Emergence of daptomycin non-susceptible coagulase-negative *Staphylococci* in patients with cardiovascular device infections

Two cases report investigated by whole genome analysis

Hideharu Hagiya, MD, PhD, Yo Sugawara, PhD, Keigo Kimura, MT, Shigeto Hamaguchi, MD, PhD, Isao Nishi, PhD, Masahiro Hayashi, PhD, Yukihiro Akeda, PhD, Kazunori Tomono, MD, PhD

Abstract

**Rationale:** Daptomycin (DAP) is a key drug for treating severe *Staphylococcus* infections. The emergence of DAP non-susceptible *Staphylococcus aureus* has been widely recognized in clinical situations, although the clinical status of DAP non-susceptible coagulase-negative *Staphylococcus* (CoNS) infections is unclear. We encountered 2 cases of cardiovascular device infections that were associated with DAP non-susceptible CoNS.

**Patient concerns:** The first case involved a 60-year-old woman with a pump pocket infection in a left ventricular assist device. DAP non-susceptible *Staphylococcus capitis subsp. ureolyticus* was isolated from a blood culture after treatment using vancomycin (10 days) and DAP (6 days). The second case involved a 71-year-old man with an aortic graft infection. DAP non-susceptible *S capitis subsp. ureolyticus* was detected in pus after treatment using vancomycin (2 weeks) and DAP (1 week) without complete removal and debridement.

**Diagnosis:** Cardiovascular device infections caused by DAP non-susceptible CoNS.

**Interventions and outcomes:** Whole genome sequencing of these strains revealed multiple mutations in genes that are related to DAP non-susceptibility in *S aureus*, which created amino acid substitutions in *mprF, dltAB, dltD, rpoC, yycG, cls2, pgsA,* and *vraSR.* To the very best of our knowledge, the substitution patterns were not identical to those previously reported in DAP non-susceptible *S aureus*.

**Lessons:** Clinicians should be cautious regarding the emergence of DAP non-susceptible CoNS, especially in cases with implanted prosthetic devices, inadequate debridement, and prior usage of vancomycin and DAP. Further studies are needed to understand the relevance of these genetic changes and DAP non-susceptibility in CoNS strains.

**Abbreviations:** ANI = average nucleotide identity, CoNS = coagulase-negative *Staphylococcus*, CRP = C-reactive protein, CT = computed tomography, DAP = daptomycin, MIC = minimum inhibitory concentration, VCM = vancomycin.

**Keywords:** amino acid substitution, antimicrobial resistance, daptomycin, prosthetic device, staphylococcus capitis subsp, ureolyticus, whole genome analysis

1. Introduction

Daptomycin (DAP) is part of a new class of natural cyclic lipopeptide antibiotics that are active against Gram-positive organisms. Based on its strong bactericidal effect and good pharmacokinetics, DAP is widely recommended for treating various *Staphylococcus aureus* infections, including bacteremia and endocarditis.[1] However, the emergence of DAP resistance in *S aureus* has been described in laboratory studies,[2] clinical trials,[3] and post-marketing surveillance.[4] Exposure to DAP causes *S aureus* strains to develop an altered membrane potential and a more positive membrane surface charge, which leads to DAP resistance.[5]

In contrast, coagulase-negative *Staphylococcus* (CoNS) rarely exhibits decreased susceptibility to DAP, as a previous study[6] has indicated that DAP was active for 99.8% of CoNS isolates at a susceptibility breakpoint of ≤ 1 μg/mL. In culture results, CoNS are often considered contaminants, although their roles as real pathogens have been recognized in various clinical situations.[7] Furthermore, CoNS strains can have elevated minimum
inhibitory concentration (MIC) values for glycopeptides, which may be related to poor clinical outcomes. However, given the rarity of DAP non-susceptible CoNS infections, its clinical course, incidence, and genetic background remain unclear. We recently encountered 2 cases of DAP non-susceptible CoNS infections that involved patients with implanted cardiovascular devices and used whole genome sequencing to identify amino acid substitutions that could be responsible for the DAP non-susceptibility.

2. Case presentation

2.1. Case 1

A 60-year-old woman with a history of hypertrophic cardiomyopathy had undergone implantation of a left ventricular assist device 2 years ago. Eighteen months later, the device suddenly malfunctioned and the patient underwent an emergent exchange surgery. She subsequently developed a surgical site infection that was treated using intravenous ampicillin/sulbactam.

Three months later, the surgical wound infection recurred and the patient was re-hospitalized with a low-grade fever and tenderness directly around the pump. Her serum level of C-reactive protein (CRP) was slightly elevated (1.39 mg/dL) and gallium-67 scintigraphy revealed inflammation surrounding the device pump (Fig. 1A). A blood culture was positive for methicillin-resistant Staphylococcus capitis subsp. ureolyticus. Intravenous vancomycin (VCM) was empirically initiated, although the patient remained febrile. Debridement surgery was performed for the pump pocket infection on day 7 of that admission, and the organism was also detected in a culture of the purulent tissue. The VCM treatment was switched to DAP (350 mg/day [5.7 mg/kg]) on day 10 because of drug-induced neutropenia. Although the patient’s condition seemed favorable, a high fever re-emerged on day 16 and 2 blood cultures were again positive for S. capitis subsp. ureolyticus, with drug tests revealing non-susceptibility to DAP (Table 1). Thus, the DAP treatment was discontinued and the patient did not experience recurrence after a 1-month course of combination therapy using clindamycin and rifampicin.

2.2. Case 2

A 71-year-old man who had undergone thoracic endovascular aortic repair 7 years previously was admitted to a hospital with a complaint of fever, back pain, and bloody sputum. Based on the results from positron emission tomography-computed tomography (CT), the patient was diagnosed with an aortic graft infection and underwent CT-guided drainage for a peri-aortic abscess. The pathogen was unclear and he received empirical antimicrobial treatments using meropenem and DAP for 1 week (dose unknown), ampicillin and sulbactam for 3 weeks, VCM for 2 weeks, and oral levofloxacin for 4 weeks. The patient was discharged with a prescription for oral levofloxacin and followed-up at an outpatient clinic. Three months later, his condition seemed to be improving, and a repeat chest CT showed a reduction in the size of the abscess. However, a blood culture performed during this admission was positive for the same strain of S. capitis subsp. ureolyticus, with drug tests confirming non-susceptibility to DAP (Table 1). Thus, the DAP treatment was discontinued, and the patient received a 1-month course of combination therapy using clindamycin and rifampicin.

---

Figure 1. Radiological findings. Gallium-67 scintigraphy revealed uptake at the site of a cardiac pump in Case 1 (A) and in the peri-aortic space in Case 2 (B). Contrast-enhanced computed tomography revealed a massive abscess surrounding the aortic graft in Case 2 (C).
been described in our previous report.[9] The sequence reads were on brain heart infusion broth (BD Bacto, Franklin Lakes, NJ, USA) and genomic bacterial isolates were cultured overnight in brain heart infusion. The results might be associated with the DAP non-susceptibility. The systems (Illumina, San Diego, CA, USA) to identify mutations that were performed using the MiSeq 2.4. Whole genome analysis

1.5

/ C20 49326). MIC of the reference strain for DAP was confirmed using the E-test (bioMe"rieux, Marcy l’Etoile, France) on cation-

A recent study from the SENTRY Antimicrobial Surveillance Program (283 hospitals in 42 countries during 2002–2010) revealed that 99.8% of > 22,000 isolates of CoNS strains were susceptible to DAP, with only a few CoNS strains (eg, S sciu, S auricularis, S warneri, and S capitis) having elevated MIC90 values relative to those of other Staphylococcus spp.[6]

3. Discussion

We encountered 2 clinical cases of DAP non-susceptible CoNS infections. Although DAP non-susceptible strains of S aureus have been noted in various clinical settings, these cases rarely involve CoNS. Based on the guidelines from the European Committee on Antimicrobial Susceptibility Testing[15] and the Clinical and Laboratory Standards Institute,[16] DAP susceptibility in Staphylococcus spp. is defined as an MIC of ≤ 1 mg/L and strains with an MIC of > 1 mg/L are considered DAP non-susceptible. We measured the MIC values using a MicroScan WalkAway 96 Plus (Beckman Coulter, Brea, CA, USA) and confirmed the results using the E-test (bioMe’rieux). A recent study from the SENTRY Antimicrobial Surveillance Program (283 hospitals in 42 countries during 2002–2010) revealed that 99.8% of > 22,000 isolates of CoNS strains were susceptible to DAP, with only a few CoNS strains (eg, S sciu, S auricularis, S warneri, and S capitis) having elevated MIC90 values relative to those of other Staphylococcus spp.[6]

Treatment using VCM or DAP usually precedes the development of DAP resistance in S aureus.[17] and both of our patients had received these drugs before the emergence of DAP non-susceptible CoNS (Case 1: 10 days of VCM and 6 days of DAP, Case 2: 2 weeks of VCM and 1 week of DAP). A previous study has indicated that DAP non-susceptible S aureus emergence could be predicted by a low dosage of DAP, persistent infection, and high bacterial loads,[18] which indicates that an adequate dose of DAP should be administered to avoid resistance. The safety of high-dose DAP (≥ 6 mg/kg) has been widely recognized,[19] and the administration of a high DAP dose is recommended, especially in refractory cases. Moreover, both of our cases involved prosthetic devices (a left ventricular assist device in Case 1 and aortic graft in Case 2), and the difficulty that is associated with debridement and removal of these foreign bodies might also

Table 1

| Minimum inhibitory concentration (µg/mL) | Case 1 | Case 2 |
|----------------------------------------|--------|--------|
| Vancomycin                             | ≤1     | 2      |
| Daptomycin                             | ≤0.5   | 2 [3]  |

The minimum inhibitory concentrations were determined using a MicroScan WalkAway 96 Plus (Beckman Coulter, Brea, CA, USA). Results of the E-tests for daptomycin are shown in brackets.

Table 2

Substitutions of amino acids based on the whole genome sequencing.

| Genes            | Case 1                  | Case 2 |
|------------------|-------------------------|--------|
| mprF             | –                       | L336F  |
| dbtABCd          | G119R (dbt)             | multiple* |
| ppoB             | –                       | –      |
| ppoC             | –                       | N341D  |
| yycF             | –                       | –      |
| yycG             | V220F                   | N183I  |
| cls2             | I70T                    | D226S  |
| pgsA             | –                       | D187E  |
| vraSR            | T175S                   | –      |

Amino acid changes in the associated genes of the 2 clinical strains were compared to those of the reference strain (Staphylococcus capitis subsp. ureolyticus, ATCC 49326).

Multiple mutations in dltA (E17D, S227F, K431R, M438T, D187E) and dltB (161W, F237L).

**Table 2**

| Genes            | Case 1                  | Case 2 |
|------------------|-------------------------|--------|
| mprF             | –                       | L336F  |
| dbtABCd          | G119R (dbt)             | multiple* |
| ppoB             | –                       | –      |
| ppoC             | –                       | N341D  |
| yycF             | –                       | –      |
| yycG             | V220F                   | N183I  |
| cls2             | I70T                    | D226S  |
| pgsA             | –                       | D187E  |
| vraSR            | T175S                   | –      |

Amino acid changes in the associated genes of the 2 clinical strains were compared to those of the reference strain (Staphylococcus capitis subsp. ureolyticus, ATCC 49326).

Multiple mutations in dltA (E17D, S227F, K431R, M438T, D187E) and dbtABC (C0/C0/C0).

**Table 2**

Substitutions of amino acids based on the whole genome sequencing.

| Genes        | Case 1                  | Case 2 |
|--------------|-------------------------|--------|
| mprF         | –                       | L336F  |
| dbtABCd      | G119R (dbt)             | multiple* |
| ppoB         | –                       | –      |
| ppoC         | –                       | N341D  |
| yycF         | –                       | –      |
| yycG         | V220F                   | N183I  |
| cls2         | I70T                    | D226S  |
| pgsA         | –                       | D187E  |
| vraSR        | T175S                   | –      |

Amino acid changes in the associated genes of the 2 clinical strains were compared to those of the reference strain (Staphylococcus capitis subsp. ureolyticus, ATCC 49326).

Multiple mutations in dltA (E17D, S227F, K431R, M438T, D187E) and dbtABC (C0/C0/C0).

**Table 2**

Substitutions of amino acids based on the whole genome sequencing.

| Genes        | Case 1                  | Case 2 |
|--------------|-------------------------|--------|
| mprF         | –                       | L336F  |
| dbtABCd      | G119R (dbt)             | multiple* |
| ppoB         | –                       | –      |
| ppoC         | –                       | N341D  |
| yycF         | –                       | –      |
| yycG         | V220F                   | N183I  |
| cls2         | I70T                    | D226S  |
| pgsA         | –                       | D187E  |
| vraSR        | T175S                   | –      |

Amino acid changes in the associated genes of the 2 clinical strains were compared to those of the reference strain (Staphylococcus capitis subsp. ureolyticus, ATCC 49326).

Multiple mutations in dltA (E17D, S227F, K431R, M438T, D187E) and dbtABC (C0/C0/C0).

**Table 2**

Substitutions of amino acids based on the whole genome sequencing.

| Genes        | Case 1                  | Case 2 |
|--------------|-------------------------|--------|
| mprF         | –                       | L336F  |
| dbtABCd      | G119R (dbt)             | multiple* |
| ppoB         | –                       | –      |
| ppoC         | –                       | N341D  |
| yycF         | –                       | –      |
| yycG         | V220F                   | N183I  |
| cls2         | I70T                    | D226S  |
| pgsA         | –                       | D187E  |
| vraSR        | T175S                   | –      |

Amino acid changes in the associated genes of the 2 clinical strains were compared to those of the reference strain (Staphylococcus capitis subsp. ureolyticus, ATCC 49326).

Multiple mutations in dltA (E17D, S227F, K431R, M438T, D187E) and dbtABC (C0/C0/C0).
have contributed to the emergence of DAP non-susceptible isolates. There are some possible mechanisms for the increased MIC of DAP in our isolates. As have reported in S. aureus, a positive charge at the membrane surface\textsuperscript{10} and a thickening of the cell wall\textsuperscript{45} could be associated with DAP-non-susceptibility, with major mutations thought to involve mprF, dltABCD, rpoB/rpoC, and yycF/yycG\textsuperscript{11,14}. To the best of our knowledge, only 1 report has described whole genome analysis of multidrug-resistant S. capitis subsp. ureolyticus, although that report did not describe any genetic changes that were potentially responsible for the DAP non-susceptibility.\textsuperscript{22} In this context, our molecular analysis detected multiple amino acid substitutions in these possibly responsible genes for both isolates (vs the publicly available isolate). Interestingly, both of the S. capitis subsp. ureolyticus isolates had amino acid changes commonly in dltABCD, yycG, and els2, which is thus speculated to be majorly related to DAP non-susceptibility in CoNS. Of note, these substitution patterns were not completely identical to the previously reported patterns for DAP non-susceptible S. aureus strains.\textsuperscript{11–14} Thus, they would be novel to be reported in the literature and also might be characteristic to CoNS strains. However, we are unable to conclude an association between the DAP non-susceptibility and these genetic changes, given the lack of available isolates before the DAP exposure. Further molecular analysis is needed to identify the genetic variant(s) that are responsible for DAP non-susceptibility in CoNS.

In conclusion, we encountered 2 cases of DAP non-susceptible CoNS infections. Both cases were associated with artificial device infections, and emergences of DAP non-susceptible strains were preceded by the administration of VCM and DAP. In general, CoNS infrequently causes refractory infections and thus has limited opportunity to become non-susceptible to DAP. The present cases demonstrated that CoNS can also develop DAP non-susceptibility in the specific situations described above. Our whole genome sequencing detected several amino acid substitutions in proteins that may be responsible for DAP non-susceptibility in S. aureus. Resistance in VCM emerged after 4 decades of its clinical use, while DAP resistance occurred shortly after its debut. In this era of antibiotic shortage, emergence of DAP-resistant strain is of great concern to clinicians and should be closely monitored in various bacterial species.

Author contributions
Conceptualization: Hideharu Hagiya.
Investigation: Yo Sugawara, Keigo Kimura, Isao Nishi, and Masahiro Hayashi.
Resources: Masahiro Hayashi.
Supervision: Shigeto Hamaguchi, Yukhiro Akeda, and Kazunori Tomono.
Writing – original draft: Hideharu Hagiya.
Writing – review and editing: Shigeto Hamaguchi, Yukhiro Akeda, and Kazunori Tomono.

References
[1] Liu C, Bayer A, Cosgrove SE, et al. Clinical practice guidelines by the infectious diseases society of america for the treatment of methicillin-resistant Staphylococcus aureus infections in adults and children. Clin Infect Dis 2011;52:e18–55.
[2] Rose WE, Rybak MJ, Tsui BT, et al. Correlation of vancomycin and daptomycin susceptibility in Staphylococcus aureus in reference to accessory gene regulator (agr) polymorphism and function. J Antimicrob Chemother 2007;59:1190–3.
[3] Fowler VG Jr, Bouche HW, Corey GR, et al. Daptomycin versus standard therapy for bacteraemia and endocarditis caused by Staphylococcus aureus. N Engl J Med 2006;355:653–45.
[4] Sader HS, Moet GJ, Farrell DJ, et al. Antimicrobial susceptibility of daptomycin and comparator agents tested against methicillin-resistant Staphylococcus aureus and vancomycin-resistant enterococci: trend analysis of a 6-year period in US medical centers (2005–2010). Diagn Microbiol Infect Dis 2011;70:412–6.
[5] Kaatz GW, Lundstrom TS, Seo SM. Mechanisms of daptomycin resistance in Staphylococcus aureus. Int J Antimicrob Agents 2006;28:280–7.
[6] Sader HS, Jones RN. Antimicrobial activity of daptomycin in comparison to glycopeptides and other antimicrobials when tested against numerous species of coagulase-negative Staphylococci. Diagn Microbiol Infect Dis 2012;73:212–4.
[7] von Eiff C, Peters G, Heilmann C. Pathogenesis of infections due to coagulase-negative staphylococci. Lancet Infect Dis 2002;2:677–85.
[8] Taconielli E, Tumbarello M, Donati KG, et al. Glycopeptide resistance among coagulase-negative staphylococci that cause bacteremia: epidemiological and clinical findings from a case-control study. Clin Infect Dis 2001;33:1628–35.
[9] Sugawara Y, Akeda Y, Sakamoto N, et al. Genetic characterization of blaNDM-harboring plasmids in carbapenem-resistant Escherichia coli from Myzus persicae (SPPS) One 2017;12:e0184720.
[10] Yoon SH, Ha SM, Kwon S, et al. Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. Int J Syst Evol Microbiol 2017;67:1613–7.
[11] Steed ME, Hall AD, Salimnia H, et al. Evaluation of daptomycin non-susceptible Staphylococcus aureus for stability, population profiles, mprF mutations, and daptomycin activity. Infect Dis Ther 2013;2:187–200.
[12] Mehta S, Cuiropr AX, Plata KB, et al. VraSR two-component regulatory system contributes to mprF-mediated decreased susceptibility to daptomycin in vivo-selected clinical strains of methicillin-resistant Staphylococcus aureus. Antimicrob Agents Chemother 2012;56:92–102.
[13] Friedman L, Alder JD, Silverman JA. Genetic changes that correlate with reduced susceptibility to daptomycin in Staphylococcus aureus. Antimicrob Agents Chemother 2006;50:2137–45.
[14] Pelag AI, Miyakis S, Ward DV, et al. Whole genome characterization of the mechanisms of daptomycin resistance in clinical and laboratory derived isolates of Staphylococcus aureus. PLoS One 2012;7:e28316.
[15] European Committee on Antimicrobial Susceptibility Testing (EUCAST). Breakpoint tables for interpretation of MICs and zone diameters. Version 6.0. 2018. Available at: http://www.eucast.org/clinical_breakpoints. Accessed May 5, 2018
[16] Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing. CLSI document M100-S26. 2016. CLSI, Wayne, PA.
[17] Pillai SK, Gold HS, Sakoulas G, et al. Daptomycin nonsusceptibility in Staphylococcus aureus with reduced vancomycin susceptibility is independent of alterations in mprF. Antimicrob Agents Chemother 2007;51:2223–5.
[18] Moise PA, North D, Steenbergen JN, et al. Susceptibility relationship between vancomycin and daptomycin in Staphylococcus aureus: facts and assumptions. Lancet Infect Dis 2009;9:617–24.
[19] Falcone M, Russo A, Venditti M, et al. Considerations for higher doses of daptomycin in critically ill patients with methicillin-resistant Staphylococcus aureus bacteremia. Clin Infect Dis 2013;57:1568–76.
[20] Rubio A, Moore J, Varoglu M, et al. LC-MS/MS characterization of phospholipid content in daptomycin-susceptible and-resistant isolates of Staphylococcus aureus with mutations in mprF. Mol Membr Biol 2012;29:1–8.
[21] Cui L, Tominae A, Neoh H, et al. Correlation between reduced daptomycin susceptibility and vancomycin resistance in vancomycin-intermediate Staphylococcus aureus. Antimicrob Agents Chemother 2006;50:1079–82.
[22] Li X, Lei M, Song Y, et al. Whole genome sequence and comparative genomic analysis of multidrug-resistant Staphylococcus capitis subsp. urealyticus strain LNZR-1. Gut Pathog 2014;6:45.