Emergence of Daptomycin-Resistant *Staphylococcus aureus* during Treatment

Hideharu Hagiya¹, Yuto Haruki², Taeko Uchida³, Tomoko Wada²,³, Sumiko Shiota³, Tomoharu Ishida¹, Hiroko Ogawa¹, Tomoko Murase⁴ and Fumio Otsuka¹

Abstract

A 68-year-old man with persistent bacteremia accompanying a large iliopsoas abscess, vertebral osteomyelitis, discitis and central venous port infection caused by methicillin-resistant *Staphylococcus aureus* (MRSA) was admitted to our hospital. During the course of treatment, the emergence of a daptomycin (DAP)-resistant MRSA strain was confirmed; the minimum inhibitory concentration was 1 to 2 μg/mL for vancomycin and more than 1 μg/mL for DAP. Although the bacterial cell wall was not significantly thickened, an increased positive surface charge and single-nucleotide polymorphism within *mprF* have been confirmed in DAP-resistant strains. Still rare, but clinicians need to be cautious of the emergence of DAP-resistant MRSA during treatment.

Key words: bacterial surface charge, cell wall thickness, creeping, *mprF*, single-nucleotide polymorphism (SNP), *Staphylococcus aureus* bacteremia (SAB)

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Introduction

Daptomycin (DAP), a novel cyclic lipopeptide with a unique structure, is active against Gram-positive bacteria, particularly methicillin-resistant *Staphylococcus aureus* (MRSA) (1). In the presence of an adequate calcium concentration, the lipophilic tail of DAP inserts itself into the bacterial cell membrane, leading to the effusion of potassium ions and subsequent depolarization of the bacterial membrane. Due to its high bactericidal effect and preferable pharmacokinetics, DAP is widely recommended for the treatment of various infections, such as soft tissue infections, musculoskeletal system infections, bacteremia and endocarditis (2). However, the emergence of DAP-resistant strains during treatment has recently been noted (3, 4), although this issue is still not well described in Japan. We experienced such a phenomenon while treating a patient suffering from persistent MRSA bacteremia with vancomycin (VCM) and DAP. The clinical course of the current case and the results of an investigation of possible mechanisms of resistance are discussed in this article.

Case Report

A 68-year-old man (height, 161 cm; weight, 46 kg) with prolonged lenteric diarrhea was transferred to our hospital because of a high fever and lumbar pain persisting for four days. The patient exhibited exocrine pancreatic insufficiency, which had been managed with complete fasting and total parenteral nutrition given through a central venous reservoir for the past month.

On admission, the patient’s vital signs were as follows; blood pressure, 97/59 mmHg; heart rate, 73 beats/min; oxygen saturation, 97% (oxygen, 2 L/min); respiratory rate, 16/min; body temperature, 36.9°C. A physical examination showed lumbar tenderness; otherwise, there were no remarkable findings. In addition, there were no petechiae in the palpebral conjunctiva, cardiac murmurs, skin rashes or inflammatory changes at the insertion site of the central ve-
Over, contrast-enhanced computed tomography (CT) revealed a heterogeneous enhancement of vertebral bodies and disks through Th12 to L3 (B).

Laboratory testing showed a highly inflammatory state (serum C-reactive protein level, 14.4 mg/dL). Moreover, contrast-enhanced computed tomography (CT) revealed a left iliopsoas abscess (Fig. 1A), and magnetic resonance imaging demonstrated thoracic discitis. The lesions were independent and had no direct link with each other. There were no pulmonary lesions, and the results of a transthoracic echocardiograph were not indicative of valvular regurgitation or vegetation. Transesophageal echocardiography was not performed. Percutaneous CT-guided drainage of the iliopsoas abscess was conducted on the day of admission, and the intravenous administration of meropenem and VCM (1.5 g/day) was initiated empirically. The serum trough level of VCM was 25.5 μg/mL on day 4, and the antibiotic therapy was converted to DAP (270 mg/day; approximately 6 mg/g/day) was initiated empirically. The serum trough level of VCM alone on day 5, considering the superior tissue permeability of this drug. However, follow-up blood cultures obtained on days 5 and 7 were again positive for MRSA. The results of susceptibility testing for MRSA revealed that the strain was susceptible to both VCM and DAP (Table), while that of VCM was also elevated (1 to 2 μg/mL), although it remained within the susceptible category. The MICs of other antibiotics were as follows: ampicillin, 8 μg/mL; cefazolin, >32 μg/mL; gentamicin, 8 μg/mL; levofloxacin, >4 μg/mL; clindamycin, >4 μg/mL; minocycline, >8 μg/mL; trimethoprim-sulfamethoxazole, <0.5 μg/mL; teicoplanin <2 μg/mL; and linezolid, <1 μg/mL.

Linezolid was alternatively administered, and the persistent bacteremia was confirmed to have disappeared on day 30; however, the vertebral osteomyelitis and discitis largely remained (Fig. 1B). The patient’s general condition gradually deteriorated and he finally died on day 135. The clinical course of this case is summarized in Fig. 2.

**Table. Minimum Inhibitory Concentrations of Vancomycin and Daptomycin for MRSA Strains Isolated from Blood Culture.**

<table>
<thead>
<tr>
<th>Day 5</th>
<th>Day 17</th>
</tr>
</thead>
<tbody>
<tr>
<td>VITEK 2</td>
<td>≤0.5</td>
</tr>
<tr>
<td>VCM</td>
<td>WalkAway</td>
</tr>
<tr>
<td>DAP</td>
<td>WalkAway</td>
</tr>
<tr>
<td>E test</td>
<td>0.25</td>
</tr>
</tbody>
</table>

**MIC:** minimum inhibitory concentration, MRSA: methicillin-resistant *Staphylococcus aureus*, VCM: vancomycin, DAP: daptomycin, n.p.: not performed

Measurement of MICs (μg/mL) of VCM and DAP was performed by three methods: VITEK 2 (SYSMEX, bioMérieux, Tokyo, Japan), MicroScan WalkAway (Siemens Healthcare Diagnostics, Tokyo, Japan) systems and E test (SYSMEX, bioMérieux, Tokyo, Japan). The strain obtained on day 5 was susceptible to both VCM and DAP, but MICs were confirmed to be elevated to resistant category on day 17. Antimicrobial susceptibility testing was based on guidelines from the Clinical and Laboratory Standards Institute (M100-S23).

**Bacterial analysis**

We confirmed the identification of the Pre-Abx and Post-Abx strains using the phage open-reading frames typing (POT) method. This method has equal utility to that of pulsed-field gel electrophoresis (PFGE) in discriminating *S. aureus* subtypes and has been applied for surveillance during MRSA outbreaks (5, 6). The POT pattern was determined to be (93-159-111) using the Cica Geneus Staph POT kit (Kanto Chemical, Tokyo, Japan). According to the manufacturer’s instructions, it was predicted that the strains contained the SCCmeC IIa element and were NY/Japan clones.

We then compared the strain obtained from the initial blood culture (Pre-Abx) with that obtained 17 days after treatment (Post-Abx) in order to determine the mechanism underlying the elevated MIC value for DAP.

First, changes in the bacterial cell wall thickness were examined in the two strains. The samples were fixed in 1% glutaraldehyde for 1 hour at 4°C and subsequently embed-
Antibiotic therapy

<table>
<thead>
<tr>
<th>MEPM</th>
<th>DAP</th>
<th>LZD</th>
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<tr>
<td>1.5 g/day</td>
<td>270 mg/day (6 mg/kg/day)</td>
<td>1200 mg/day</td>
</tr>
</tbody>
</table>

Surgical Intervention

Blood culture

Day 5

Day 17

Day 30

Continuous irrigation

Figure 2. Clinical course of the patient. Antibiotic therapy with MEPM and VCM was empirically initiated for five days and then converted to DAP alone. Nevertheless, MRSA was repeatedly isolated from the blood, and a strain with reduced susceptibility to DAP was detected on day 17. DAP had been given for 12 days by that time. After starting LZD, the persistent MRSA bacteremia disappeared. White arrow: Drainage of the iliopectineous abscess, Black arrow: Discocytomy for thoracic discitsis, ★ Positive blood culture, ☆ Negative blood culture. CRP: C-reactive protein, BT: Body temperature, VCM: Vancomycin, DAP: Daptomycin, MRSA: Methicillin-resistant Staphylococcus aureus, LZD: Linezolid, MEPM: Meropenem. Continuous line: CRP, dotted line: BT.

ded after fixation in 1% osmium tetroxide for 1 hour at 4°C and dehydration. The thickness of the cell wall was measured by an investigator using transmission electron microscopy. In each case, five bacterial cells were randomly selected and five points of the cell walls were measured. The results showed no significant differences in the cell wall thickness between the two strains (Fig. 3).

Second, the changes in the bacterial surface charge were measured. Using a cytochrome c binding assay (7), we compared the relative surface charge of the Pre-Abx with Post-Abx strains. The Post-Abx cells bound to a lower amount of positively charged cytochrome c than the Pre-Abx cells. This result shows that the Post-Abx strain exhibited a significantly more relative positive surface charge than the Pre-Abx strain (p value <0.05) (Fig. 4).

Third, we investigated single-nucleotide polymorphisms (SNPs) in the multipptide resistance factor (mprF) gene, which has been reported to be observed in most DAP non-susceptible MRSA strains (8). We determined the DNA sequence of the whole region of the mprF in the Post-Abx strain and compared it to that of the Pre-Abx strain. We observed one nucleotide change (C to T) in the Post-Abx strain and found that this mutation occurred at position 1364645 in the S. aureus N315 sequence (GenBang accession no BA000018.3). This point mutation led to the amino acid substitution Thr345 to Ile (T345I) in MprF.

Discussion

In this report, we describe the emergence of a DAP-resistant MRSA strain shortly after the initiation of antibiotic therapy. It has been previously shown that the DAP-resistant strain occurs as an in vitro phenomenon by stimulating S. aureus with DAP (9). However, the in vivo development of DAP-resistant strains during treatment has also been reported recently (3, 10-13).

According to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and Clinical and Laboratory Standards Institute (CLSI, M100-S23), strains with an MIC breakpoint of >1 μg/mL are currently defined as resistant or non-susceptible to DAP. In strains with a higher MIC of DAP (>2 μg/mL), the cure rate has been reported to be decreased (14). Although VCM-resistant strains emerged only after four decades of clinical use, resistance to DAP developed shortly after its debut (15). Therefore, the emergence of a DAP-resistant strain of MRSA during treatment is of great concern to clinicians.

The mechanisms of DAP resistance have not been fully elucidated, although a few theories involving the cell wall or cell membrane have been proposed. In addition, it is considered that genetic alterations may be related to creeping of the MIC during treatment, as an increase in the MIC first
cell wall

Figure 3. Results for the measurements of the cell wall thickness using transmission electron microscopy. The cell wall thickness of the MRSA strain was examined. A total of 25 points in the cell wall were examined in each sample. The strain obtained from the initial blood culture is labeled Pre-Abx, and the strain revealing a resistant propensity to VCM and DAP (obtained on day 17) is labeled Post-Abx. The median value of each strain was 23.87 nm (range: 18.37 to 28.44 nm) and 23.05 nm (range: 16.79 to 27.68 nm), respectively. There were no significant differences between the cell wall thicknesses of the strains (Mann-Whitney U test). The central lines in the boxes show the median value and the error bars indicate the standard deviation.

Cell wall thickness (nm)

Pre-Abx  Post-Abx

30
25
20
15
10
5
0

p=0.79

Figure 4. Comparison of the relative cell surface charge between the Pre-Abx and Post-Abx strains. The cells were incubated with 0.5 mg/mL of cytochrome c for 10 minutes and the amount of free (unbound) cytochrome c in the supernatant was quantified as the absorbance at 550 nm (O.D. 500). A higher amount of unbound cytochrome c meant that the cells possessed a more positive surface charge. Shown is the mean +/- standard deviation (n=3). *, p<0.05.

appears shortly after drug exposure and continues for a prolonged time (12).

First, cell wall thickening is considered to be a possible mechanism (16). With a thickened cell wall, the permeability of DAP into the bacterial cell membrane may decrease (9, 17). Although this mechanism has been proposed to play a major role in DAP-resistance, cell wall thickening does not necessarily exist in DAP-resistant MRSA strains (18). In the current case, transmission electron microscopy did not show any significant changes in the thickness of the bacterial cell wall.

Second, changes in the electrical charge on the bacterial cell surface can be associated with decreased susceptibility. An increased positive surface charge among DAP-resistant strains has previously been reported (19). In this study, we detected the same phenomenon in our strain; i.e., the Post-Abx strain possessed a significantly increased positive cell surface charge compared with the Pre-Abx strain (Fig. 4).

Third, mutations in genes such as mprF, yycG, rpoB and rpoC are known to be responsible for regulating the surface charge of the bacterial membrane (20). In the present case, the sequencing analysis of the mprF of Post-Abx strain showed a point mutation leading to the amino acid substitution Thr345 to Ile (T345I), which has been observed in several DAP-non susceptible MRSA strains derived from both patients and laboratories (9, 20-22). Since we only investigated the sequence in mprF, there might be other factors, such as SNPs in yycG, rpoB or rpoC, as listed above, in which the Post-Abx strain exhibited a higher positive surface charge than the Pre-Abx strain.

Lastly, another explanation includes the existence of a pre-existing resistant subpopulation (23). These subpopulations may selectively remain with DAP treatment, and some reports have shown that VCM can select for resistant subpopulations and thus induce creeping of the MIC (24, 25); i.e., DAP-resistant strains may emerge during treatment with VCM. In such cases, the duration of VCM therapy prior to the onset of DAP resistance has been reported to range from one to 148 days (26). In our case, VCM and DAP were administered for a relatively shorter period, five and 12 days, respectively, before the emergence of the resistant strain.
There are some additional mechanisms that may be associated with the emergence of DAP-resistance, such as changes in cellular metabolism or enhanced cell wall turnover (10, 16).

Hence, whatever the mechanism of resistance, more attention should be paid when VCM or DAP is administered for MRSA infection (12, 26, 27). In addition to the prior use of these antibiotics, predictors of DAP-resistance include persistent bacteremia and a high bacterial load (27). Antimicrobial susceptibility testing should also be performed repeatedly during the treatment course, especially in refractory cases. In the present case, the bacterial load was assumed to be high as a result of multiple foci: the iliopsoas abscess, vertebral osteomyelitis, discitis and central venous reservoir infection.

Various strategies have been proposed to prevent the emergence of DAP resistance. The use of a higher dose (8-10 mg/kg) of DAP may decrease the emergence of resistance compared with that of the usual dose (6 mg/kg/day) (28, 29), and early surgical intervention may need to be performed aggressively in order to reduce the level of bacterial inoculation (30, 31). In the present case, although the dose of DAP (approximately 6 mg/kg) might have been small compared with the bacterial load, surgical therapy was performed relatively early in the clinical course. Importantly, the central venous port, a possible bacterial reservoir, should have been extracted earlier.

MIC measurements for DAP can be unreliable if the calcium concentration is not adjusted. In the present case, the MIC value was tested using the VITEK 2, MicroScan WalkAway device and E test. Although the calcium concentration of VITEK 2 is not currently disclosed, the reliability of this method has been demonstrated compared to that of the Clinical and Laboratory Standards Institute (CLSI) broth microdilution reference method (32). An appropriate calcium concentration in the media for DAP susceptibility testing has been reported to be 50 μg/mL (33), while that of the MicroScan WalkAway device and E test is set at 47 and 40 μg/mL, respectively. Therefore, we consider the above results to be trustworthy; i.e., the MRSA strain isolated in our patient had indeed become resistant to DAP during treatment.

In summary, a fatal case of DAP-resistant MRSA infection was herein presented. An increased positive bacterial surface charge and single-nucleotide polymorphism within nmrF were detected in the resistant organism. Prior VCM or DAP therapy, high-inoculum infection and the use of a comparatively low dose are assumed to be possible scenarios accounting for the emergence of DAP resistance. Close monitoring for susceptibility of MRSA is needed while treating patients with VCM or DAP.

The authors state that they have no Conflict of Interest (COI).

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References


