Background: Bacterial cultures of cardiovascular implantable electronic devices removed from patients without clinical infection are often positive, and the cultured bacteria are different from those at the time of clinical infection. This discrepancy has not been adequately explained. We hypothesized that the cause is bacterial contamination at operation and compared the results of bacterial cultures between patients with de novo pacemaker implantation and those with pacemaker replacement.

Methods and Results: We prospectively enrolled consecutive 100 patients who underwent cardiac pacemaker implantation (49 de novo implantations, 51 replacements). We took swab cultures from inside the generator pocket (1) immediately after the creation of new pocket or removal of old generator, (2) after connection of leads to new generator, and (3) after pocket lavage. Swab cultures were positive in 272 (45%) of 600 samples. The majority of the cultured bacteria were Propionibacterium species. No statistical difference was detected between de novo implantations and replacements in the positive ratio of swab cultures. The positive ratio was not correlated with the number of previous device replacements.

Conclusions: The positive ratio of swab cultures was not different between new implantations and replacements, suggesting that a positive culture merely indicates contamination of bacteria during operation rather than colonization. (Circ J 2015; 79: 1712–1718)

Key Words: Bacterial colonization; Cardiovascular implantable electronic devices; Infection

Infection related to cardiovascular implantable electronic devices (CIED) is a serious complication with high mortality and morbidity, necessitating removal of the device and prolonged antibiotic therapy. However, using sonication fluid and conventional swabs from removed CIED, Rohacek et al demonstrated positive bacterial cultures in approximately 40% of patients and suggested that bacteria do not always cause clinical infection, some colonizing CIED without clinical signs of infection. The results of another report by Mason et al on asymptomatic colonization are consistent with that report. We hypothesized that it might be the result of misinterpretation of positive bacterial cultures. In other words, positive cultures might be the result of contamination rather than colonization. Because the previous 2 reports did not have a control group to differentiate contamination and colonization, we examined and compared the results of bacterial cultures between patients with de novo pacemaker implantation and those with pacemaker replacement. If colonization exists, the positive ratio of the cultures should be higher in patients with replacement than in those with de novo implantation.
Bacterial Culture of CIED

Implantation and replacement of devices were performed in the catheterization laboratory. The level of cleanliness of the catheterization laboratory was similar to that of the operating room, as it satisfied Class II conditions of the Healthcare Engineering Association of Japan HEAS-02-1998 and Class 8 conditions of ISO14644-1. The patients came to the catheterization laboratory after being shaved and swabbed with 4% chlorhexidine solution. We lavaged each patient’s skin twice with 0.5% chlorhexidine gluconate and 7.5% povidone-iodine solution using sterile cloths. Operators and assistants wore surgical gowns and double sterile gloves. Surgical drapes coated with povidone-iodine (3M™Ioban™2 antimicrobial incise drapes, Minnesota Mining and Manufacturing Company, St. Paul, MN, USA) were used to reduce the risk of contamination from skin flora during the procedure. Antibiotic (1 g cefazoline) was routinely administered immediately before and at 6 h after operation.

<table>
<thead>
<tr>
<th>Table. Characteristics of the Study Patients Undergoing De Novo Pacemaker Implantation or Device Replacement</th>
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<tr>
<td>Age, years</td>
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<tr>
<td>Male, n (%)</td>
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<td>Body mass index, kg/m²</td>
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<td>Serum albumin, g/dl</td>
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<td>Baseline disease, n (%)</td>
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<td>Prosthetic heart valve</td>
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<td>Atrial fibrillation</td>
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<td>Corticosteroid</td>
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<td>Reason for pacemaker implantation, n (%)</td>
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<td>Atrioventricular block</td>
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<td>Atrial fibrillation with bradycardia</td>
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<td>History of prior pacemaker operations, n (%)</td>
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<td>De novo implantation</td>
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<td>Operation time (min)</td>
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<tr>
<td>Lead replacement</td>
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<td>Temporary pacing</td>
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Renal insufficiency is defined as estimated glomerular filtration rate <30 ml/min/1.73 m². Patients with diabetes mellitus are defined as those with hemoglobin A1c >6.5% or already taking diabetic medication.

The study protocol was approved by the Osaka Police Hospital ethical committee and all enrolled patients gave written informed consent.
Under continuous ECG monitoring, we injected local anesthetic (1% xylocaine), made a skin incision in the upper chest, and created a subcutaneous pocket using an electrosurgical knife. We inserted a sheath into an axillary vein and advanced a pacer lead wire through it to the heart. The lead was firmly attached to the heart muscle with a screw device. After checking the pacing and sensing thresholds, we connected the lead wire to the pulse generator, and lavaged the pocket with saline solution. After lavage, we placed the lead and device into the pocket and sutured the subcutaneous tissue and cutis vera.

In the case of pacemaker replacement, after opening the pocket, we removed the old device and checked pacing and sensing thresholds of the leads. If the threshold was inadequate, we implanted a new lead. After connecting the lead to a new generator, we lavaged the pocket with saline solution, placed the lead and device in the pocket, and sutured the subcutaneous tissue and dermis just below dermal-epidermal junction by using polydioxanone filament. We finally applied skin adhesives (DERMABOND HV, ETHICON, USA) to make a microbial barrier and wound closure integrity.

**Swab Culture**
Cotton-tipped swabs (BD BBL™ CulturesSwab™ EZ II, Becton, Dickinson & Co, Franklin Lakes, NJ, USA) were used to obtain 2 swab cultures from inside the generator pocket at 3 time points during the procedure, thus 6 samples from each patient. The 1st time point was immediately after the creation of a new pocket or removal of old generator; the 2nd time point was after connection of the leads to a new generator; the 3rd time point was after pocket lavage.

A smear of each swab with Gram staining was examined microscopically before the swabs were placed in Amiesagar, inoculated onto sheep blood agar, chocolate agar, and MacConkey agar and incubated aerobically for 48h. An additional sheep blood agar plate was incubated anaerobically for 5 days. Thioglycolate was used for enrichment. Organisms were identified by standard microbiological methods.

We compared the outcomes of swab cultures between patients undergoing de novo implantation and those receiving a replacement, and between before and after pocket lavage. We investigated the association between the outcome of swab cultures and patients' characteristics. When clinically apparent infection occurred during follow-up, cultured bacterial species at the time of pacemaker implantation and those at the time of infection were compared.

**Clinical Outcome**
After pacemaker implantation, patients were observed for approximately 1 week in hospital and checked for correct operation of the pacing and for any complications such as lead dislodgement, hematoma or signs of infection. After discharge, patients were routinely seen every 4 months to control device function and to check for signs of infection.

Infection was diagnosed as either local infection of the generator pocket (acute inflammation with redness, local warmth, pain, swelling and/or purulent drainage through incision or through skin erosion, but without systemic inflammatory symptoms) or pacemaker-associated infectious endocarditis, defined by the Duke criteria. 16

**Statistical Analysis**
Data were presented as mean±SD, number and percentage, and odds ratio with a 95% confidential interval. A Mantel-Haenszel test was used for univariate analysis to investigate the parametric risk factors of positive culture. Fisher’s exact test or Chi-square test was used for non-parametric factor evaluation. Multivariate analysis was performed using a conditional logistic regression model including all factors that were significantly associated with positive culture by univariate analysis. P<0.05 was regarded as statistically significant. Analysis was performed with SPSS version 16.0 J for Windows (SPSS inc, Chicago, IL, USA).

**Results**
**Patients' Characteristics**
Of the 100 enrolled patients, 49 received their first device and 51 underwent replacement of their pacemaker. The patients’ characteristics are presented in Table. There was no statistical difference in the patients’ age, sex or body mass index, but serum albumin level was lower in the patients with a first device than in those with a replacement. The patients undergoing de novo implantation had coronary artery disease, antiplatelet therapy, and prosthetic heart valves more frequently than those with a replacement device.

**Culture Results and Bacterial Species**
In no patients were bacteria detected by microscopic examina-
Bacterial Culture of CIED

At least 1 sample was positive at the 1st time point in 39 (39%) patients. The cultures were positive in 83 (42%), 90 (45%), and 94 (47%) samples at 1st, 2nd, and 3rd time points, respectively. The ratio of positive culture increased at the 3rd time point compared with the 1st time point (P=0.049); however, the difference was not statistically significant between the 1st and 2nd (P=0.30) or between the 2nd and 3rd time points (P=0.35) (Figure 5).

The positive ratio for any bacteria, Propionibacterium species or coagulase-negative Staphylococci (CNS), was not different between the samples from de novo implantation patients and those undergoing device replacement.

No statistical difference between de novo implantations and replacements was detected in the positive ratio of the swab cultures for Propionibacterium species (45% vs. 47%, P=0.8) or for coagulase-negative Staphylococci (14% vs. 24%, P=0.1) (Figures 2,3). The positive ratio for any bacteria was not correlated with the number of previous device replacements (Figure 4).

Culture Results and Sample Timing
At least 1 sample was positive at the 1st time point in 39 (39%) patients. The cultures were positive in 83 (42%), 90 (45%), and 94 (47%) samples at 1st, 2nd, and 3rd time points, respectively. The ratio of positive culture increased at the 3rd time point compared with the 1st time point (P=0.049); however, the difference was not statistically significant between the 1st and 2nd (P=0.30) or between the 2nd and 3rd time points (P=0.35) (Figure 5).

The positive ratio for any bacteria, Propionibacterium species or coagulase-negative Staphylococci, was not different...
A 74-year-old male patient developed a clinically apparent pacemaker infection at 397 days after pacemaker replacement. The patient underwent pacemaker (DDD mode) implantation in May 2007 for sick sinus syndrome and atrioventricular block, and then pacemaker replacement in September 2012 because of insufficient battery charge. In October 2013, he presented with the chief complaint of erythema and swelling around the wound. Emergency removal of the pacemaker and leads was performed on the same day, and pacemaker re-implantation was performed after 1 month of antibiotic therapy. Although *P. acnes* had been cultured at the time of pacemaker replacement, coagulase-negative *Staphylococci* were cultured at the time of infection. Smear had revealed no bacteria at the time of pacemaker replacement, but there were Gram-positive cocci and white blood cells at the time of infection.

**Discussion**

In the present study, we demonstrated that approximately one-half of the patients who received a first pacemaker or replacement had positive results for bacterial culture swabs from the pacemaker pocket and that there was no significant difference in the positive ratio of cultures between the 2 groups. Therefore, the positive cultures might be the result of contamination during operation rather than bacterial colonization.

**Interpretation of Culture Results**

Our results for the patients undergoing pacemaker replacement were consistent with 2 previous reports. Rohacek et al. reported that approximately 40% of swab cultures were positive and that the majority of cultured bacteria were *Propionibacterium* species, represented by *P. acnes*. Mason et al. reported that 21% of patients had positive cultures, and almost all of them were *P. acnes*.
were skin flora. However, we demonstrated, for the first time, that the positive ratio of cultures was not different between patients with de novo pacemaker implantation and those with a replacement and that the majority of cultured bacteria were \textit{Propionibacterium} species normally present on human skin, and in sebaceous glands, and hair follicles. Therefore, these results could be significantly influenced by contamination rather than colonization, and the previous reports might have overestimated the bacterial colonization. Even if colonization exists, it is likely to be much less frequent than previously reported.

\textbf{Contamination, Colonization and Infection}

We know from studies of surgical site infection that the essential difference between contamination and colonization is the concentration of organisms in the wound.\textsuperscript{17,18} An infected wound contains a larger number of microorganisms than a contaminated wound. Although approximately one-half of the patients in the present study had positive culture results, there was only 1 late case of CIED infection, related to the fact that swab culture is a qualitative evaluation and its results do not reflect the concentration of organisms in the pacemaker pocket.

In our case of CIED infection, although \textit{P. acnes} had been cultured at the time of pacemaker replacement, coagulase-negative \textit{Staphylococci} was cultured at the time of infection, a result that is consistent with the previous report by Mason et al.,\textsuperscript{9} in which no patients with asymptomatic “colonization” developed clinical infection during the follow-up period. They concluded that a significant proportion of patients had asymptomatic “colonization”, although it was not a marker of future pocket infection. We should rephrase that a significant proportion of patients had contamination of bacteria during operation, but it was not a marker of future pocket infection.

In differentiating contamination, colonization, and infection, we should consider the following questions: (1) Can the cultured bacteria infect the target organ? (2) Does the patient have clinical symptoms of infection? and (3) Were the cultured bacteria also detected in the smear?\textsuperscript{1–7,20–22}

\textbf{Effect of Lavage Procedure}

The rate of positive culture in the present study was not different between before and after lavage of the pacemaker pocket with saline solution. However, in general wound management, irrigation with warm sterile saline is used routinely and believed to be effective in decreasing the bacterial load. Proper wound

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure6.png}
\caption{Comparison of cultured bacteria between de novo implantation and replacement at different sampling time points. The positive ratio for (A) any bacteria, (B) \textit{Propionibacterium} species or (C) coagulase-negative \textit{Staphylococci} (CNS) was not different between the samples from patients undergoing de novo implantation and those having device replacement at any sampling time point.}
\end{figure}
cleansing and debridement can prevent bacterial colonization proceeding to clinical infection. The results of the swab cultures in the present study did not reflect the concentration of organisms in the pacemaker pocket, so the effect of lavage could not be evaluated correctly and remains inconclusive.

**Contamination of the Surgical Field**
The present study demonstrated a rather high incidence of contamination during operation and the frequency of causing clinical infection from contamination was 0.5% (1/55). We should realize that "uninfected CIED is sterile" might be a myth. Although contamination during operation can occur more frequently than we suppose, the *Propionibacterium* species mainly cultured at the time of pacemaker implantation would not cause clinically apparent pacemaker infection during follow-up.

**Study Limitations**
There are some important limitations in this study. Because the clinically apparent pacemaker infection during follow-up was cultured at the time of pacemaker implantation, the bacteria would not cause CIED infection or a replacement had positive results for bacterial swab cultures. The present study demonstrated a rather high incidence of contamination during operation rather than bacterial colonization.

**Conclusions**
Nearly half of the patients who underwent de novo implantation or a replacement had positive results for bacterial swab cultures from the pacemaker pocket. However, the positive ratio of the swab cultures was not different between the 2 groups of patients, suggesting that the positive cultures may be contamination during operation rather than bacterial colonization.

**Conflict of Interest**

**Funding Sources**
None.

**Financial Support**
None.

**Disclosures**

**References**