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Case Report

Left ventricular assist device-associated endocarditis involving multiple clones of *Staphylococcus aureus* with distinct antimicrobial susceptibility patterns

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ABSTRACT

The case of a patient with left ventricular assist device (LVAD)-associated endocarditis involving multiple clones of Staphylococcus aureus is presented. Different clones with distinct colony morphology were identified from blood cultures collected on the same day and showed diverse antimicrobial resistance patterns. In addition, a difference in antimicrobial susceptibility was observed even within an identical clone recovered 400 days apart due to the loss of SCCmec for methicillin and modification of the 23S rRNA target site for linezolid during a long-term treatment course.
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Introduction

Left ventricular assist device (LVAD)-associated infections hinder the long-term use of LVAD and are associated with high mortality. *Staphylococcus aureus* is the most common causative organism of LVAD-associated infections, along with coagulase-negative staphylococci (Nienaber et al., 2013). While LVAD-associated endocarditis is not common, the condition is difficult to cure and its mortality appears to reach as high as 50% (Thyagarajan et al., 2016). The case of a patient with LVAD-associated endocarditis involving multiple clones of *S. aureus* is presented here. Each clone of *S. aureus* showed different patterns of antimicrobial susceptibility, and a change of drug susceptibility in the same clone was also observed during the long-term antimicrobial treatment.

Case report

A 47-year-old man with a history of diabetes mellitus and hypertension developed acute myocardial infarction due to occlusion of the left main trunk of the coronary artery. After reperfusion obtained by percutaneous coronary intervention, hypotension persisted and implantation of a LVAD was performed. On day 89 after implantation of the LVAD, the patient experienced the first episode of bacteremia caused by methicillin-susceptible *S. aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA). On day 106 after implantation of the LVAD, a vegetation was identified between the septum and the orifice of the outflow cannula by transesophageal echocardiography, which established the diagnosis of LVAD-associated endocarditis. Despite long-term, sequential treatment with vancomycin and linezolid, the MRSA bacteremia persisted. On day 492 after implantation of the LVAD, two MRSA strains showing different colony morphology on blood agar were

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A

Isolate	Day of isolation	Colony morphology type	Minimum inhibitory concentrations							PFGE type	SCCmec	Spa type	ST
			MET	ERY	CLI	SXT	LVX	VAN	LZD				
TUM9458	89	A	0.5	>4	≤0.5	≤1/19	>4	≤0.5	1	A	NA	t539	2389
TUM9459	169	B	>2	≤0.25	≤0.5	≤1/19	≤1	1	2	B	IV	t2196	630
TUM9460	208	B	>2	≤0.25	≤0.5	≤1/19	≤1	1	2	B	IV	t2196	630
TUM9461	371	B	>2	≤0.25	≤0.5	≤1/19	≤1	1	2	B	IV	t2196	630
TUM9462	492	B	>2	≤0.25	≤0.5	≤1/19	≤1	1	2	B	IV	t2196	630
TUM9463	492	A	>2	>4	>2	≤1/19	>4	≤0.5	8	A	II	t539	2389
TUM9464	495	B	>2	≤0.25	≤0.5	≤1/19	≤1	1	2	B	IV	t2196	630
TUM9465	500	B	>2	≤0.25	≤0.5	≤1/19	≤1	1	2	B	IV	t2196	630

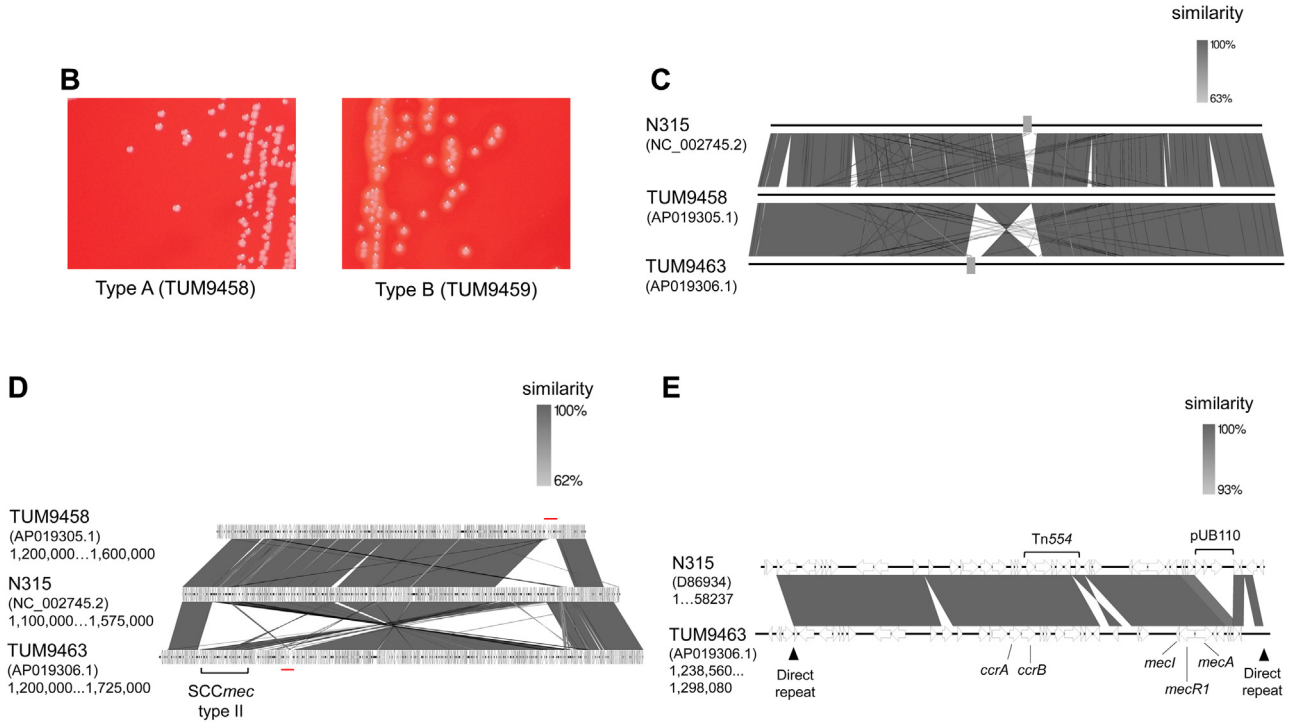

Figure 1. (A) Clinical, microbiological, and molecular characteristics of the *Staphylococcus aureus* bloodstream isolates. Days of isolation indicate the number of days between insertion of the LVAD and the isolation of the isolates. Abbreviations: MET, methicillin; ERY, erythromycin; CLI, clindamycin; SXT, trimethoprim–sulfamethoxazole; LVX, levofloxacin; VAN, vancomycin; LZD, linezolid. (B) Colony morphology of type A and type B isolates on tryptic soy agar with 5% sheep blood. Isolates of type B, not of type A, had β-hemolysis surrounding colonies. (C) Schematic presentation of the whole genomic structure of N315, TUM9458, and TUM9463 drawn using EasyFig (version 2.1). The gray box indicates SCC*mec* type II. (D) Schematic representation of part of the genomic structure of TUM9458, N315, and TUM9463 surrounding SCC*mec* type II. The red line represents a genetic fragment that was observed in both TUM9458 and TUM9463 but not in N315. The arrows indicate putative open reading frames (ORFs) and their orientations identified with DFAST (https://dfast.nig.ac.jp/help_annotation). (E) Schematic representation of the genomic structure of SCC*mec* type II of N315 and TUM9463. The arrows indicate ORFs and their orientations determined with BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) using **D86934** as a reference sequence.

recovered from blood culture. One of these strains (TUM9463) had a minimum inhibitory concentration (MIC) of 8 μg/ml for linezolid by broth microdilution method (Figure 1A and B). Linezolid had been administered for 78 days in total before this linezolid-resistant MRSA strain was detected. The patient was treated with vancomycin alone thereafter and an exchange of the LVAD pump was performed on day 520 after implantation. While the bacteremia temporarily cleared after exchange of the LVAD pump, MRSA bacteremia recurred after 6 months and the patient finally died due to a stroke 31 months after implantation of the LVAD.

Eight *S. aureus* isolates – one MSSA isolate and seven MRSA isolates – were analyzed microbiologically and genetically (Figure 1A). All isolates were confirmed as *S. aureus* by multiplex PCR method for species identification of coagulase-positive staphylococci (Sasaki et al., 2010). These isolates were divided into two groups by pulsed-field gel electrophoresis (PFGE) of *Sma*I-digested DNA fragments of genomic DNA. Isolates of PFGE group A and those of PFGE group B showed different colony morphology on tryptic soy agar with 5% sheep blood (Figure 1A and B).

Antimicrobial susceptibility was evaluated with the agar dilution method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines for linezolid (Clinical and Laboratory Standards Institute, 2015) and with BD Phoenix M50 (Becton Dickinson Diagnostic Systems, Sparks, MD) for other agents. Although one of the isolates of PFGE type A (TUM9458) was MSSA with a low MIC for linezolid, the other isolate of PFGE type A (TUM9463) was MRSA with an elevated MIC for linezolid. All isolates of PFGE type B were MRSA and had an identical antimicrobial susceptibility profile.

Genetic characteristics of the isolates were investigated by multiplex PCR for staphylococcal cassette chromosome *mec* (SCC*mec*) typing (Kondo et al., 2007), *spa* typing (https://www.spaserver.ridom.de/), and multilocus sequence typing (https://pubmlst.org/saureus/). Although both of the PFGE group A isolates

were t539 and ST2389, SCC*mec* type II was identified only for the second isolate (TUM9463). All isolates of PFGE group B were t219, ST630, and SCC*mec* type IV.

To elucidate the mechanism underlying the change in antimicrobial susceptibility between the PFGE group A isolates, whole-genome sequencing of TUM9458 and TUM9463 was performed with an Illumina MiSeq (Illumina Inc., San Diego, CA, USA) and MinION (Oxford Nanopore Technologies, Oxford, UK). The initial reads created by MiSeq were quality trimmed with the Trimmomatic tool (version 0.38) and subjected to hybrid de novo assembly using MinION reads using SPAdes (version 3.12.0) (Antipov et al., 2016). Complete genomic sequences were deposited in the NCBI database under accession numbers **AP019305.1** and **AP019306.1** for TUM9458 and TUM9463, respectively.

The overall genomic structures of TUM9458 and TUM9463 were highly similar and only six single nucleotide polymorphisms (SNPs) were identified in the common genome sequences excluding those in multiple-copy genes. Furthermore, the genomic structure of these isolates was similar to that of *S. aureus* N315 strain (accession number **NC_002745.2**), which is a representative strain of ST5, a single locus variant of ST2389 (Figure 1C). Of note, compared with TUM9458, TUM9463 had insertion of a ~103-kb sequence containing SCC*mec* type II and adjacent inversion of ~308 kb. Comparison of the genetic environment of SCC*mec* type II suggested that TUM9458 and TUM9463 evolved from a common ancestral strain resembling N315 through deletion (TUM9458) and inversion (TUM9463) events. However, the presence of a genetic fragment that was observed in both TUM9458 and TUM9463 but not in N315, also suggested the existence of a common intermediate ancestral strain (Figure 1D). Although SCC*mec* type II of TUM9463 carried most of the core structures such as the *mec* gene complex and *ccr* gene complex, and direct repeats on both ends, it lacked pUB110 in comparison with SCC*mec* type IIa of N315 (accession number **D86934**). In addition, partial modification of the content of Tn*554* was observed (Figure 1E). While guanine at position 2576 of the domain V of all five copies of 23S rRNA were wild-type in TUM9458, G2576T mutation was observed in three of the five copies of 23S rRNA in TUM9463 according to mapping of the MiSeq reads against a reference 23S rRNA sequence.

Discussion

This article describes a case of LVAD-associated endocarditis involving multiple clones of *S. aureus.* Antimicrobial susceptibility profiles of an MRSA isolate belonging to PFGE type A and MRSA isolates belonging to PFGE type B were different. In addition, while TUM9463 was linezolid-resistant MRSA, TUM9458 belonging to the same PFGE type was linezolid-susceptible MSSA. Whole-genome sequencing suggested that TUM9458 and TUM9463 were derived from a common ancestral strain. Loss of SCC*mec* was observed in *S. aureus* isolates colonizing the human nasal mucosa in a previous study (Boundy et al., 2012). On the other hand, TUM9463 had obtained the G2576T mutation in three of five copies of domain V in 23S rRNA. G2576T is a well described mutation that cumulatively leads to acquired linezolid resistance in *S. aureus* after long-term treatment with linezolid (Long and Vester, 2012).

A previous study showed that a change in antimicrobial susceptibility profiles among *S. aureus* strains isolated from the same patient over a short period of time was infrequent compared with that of coagulase-negative staphylococci, *Pseudomonas aeruginosa*, and *Enterobacteriaceae* (Thomson et al., 1989). Nevertheless, TUM9462 and TUM9463, which were isolated from blood culture on the same day, were different clones of *S. aureus* and had completely different antimicrobial susceptibility profiles. Giltner et al. reported three cases of MRSA bacteremia that were accompanied by short-term changes in drug susceptibility against daptomycin, linezolid, and vancomycin (Giltner et al., 2014). Resistant isolates were recovered only intermittently in these cases. All cases had characteristics similar to the case presented herein, namely long-term placement of an intravascular device, co-existence of endocarditis, and prolonged antimicrobial treatment.

Prolonged courses of treatment in intractable implantable device-associated infections may provide an opportunity for multiple species or multiple clones of identical species to become involved in infection. In addition, isolates derived from the same ancestral strain can differentiate the repertoire of antimicrobial resistance genes through mutational events, recombination, and deletions, which may occur spontaneously or under the selective pressure of antimicrobial treatment. The frequency of mutations and horizontal gene transfer events has been demonstrated to increase in biofilms formed in endocarditis or chronic device-associated infections (Høiby et al., 2010). The case presented here suggests the possible benefit of repeating susceptibility testing of *S. aureus* identified from blood even if they were recovered within a short period or even on the same day in patients with an endovascular infection and lack of source control.

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Ethical approval

Approval was not required for this study.

Conflict of interest

All authors report no conflicts of interest.

Author contributions

Sohei Harada designed the study, contributed to the acquisition, analysis, and interpretation of the data, and drafted the manuscript. Kotaro Aoki contributed to the acquisition, analysis, and interpretation of the data. Koh Okamoto planned the care of the patient and contributed to the acquisition, analysis, and interpretation of the data. Osamu Kinoshita, Kan Nawata, Hiroshi Yotsuyanagi, Kyoji Moriya, and Minoru Ono planned the care of the patient and contributed to the acquisition of data. Yoshikazu Ishii, Kazuhiro Tateda, and Tomoo Saga contributed to the analysis and interpretation of the data. Masakazu Sasaki contributed to the acquisition of the data. Yohei Doi revised the manuscript critically for important intellectual content. All authors read and approved the final manuscript.

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