen should be pursued vigorously by repeated cultivation of blood, tissue, or fluid samples, as well as an analysis of leads, vegetations, and pocket tissue collected in extraction procedures.[10] However, in up to 49%, microbiological analysis fails to identify a pathogen due to previous antibiotic treatment, difficult-to-cultivate microorganisms, or biofilm-associated infections.[11]

Fluorescence *in situ* hybridization (FISH), in combination with polymerase chain reaction (PCR) and sequencing (FISHseq), is a valuable diagnostic approach in identifying and visualizing the morphology, quantity, and grade of activity of microorganisms in valvular endocarditis or bloodstream infections.[12,13]

This analysis investigates the impact of FISHseq on patients with CIED infections in addition to standard microbiological diagnostics.

Methods

Study cohort and data collection

We retrospectively analysed data from 36 adult patients with CIED infections who were hospitalized for treatment at the German Heart Center Berlin between July 2016 and July 2018. All patients had received both standard microbiological diagnostics and FISHseq analysis. Patient baseline characteristics; clinical, echocardiographic, and microbiological findings; antibiotic therapy; surgical data; FISHseq results and outcome parameters including survival, need for redo surgery, and re-hospitalization rates were recorded in an electronic database. Outcome data were examined by reviewing institutional clinical databases and follow-up phone calls of the patients or their general practitioners. This analysis was approved by the ethics committee of Charité—Universitätsmedizin Berlin (EA 2/2018/18).

Perioperative diagnostics and treatment

Diagnostics and treatment were performed according to the current European and international guideline recommendation.[1] Most patients were referred from other hospitals.

If septic-haemodynamic status allowed, samples were repeatedly collected for preoperative standard microbiological cultivation before the initiation of antibiotic treatment or after an antibiotic-free period of at least 48 h. Overall, preoperative samples were analysed in 34 patients. In one patient, who was urgently admitted for surgery and died in septic shock shortly after admission, no information about preoperative sampling was retrospectively available. Another patient was admitted with traumatic lead dislocation and no clinical signs of infection. Intraoperatively, the generator pocket showed purulent infection.

In patients with isolated generator pocket infections, each one swab was collected for preoperative conventional culture.

Antibiotic treatment was initiated empirically and adapted to antibiogram if available. In most patients, the empirical treatment was chosen individually depending on the initially suspected focus. Commonly, the escalation to guideline recommended endocarditis therapy was initiated simultaneously to the transfer for urgent device explantation.

Surgical management and intraoperative microbiological sampling

Explantation of the complete device, including all transvenous leads, was performed by the same experienced cardiac surgeon in general anaesthesia. Median duration of surgery was 95.5 min [interquartile range (IQR) 70.5–143.75 min]. The lead extraction was performed by a transvenous approach with the use of rotational extraction sheaths in 28 cases and a simple polypropylene extraction sheath in 1 case. In seven cases, leads were explanted without the need for specific extraction tools due to short durations since implantation. Vacuum-assisted wound closures were used in 11 (30.6%) patients with severe pocket infections for a median of 10.0 days (IQR 6.0–12.5 days). Tissue samples and swabs were taken intraoperatively for conventional microbiological analysis and explanted CIEDs were analysed by FISHseq in all patients. *Figure 1* presents a flow chart of the clinical sampling procedure.

Fluorescence *in situ* hybridization sequencing

Segmented CIED leads were prepared: samples were fixed and embedded in cold-polymerizing resin. Two micrometres of samples were analysed by FISH as previously described.[13] First, samples were screened with pan-bacterial 16S rRNA-directed probe (EUB338) and 18S rRNA-directed probe (EUK516) to detect bacteria and Eukarya for particular yeasts. Positive FISH signals were reviewed using a nonsense probe (NON338) to exclude unspecific probe binding.[14] If microorganisms were detected, a panel of FISH probes was applied for identification on genus- or species-specific levels. DAPI (4′,6-diamidino-2-phenylindole), as a nucleic acid stain that visualizes nucleic acids, was applied as a counterstain to visualize host cell nuclei and bacteria, even if these contained too few ribosomes to elicit a positive FISH signal. The FISH signal has been shown to directly correlate to the ribosome content, and therefore the state of activity, of the bacteria.[15,16] The absence of an FISH signal indicated inactive (dead or resting) bacteria, for example in resting zones in biofilms or upon antibiotic treatment. In DAPI positive, FISH-negative cases, identification of the bacterial species was obtained by pan-bacterial PCR amplification of part of the 16S rRNA gene and sequencing of the PCR product. Both FISH and PCR data points were interpreted together.

From sections, consecutive to the ones used for FISH, DNA was extracted (Amplicor; Roche Molecular Systems Inc., Branchburg, NJ, USA), and broad-range PCR amplification and sequencing of part of the 16S rRNA gene were performed as described.[17] Sequences were analysed using the SmartGene Centroid database (SmartGene Inc., Switzerland). An example for FISHseq is presented in *Figure 2*. The time from the receipt of the sample to the first results was 36 h.

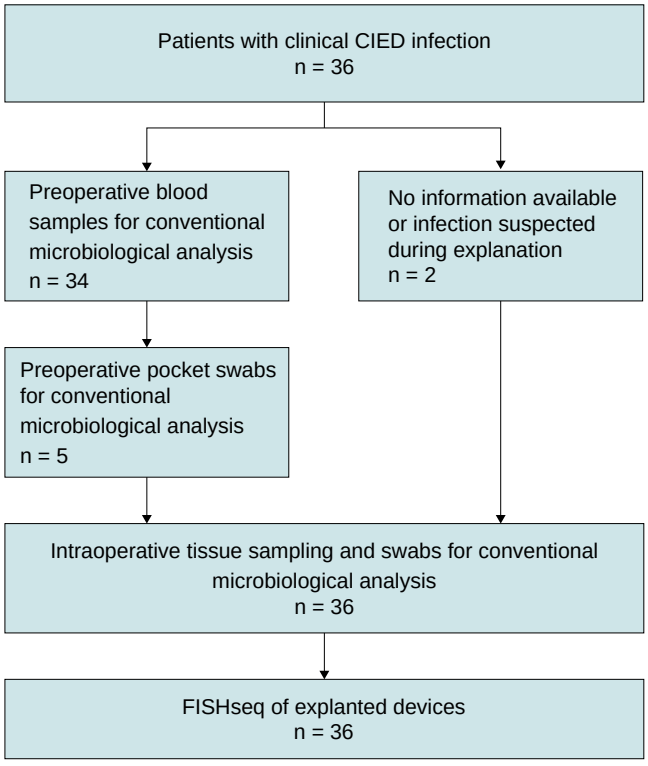


Figure 1 Flow chart of a sampling procedure. CIED, cardiac implantable electronic device; FISHseq, fluorescence *in situ* hybridization sequencing.

Results

Patient baseline characteristics

Within the observational period, 36 patients with CIED infections were treated in our institution. Eight (22.2%) patients had a PM, 11 (30.6%) had an ICD, and 17 (47.2%) had a CRT defibrillator (CRT-D) device. The mean age was 61.9 (±16.2) years; 25 (69.4%) were males. Most patients (66.7%) had undergone primary device implantation without a following procedure. Six (14%) patients had one, four (9%) patients had two, one (2%) patient had three, and one (2%) patient had four replacement or revision procedures after implantation and before current CIED infection, including CRT-D upgrading ($n = 7$), generator replacement ($n = 6$), revision due to CIED infection ($n = 5$), and lead implantation for dysfunction ($n = 3$). Five (12%) patients had a previous device infection (*Table 1*).

Cardiac implantable electronic device infections and preoperative treatment

In median, CIED infections occurred 4.7 years (IQR 1.4–9.0 years) after the last device-related procedure. Eight (22%) patients had a local generator pocket infection. In 26 (72%) patients, endoplastitis was diagnosed according to the modified Duke criteria and showed infectious vegetations on CIED leads [visualized echocardiographically: $n = 21$ right ventricular (RV) lead; $n = 4$ right atrial lead; $n = 1$ transvalvular left ventricular (LV) lead in single LV congenital heart disease]. The vegetation size was measured >10 mm in most ($n = 14$, 54%) cases. Two (6%) patients showed both a pocket infection and endoplastitis with RV vegetations.

Most (94%) patients were on antibiotic treatment ($n = 9$ single, $n = 9$ double, $n = 8$ triple, $n = 4$ quadruple, $n = 4$ quintuple) at explantation for a median of 9.0 days (IQR 4.25–16.0 days; see Supplementary material online, *Table S1*). Two patients without signs of systemic infection did not receive antibiotic treatment on the days prior to CIED explantation. One was referred for elective surgery in an antibiotic-free window and one patient was admitted from the emergency department with local pocket infection.

Pathogens identified preoperatively (by conventional microbiological cultivation) and intraoperatively (by conventional microbiological cultivation and fluorescence *in situ* hybridization sequencing)

A total of 39 preoperative blood or fluid samples and swabs were collected in 34 patients. In preoperative blood samples, pathogens were detected in 19 (56%) patients by standard microbiological cultivation. In 17 (50%) patients, blood cultures remained sterile. In patients with negative blood cultures, cultivation of pocket fluid or swabs yielded bacteria in four (12%) patients, including *Staphylococcus epidermidis* ($n = 2$), Propionibacteriales ($n = 1$), as well as *S. epidermidis* and *Candida glabrata* ($n = 1$; *Figure 3A*).

Intraoperatively, swabs, tissue samples, and lead fragments were collected and analysed by standard microbiological cultivation; explanted lead fragments of all patients were analysed by FISHseq.

Pathogens were isolated in 42% ($n = 15$) and 53% ($n = 19$) in standard microbiological cultivation and FISHseq, respectively. In 47% ($n = 17$), FISHseq failed to identify a causative microorganism. From these 17 (47%) patients, 16 (44%) showed a positive DAPI signal, indicating inactive microorganisms (*Figure 3B* and *C*). The microorganisms were

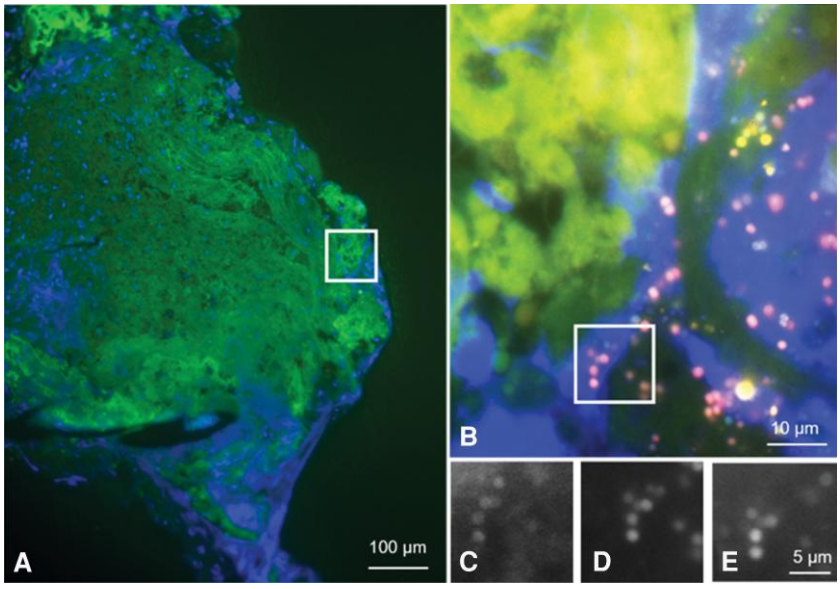


Figure 2 FISH analysis of cardiac tissue adjacent to an ICD lead with *Staphylococcus aureus* group biofilms. (*A*) Overview of the tissue with *S. aureus* group biofilms (blue—DAPI, nucleic acid stain; green—tissue background). (*B*) Magnification of the inset in *A* showing the biofilm in greater detail. Note that some cocci feature more intense FISH signals than others, indicating a different ribosome content (blue—DAPI, nucleic acid stain; green—tissue background and the *Staphylococcus* genus-specific FISH-probe STAPHY in FITC; orange—*S. aureus* group-specific FISH-probe SAU in Cy3; magenta—eubacterial probe EUB338 in Cy5). (*C–E*) Magnification of the inset in *B* displaying the separate single filter sets in black-and-white at the identical position. (*C*) *Staphylococcus* genus-specific FISH-probe STAPHY. (*D*) *Staphylococcus aureus* group-specific FISH-probe SAU. (*E*) Eubacterial probe EUB338. DAPI, 4′,6-diamidino-2-phenylindole; FISH, fluorescence *in situ* hybridization; ICD, implantable cardioverter defibrillator.

Table 1 Demographics $n = 36$

Age, years	61.9 ± 16.2
Female	11 (30.6)
BMI, kg/m^2	27.3 ± 6.1
Device type	
PM	8 (22.2)
ICD	11 (30.6)
CRT-D	17 (47.2)
Leads *in situ*	
1	7 (19.4)
2	8 (22.2)
3	16 (44.4)
4	3 (8.3)
5	2 (5.6)
Total number of device procedures	
1	24 (66.7)
2	6 (16.7)
3	4 (11.1)
4	1 (2.8)
5	1 (2.8)
Indication for PM implantation	
AV block	4 (11.1)
Sick sinus syndrome	4 (11.1)
Indication for CRT-D/ICD implantation	
Primary prevention	21 (58.3)
Secondary prevention	5 (13.9)
Expected high % of ventricular pacing	2 (5.6)
Duration implantation to explantation (years)	4.67 (1.43–9.01)
LVEF	
≥50%	9 (25.7)
41–49%	4 (11.4)
≤40%	22 (62.9)
NYHA class	
No HF	8 (22.2)
I	2 (5.6)
II	3 (7.0)
III	11 (30.6)
IV	4 (11.1)
Medical history of	
Myocardial infarction	15 (41.7)
CIED infection	5 (13.9)

AV, atrioventricular; BMI, body mass index; CIED, cardiac implantable electronic device; CRT-D, cardiac resynchronization therapy defibrillator; HF, heart failure; ICD, implantable cardioverter defibrillator, LVEF, left ventricular ejection fraction; NYHA, New York Heart Association; PM, pacemaker.

detected either in the tissue or on the device ($n = 19$) or formatted in biofilms ($n = 4$) or microcolonies ($n = 2$).

Patients in whom FISHseq did not identify a pathogen were on antibiotic treatment for 10.0 days (IQR 5.0–16.0 days), whereas patients in whom causative pathogens were identified were treated for 8.0 days (IQR 3.0–13.0 days; P=0.281).

Combined diagnostic approach

In 10 (43%) and 8 (34%) patients, in whom pathogens were detected preoperatively, conventional cultivation and FISHseq of intraoperatively collected samples confirmed preoperative microbiological results, respectively. In a combined approach, pathogens were confirmed in 11 (48%) patients.

Standard cultivation of intraoperative samples failed to identify pathogens in 21 (61%) and FISHseq in 17 (47%) patients. All patients were on antibiotic treatment immediately before surgery. Failure of pathogen confirmation remained 39% in a combined approach.

In one patient in whom *Proteus vulgaris* was isolated in preoperative blood cultures, both standard cultivation and FISHseq of intraoperative samples yielded *Staphylococcus haemolyticus/capitis*. In one patient with preoperatively detected *S. capitis* and *S. epidermidis*, cultivation of intraoperative samples identified Propionibacteriales.

In patients in whom no pathogen was identified preoperatively, analysis of intraoperative samples identified new pathogens in three (23%) and eight (62%) patients by conventional microbiological cultivation and FISHseq, respectively. A combined approach did not exceed the rate, compared with the FISHseq results (*Figure 4*).

Overall, the number of patients in whom no pathogen was identified, was reduced from 13 (36%) preoperatively to 10 (28%) by standard microbiological cultivation of intraoperative samples and to 5 (14%) by FISHseq (*Figure 5*).

Patients' outcome

After CIED explantation, most (86%) patients were discharged to another hospital and four (9%) patients were discharged home.

Two (5.6%) patients were re-hospitalized due to persisting or recurrent device infections. One patient with restrictive cardiomyopathy secondary to infectious pericarditis had a complete PM explantation for RV lead endoplastitis and re-implantation of an epicardial LV lead with a generator in contralateral position. Five months later, the patient was hospitalized in septic shock due to a severe CIED re-infection. Despite an adequate antibiotic treatment and PM explantation, the patient died in prolonged shock. In another patient, an ICD was explanted for endoplastitis and reimplanted after 3 months. Five weeks later, the patient presented with temperature and swelling of the generator pocket; in echocardiography, lead vegetations were detected. Again an explantation and a consecutive implantation of a subcutaneous ICD were performed. No signs of infection reoccurred.

Overall, in-hospital mortality, 30-day mortality, and 1-year mortality after CIED infections were 2.8, 22.2, and 52.8%, respectively. Of all patients who died within 1 year, 16 (89%) patients had CRT-D or ICD device infections. One (2.8%) patient died during the hospital stay due to septic shock. Within 30 days, four (11%) patients died of heart failure (HF) and three (8%) patients died due to septic shock. Causes of death within 1 year after CIED explantation were HF, septic shock, and cerebral haemorrhage in five (14%), two (6%), and one (3%) patients, respectively. In three (8%) patients, the cause of 1-year mortality was unknown.

Discussion

Cardiac implantable electronic device infections represent a life-threatening complication after PM, ICD, or CRT-D implantation. Fluorescence *in situ* hybridization sequencing is a valuable tool for the identification, quantification, and description of the activity state of microorganisms. DAPI stain additionally visualizes dead or resting pathogens.

In a previous work, our group found that FISHseq yielded interpretable results in 100% ($n = 61$) of patients with driveline infections of ventricular assist devices; only five (8%) patients were on long-term

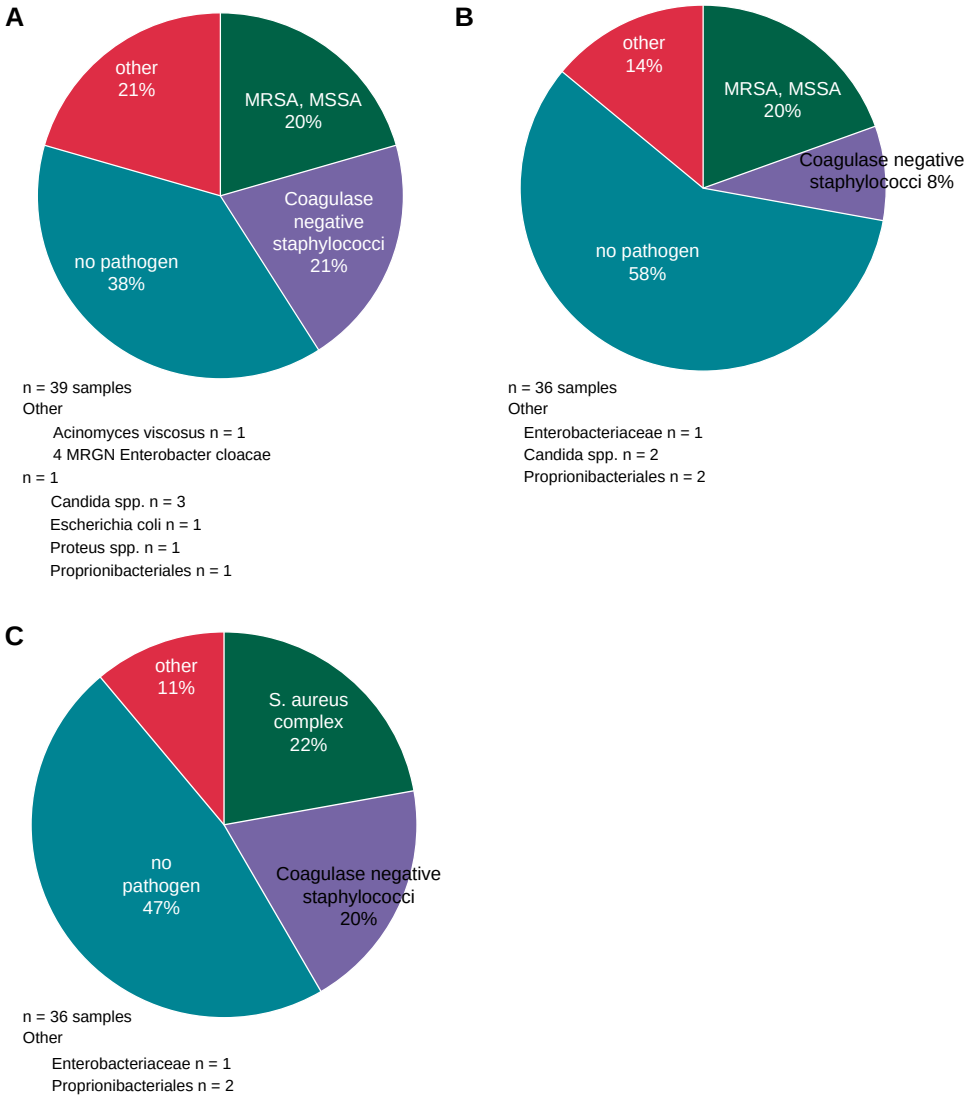


Figure 3 Pathogens identified (*A*) preoperatively by cultivation of blood, pocket fluid, or pocket swabs; (*B*) intraoperatively by cultivation of tissue or swabs; and (*C*) intraoperatively by FISHseq analysis. FISHseq, fluorescence *in situ* hybridization sequencing.

antibiotic treatment when explanted.[18] In our CIED cohort, the rate of microorganisms identified by FISHseq was lower. However, preoperative blood cultures were positive in 64% of patients, enabling antibiogram-guided antibiotic treatment. DAPI identified pathogens in all but one patients, resulting in an overall positive FISHseq detection rate of 97% ($n = 35$).

Comparing the results from the analyses of intraoperative samples by conventional microbiological cultures and FISHseq, we found that FISHseq identified more causative pathogens. For the confirmation of preoperatively known bacteria, a combined approach was most efficient. Interestingly, for blood culture–negative infections, a combined approach did not exceed the rate of microorganisms identified, compared with FISHseq alone. This finding suggests the presence of biofilms or microcolonies, which were detected in two blood culture–negative patients in our cohort. Since biofilm infections are poorly detectable by standard cultivation and frequently lead to failure of antibiotic treatment and relapsing infections, detection of the formation of the microorganisms is of particular importance.[19]

Most (67%) patients in our cohort had HF with reduced rejection fraction (HFrEF) and ICD or CRT-D infections. In this population, infections do not only carry the risk of acute sepsis or septic shock but also trigger decompensation of HF. Alarmingly, combined sepsis and decompensated HF increases mortality rates to up to 90%.[20] In our cohort, progression of HF caused 47% of all deaths within 1 year and occurred exclusively in patients with previously known severe structural cardiac diseases (HFrEF in eight of nine patients and one patient with malignant arrhythmias). Consequently, these patients should be screened intensely for signs of HF progression following CIED infection.

Detection of the full spectrum of causative pathogens is crucial for the initiation of an effective treatment. Additionally, in patients with advanced HF, complete recovery from infection would be essential to implement long-term advanced HF treatments, including mechanical circulatory support or heart transplantation.

In line with our data, bacteria of the skin flora are common pathogens causing CIED infections.[21] Still, when identifying these in culture (especially when yielded from pocket swabs), uncertainty about the

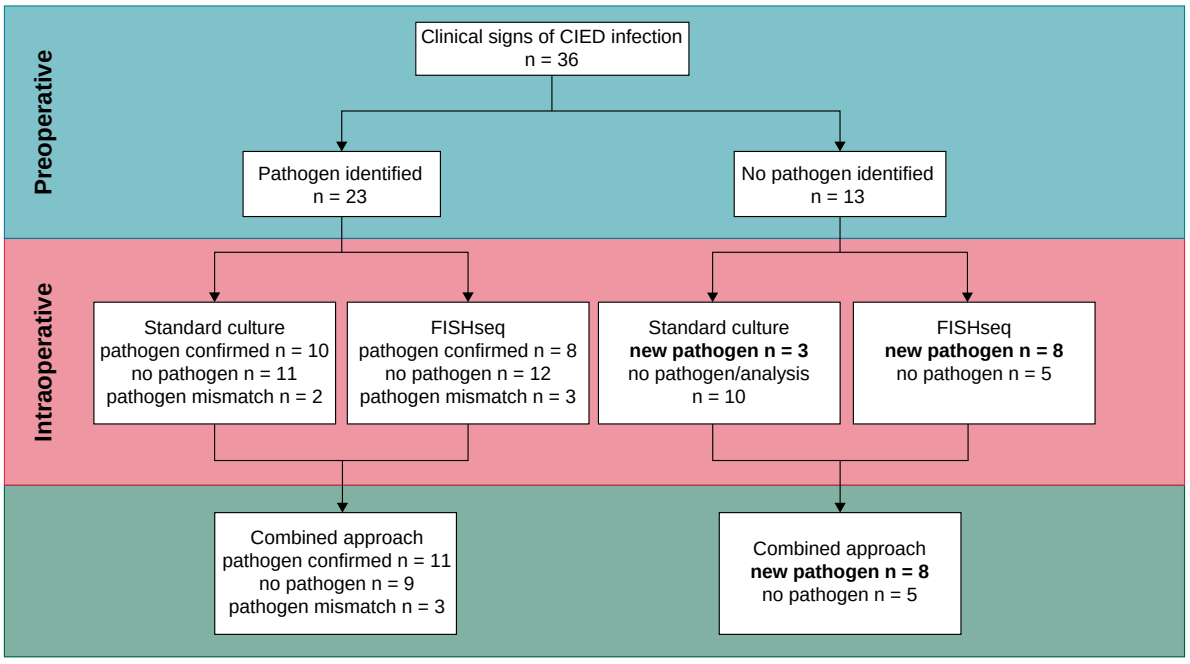


Figure 4 Pathogen identification in preoperative and intraoperative settings. CIED, cardiac implantable electronic device; FISHseq, fluorescence *in situ* hybridization sequencing.

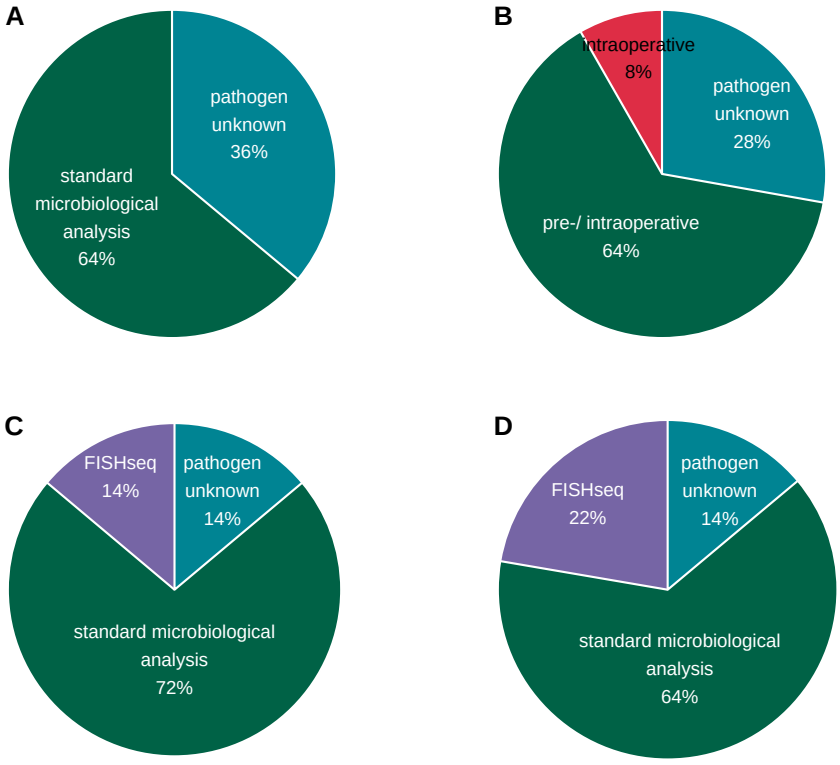


Figure 5 Rate of pathogens identified (*A*) preoperatively; (*B*) by standard microbiological culture of pre- or intraoperative samples; (*C*) by FISH/PCR and standard microbiological analysis of pre- or intraoperative samples; and (*D*) by FISH/PCR and standard microbiological analysis of preoperative samples. FISH, fluorescence *in situ* hybridization; FISHseq, fluorescence *in situ* hybridization sequencing; PCR, polymerase chain reaction.

reliability of the results often remains. An advantage of FISHseq is the ability to reliably differentiate between contamination and infection.[22]

However, currently, FISHseq is unable to provide antibiotic resistance testing.

Study limitations

This analysis was performed in a non-consecutive single-centre cohort. Data were derived from clinical routine and were analysed retrospectively, limiting the availability of external follow-up data, especially in cases of deceased patients. Clinical, randomized controlled studies are needed to compare the clinical impact (change of antibiotic regime) and outcome in patients who receive FISHseq and/or conventional diagnostics.

Conclusions

Fluorescence *in situ* hybridization sequencing is a valuable tool to detect causative microorganisms in CIED infections. In our cohort, the combination of FISHseq with preoperative conventional cultivation showed the highest efficacy in detecting pathogens. Additional cultivation of intraoperative tissue samples or swabs yielded more confirmation of pathogens known from preoperative culture. Further research is desirable to investigate the impact of FISHseq diagnostics on clinical outcome in a prospective, randomized controlled cohort with CIED infections.

Supplementary material

Supplementary material is available at *Europace* online.

Conflict of interest: A.M. and J.K. are employees and hold shares of MoKi Analytics GmbH in addition to being employed by Charité, and A.M. is the founder and head of the private practice Moter Diagnostics. V.F. declares relevant (institutional) financial activities outside the submitted work with the following commercial entities: Medtronic GmbH, Biotronik SE & Co., Abiomed GmbH, Abbott GmbH & Co. KG, Boston Scientific, Edwards Lifesciences, Berlin Heart, Novartis Pharma GmbH, JOTEC/CryoLife GmbH, LivaNova, and Zurich Heart in relation to educational grants (including travel support), fees for lectures and speeches, fees for professional consultation, research, and study funds. C.S. declares payment to his institution related to his activity as speaker fees, honoraria, consultancy, advisory board fees, investigator, committee member from Angiodynamics, Abiomed, Atricure, Medtronic, Spectranetics, Biotronik, Liva Nova (Sorin) and Cook Medical, and departmental or institutional research funding from Cook Medical, Hylomorph. F.S. received institutional grants from Novartis, Abbott, non-financial support from Medtronic, and institutional fees (speaker honoraria) from Orion Pharma outside of the submitted work.

Data availability

The data underlying this article are available in the article and in its online Supplementary material.

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