



Original article

Antimicrobial susceptibility and molecular characteristics of methicillin-resistant *Staphylococcus aureus* in a Japanese secondary care facility



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ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA) is prevalent in Japan, and the *Staphylococcus* cassette chromosome *mec* (SCCmec) type II is common among hospital-acquired MRSA isolates. Information pertaining to MRSA characteristics is limited, including SCCmec types, in primary or secondary care facilities. A total of 128 MRSA isolates (90 skin and soft tissue isolates and 38 blood isolates) were collected at a secondary care facility, Kawatana Medical Center, from 2005 to 2011. Antimicrobial susceptibility testing for anti-MRSA antibiotics and molecular testing for SCCmec and virulence genes (*tst*, *sec*, *etb*, *lukS/F-PV*) were performed. Strains positive for *lukS/F-PV* were analyzed by multilocus sequence typing and phage open-reading frame typing. SCCmec typing in skin and soft tissue isolates revealed that 65.6% had type IV, 22.2% had type II, 8.9% had type I, and 3.3% had type III. In blood isolates, 50.0% had type IV, 47.4% had type II, and 2.6% had type III. Minimum inhibitory concentrations, MIC₅₀/MIC₉₀, against vancomycin, teicoplanin, linezolid, and arbekacin increased slightly in SCCmec II isolates from skin and soft tissue. MICs against daptomycin were similar between sites of isolation. SCCmec type II isolates possess *tst* and *sec* genes at a greater frequency than SCCmec type IV isolates. Four *lukS/F-PV*-positive isolates were divided into two clonal patterns and USA300 was not included. In conclusion, SCCmec type IV was dominant in blood, skin, and soft tissue isolates in a secondary care facility in Japan. Because antimicrobial susceptibility varies with the SCCmec type, SCCmec typing of clinical isolates should be monitored in primary or secondary care facilities.

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1. Introduction

Since the first report of methicillin-resistant *Staphylococcus aureus* (MRSA) in 1961 [1], the prevalence of MRSA has been increasing worldwide. Although MRSA infections have been observed widely in nosocomial settings, severe infections related to community-acquired (CA)-MRSA have been recognized mainly in

the United States [2]. Clinically, CA-MRSA infections typically occur as skin and soft tissue infections and sequentially develop into pulmonary abscesses or necrotic pneumonia. Severe CA-MRSA infections have also been reported in France and Australia [3,4]; moreover, CA-MRSA is becoming a worldwide concern.

Most CA-MRSA strains are characterized by the presence of *Staphylococcus* cassette chromosome *mec* (SCCmec) IV, and they frequently possess the Panton-Valentine leukocidin (*lukS/F-PV*) gene. In contrast, hospital-acquired MRSA strains have the SCCmec II cassette and are often positive for toxic shock syndrome toxin-1 (TSST-1). Japan is known as a high prevalence country for MRSA, and the majority of MRSA strains are considered to be hospital-acquired. In Japan, the percentage of MRSA among all *S. aureus*

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strains is reported to be about 50–60% [5,6]; however, severe infections due to CA-MRSA have rarely been reported in Japan [7,8].

Anti-MRSA antibiotics, such as vancomycin (VCM), teicoplanin (TEIC), linezolid (LZD), and daptomycin (DAP), have potent activities against MRSA. In addition, arbekacin (ABK), an aminoglycoside, is also available as an anti-MRSA antibiotic in Japan. MRSA strains resistant to anti-MRSA antibiotics have not emerged in Japan; however, information about the antimicrobial susceptibilities of strains is important for selecting antibiotics in clinical settings. Treatment failure can occur in patients with bloodstream infections due to strains with a VCM minimum inhibitory concentration (MIC) of 2.0 µg/mL, as reported previously [9]. Thus, a mild shift in MIC values can affect clinical outcomes.

In addition to a difference in pathogenicity, MRSA strains have different antimicrobial susceptibilities between the SCCmec II and IV strains. Some SCCmec IV strains have been known to show good susceptibility to non-anti-MRSA antibiotics, such as clindamycin, erythromycin, and levofloxacin, compared to SCCmec II strains [10,11]. However, there is less information about anti-MRSA antibiotics. Our previous studies on MRSA bloodstream infections revealed the possibility of a temporal change in SCCmec type II and SCCmec type IV proportions in a tertiary hospital [12,13]. Studies on the distribution of SCCmec types have been performed mainly in tertiary hospitals in Japan, but SCCmec types in primary or secondary care facilities are unknown. Because a change in the microbiological background can affect therapeutic strategies, we should understand trends in antimicrobial susceptibilities.

In this study, we monitored the microbiological characteristics of clinical MRSA isolates in a secondary medical care facility. We compared the SCCmec types and antimicrobial susceptibilities of isolates from blood culture with those from skin and soft tissue.

2. Materials and methods

2.1. Collection of clinical isolates

MRSA isolates from skin and soft tissue and from blood were obtained from the National Hospital Organization Nagasaki Kawatana Medical Center between 2005 and 2011. The facility offers secondary medical care and has 310 beds. The percentage of MRSA isolates among all *S. aureus* in the facility was about 60% during the study period. When several isolates were obtained from the same patient, only one isolate was selected. All isolates were identified by the VITEK II System (bioMérieux, Marcy l'Etoile, France). The antimicrobial susceptibility testing and molecular analyses were performed at Nagasaki University Hospital.

2.2. Antimicrobial susceptibility testing

The MICs of VCM, TEIC, ABK, LZD, and DAP were determined using a broth dilution antimicrobial susceptibility test according to the procedures outlined by the Clinical and Laboratory Standard Institute (CLSI) [14].

2.3. SCCmec typing and the detection of virulence genes

Bacterial DNA was extracted by heat lysis using Chelex (Bio-Rad Laboratories, Hercules, CA, USA). SCCmec types I–IV, *nuc*, *mecA*, *vanA*, toxin shock toxin (*tsst*), enterotoxin type C (*sec*), exfoliative toxin type b (*etb*), and *lukS/F-PV* were amplified by multiplex real-time polymerase chain reaction (PCR) as previously reported [15]. The amplifications of *nuc* and *mecA* were used for confirmation of MRSA. The PCR products were analyzed using 2% agarose gel electrophoresis and were visualized by staining with ethidium bromide.

2.4. Identification of the clonal complexes of the isolates

Molecular typing of MRSA was performed using POT-kit (Kanto Chemical co., Tokyo, Japan), which is a new technology to discriminate MRSA types based on a determination of the conservation patterns of small genomic islets, according to the manufacturer's instructions [16]. Phage open reading frame types (POT) were determined by the three scores of POT1–POT2–POT3 calculated by the manufacturer's instructions.

2.5. Multilocus sequence typing (MLST)

The MLST was performed according to the method described by Enright et al. [17]. Sequence types (STs) were assigned to clusters using the MLST database (<http://www.mlst.net>).

3. Results

3.1. Baseline characteristics of the study population

A total of 128 MRSA isolates were obtained in the study. Of the 128 isolates, 90 were isolated from skin and soft tissue and 38 were isolated from blood. The baseline characteristics of patients were shown in Table 1.

3.2. SCCmec types in the skin and soft tissue isolates and the blood isolates

SCCmec types were analyzed in all isolates (Table 2). The percentages of SCCmec types I, II, III, and IV among the skin and soft tissue isolates were 8.9%, 22.2%, 3.3%, and 65.6%, respectively. In contrast, among the blood isolates, the percentages of SCCmec types I, II, III, and IV were 0.0%, 47.4%, 2.6%, and 50.0%, respectively. SCCmec type I was detected only in the isolates from skin and soft tissue. The prevalence of SCCmec type II in the isolates from blood was twice as frequent as that in the isolates from skin and soft tissue.

3.3. Antimicrobial susceptibilities of the isolates

The MICs of the 128 isolates were measured (Fig. 1). DAP showed the most potent activity against all of the isolates, with MIC₅₀/MIC₉₀s of 0.25/0.5 µg/mL. The MICs against VCM, TEIC, and ABK seemed to be slightly higher in the skin and soft tissue isolates than in the blood isolates. The MIC₉₀s against VCM, TEIC, and ABK in the skin and soft tissue isolates were 2.0 µg/mL, while those in the blood isolates were 1.0 µg/mL. The isolates with MICs of 2.0 µg/mL against VCM were not observed in the blood isolates, but were observed in 13.3% of the skin and soft tissue isolates. The MIC₉₀s against LZD were similar from both sample sources; however, the MIC₅₀s were 2.0 µg/mL in the skin and soft tissue isolates and 1.0 µg/mL in the blood isolates. There were no isolates resistant to all of the antimicrobials tested.

Next, the susceptibilities of each SCCmec type were analyzed (Fig. 2). The distribution of MIC values against DAP was similar between the blood and the skin and soft tissue isolates in both SCCmec types, but the MIC values in SCCmec II was slightly elevated.

Table 1
Baseline characteristics of patients.

	Skin and soft tissue, n = 90	Blood, n = 38	Total, n = 128
Age (mean ± SD)	62.2 ± 28.0	75.2 ± 15.5	68.7 ± 25.7
Male (%)	53.3	68.4	57.8
Inpatient (%)	67.8	92.1	75.0

Table 2
SCCmec types of the 128 MRSA strains.

	SCCmec type, n (%)			
	I	II	III	IV
Skin and soft tissue, n = 90	8 (8.9)	20 (22.2)	3 (3.3)	59 (65.6)
Blood, n = 38	0	18 (47.4)	1 (2.6)	19 (50.0)
Total, n = 128	8 (6.3)	38 (29.7)	4 (3.1)	78 (60.9)

In tests with VCM, TEIC, and ABK, the MIC values in SCCmec IV were similarly distributed in both the blood and the skin and soft tissue isolates. However, MIC values in SCCmec II were different in tests with TEIC and ABK, with the blood isolates showing greater susceptibility to both antimicrobials than the skin and soft tissue isolates. In tests with LZD, the most common MIC value of both

SCCmec types in the blood isolates was 1.0 µg/mL, but that in the skin and soft tissue isolates was 2.0 µg/mL.

3.4. Analysis of virulence genes

Because the virulence factors of a specific *S. aureus* strain can be associated with clinical findings, the characteristics of virulence genes were molecularly analyzed (Table 3). The most common gene was *tst* in isolates from both groups (41.1% in the skin and soft tissue isolates and 47.4% in the blood isolates). Similarly, *sec* was frequently observed, and the percentages in the skin and soft tissue isolates and the blood isolates were 40.0% and 34.2%, respectively. However, *etb* and *lukS/F-PV* were rare and observed only in the skin and soft tissue isolates (*etb*, 6.7%; *lukS/F-PV*, 4.4%). All virulence genes tested were observed more commonly in the skin and soft tissue isolates. There were no *vanA* positive isolates in this study.

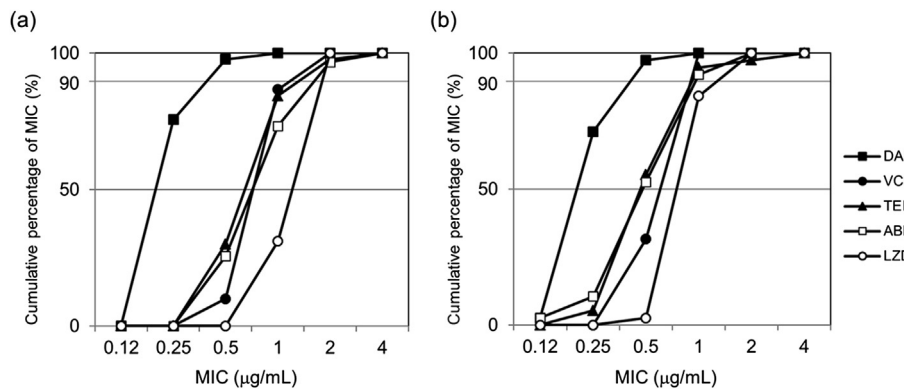
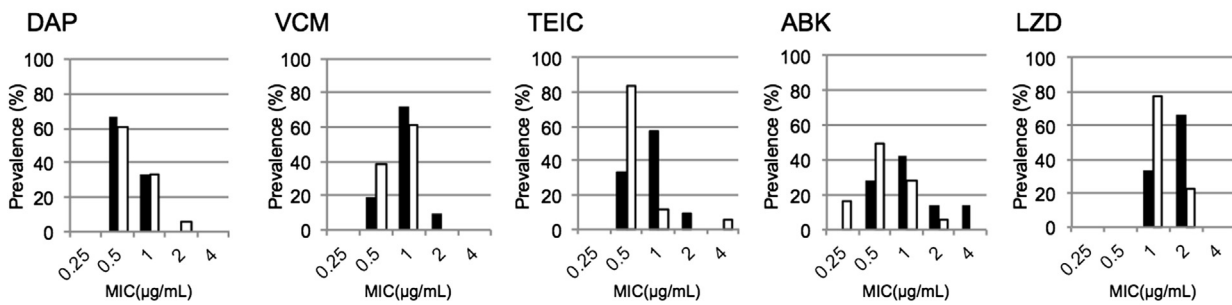


Fig. 1. Cumulative minimum inhibitory concentration curves. (a) Skin and soft tissue isolates: The MIC₉₀ values for VCM, TEIC, ABK, LZD, and DAP were 1, 1, 1, 2, and 0.5 µg/mL, respectively. (b) Blood isolates: The MIC₉₀ values for VCM, TEIC, ABK, LZD, and DAP were 1, 2, 2, 2 and 0.5 µg/mL, respectively.

(a) SCCmec II



(b) SCCmec IV

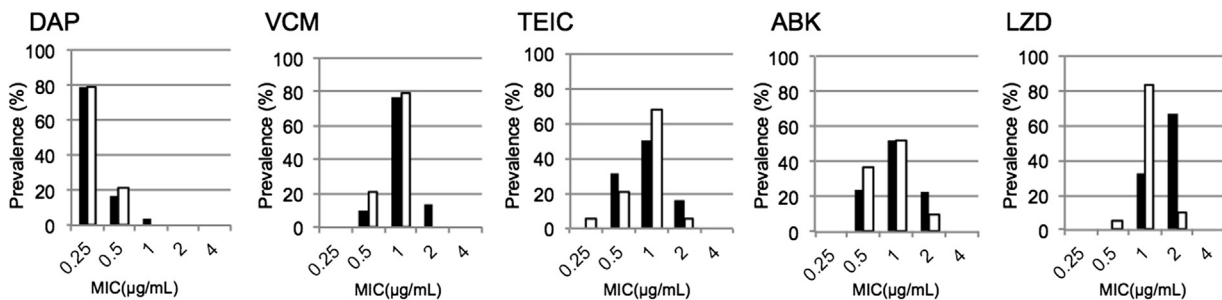


Fig. 2. MIC values according to SCCmec type. The histogram of susceptibilities to anti-MRSA antibiotics of SCCmec II isolates (a) and SCCmec IV isolates (b). Black and white bars represent the skin and soft tissue isolates and the blood isolates, respectively.

Table 3
Virulence genes of the 128 MRSA strains.

SCCmec type	Skin and soft tissue, n (%)				Blood, n (%)			
	<i>tst</i>	<i>sec</i>	<i>etb</i>	<i>lukS/F-PV</i>	<i>tst</i>	<i>sec</i>	<i>etb</i>	<i>lukS/F-PV</i>
I	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	—	—	—	—
II	16 (80.0)	14 (70.0)	1 (5.0)	0 (0.0)	12 (66.7)	8 (44.4)	0 (0.0)	0 (0.0)
III	2 (66.7)	2 (66.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
IV	19 (32.2)	20 (33.9)	5 (8.5)	4 (6.8)	6 (31.6)	5 (26.3)	0 (0.0)	0 (0.0)
Total	37 (41.1)	36 (40.0)	6 (6.7)	4 (4.4)	18 (47.4)	13 (34.2)	0 (0.0)	0 (0.0)

3.5. Genotyping by phage open reading frame typing and multilocus sequence typing

To analyze the relationship between the four *lukS/F-PV*-positive isolates, the isolates were compared by two different molecular typing methods, POT and MLST. The four isolates were divided into two groups, with each group including two isolates. The POT score and the MLST score of one group was 106–127–113 and ST8, and the other was 110–1–137 and ST30.

4. Discussion

The epidemiological background of MRSA clinical isolates is important for making clinical decisions and determining infection control strategies. We previously reported clinical characteristics and MRSA SCCmec typing in MRSA bloodstream infections in Nagasaki University Hospital [12,13]. In the present study, we examined antimicrobial and molecular characteristics of MRSA clinical isolates in Kawatana Medical Center, a secondary care facility. Because the Kawatana Medical Center is located about 56 km (35 miles) away from Nagasaki University Hospital, We believe that these two hospitals have different patient profiles.

Japan is known to have a high prevalence of MRSA. MRSA infections observed in hospital settings have been recognized as primarily SCCmec type II MRSA. However, SCCmec type II MRSA represented less than half of the blood isolates in the present study, while half of these isolates were SCCmec type IV. During the study period, the prevalence of SCCmec type IV in the blood isolates varied according to the year; however, there were some periods with a high detection rate also (data not shown). The high percentage of SCCmec type IV among blood isolates is similar to two previous reports from Nagasaki University Hospital in which the percentages of SCCmec type IV increased from 18.2% to 32.0% [12,13]. Although we did not analyze the clinical characteristics of the patients, we speculate that secondary bloodstream infections after soft tissue and skin infections occurred in only a small proportion of these MRSA cases, because medical illnesses, such as respiratory diseases and neuromuscular disorders, and surgical cases dominate the inpatient ward of Kawatana Medical Center. The possibility of catheter-related infections should also be considered. To determine the reason for the increase in SCCmec IV MRSA in blood isolates, a molecular approach to typing of blood isolates and isolates from other sources will be required.

There were small differences in antimicrobial susceptibilities according to the sources of MRSA isolates. The isolates with MICs of 2.0 µg/mL to VCM were not recovered from blood; however, the presence of less susceptible strains in the skin and soft tissue suggests that patients with secondary MRSA bacteremias resulting from skin and soft tissue infections should be carefully treated. In the present study, we did not address the reason for the mild elevation of MICs against anti-MRSA antibiotics among SCCmec type II MRSA isolates from skin and soft tissue. The use of VCM can lead to reduced susceptibility in patients with persistent or

recurrent MRSA infections, but strains with reduced VCM susceptibility are not frequently observed [18]. Considering that the rates of virulence genes in SCCmec type II isolates were different for each sample, the major clones in the isolates from each sample could also differ, rather than a clonal strain having developed reduced susceptibilities to glycopeptides and aminoglycosides.

SCCmec type II strains commonly possess *tst* and *sec* genes [19]. In the present study, the isolates with *tst* or *sec* genes were predominantly SCCmec type II, in contrast to a previous report [20]. Correspondingly, these genes were found in SCCmec type IV isolates at a lower rate in this study than in the earlier report [20]. These findings can reflect a geographical difference. A geographical difference in the prevalence of *lukS/F-PV*-positive strains has been recognized. These strains are highly prevalent in the United States, while Japan is a low prevalence country, with only 1.6–10% of strains reported as *lukS/F-PV*-positive. Therefore, the percentage of *lukS/F-PV*-positive isolates in the present study is low compared with reports from other countries.

POT is a new molecular-based technology to analyze the genetic relatedness of MRSA strains. POT can separate different strains more accurately and reliably than repetitive-sequence-based PCR [21]. The sequence types of *lukS/F-PV* positive strains in this study were ST8 and ST30. ST8 is known to be the sequence type of the pandemic clone, USA300, but POT scores of the isolate were different from those of USA300 (POT 106–77–113). Because ST8 and ST30 have both been observed in Japan, *lukS/F-PV* positive strains in this study could be domestic strains rather than the imported USA300.

In conclusion, the characteristics of MRSA isolates from blood and from skin and soft tissue were evaluated in a secondary medical care facility. MRSA clone USA300 was not detected in this study, but SCCmec type IV was dominant in both the blood and the skin and soft tissue isolates. A mild elevation in MIC values against anti-MRSA antibiotics, except for LZD, was observed in the SCCmec II isolates from skin and soft tissue. Thus, the continuous monitoring of MIC values in clinical MRSA isolates is important for managing the treatment and control of MRSA.

Conflict of interest

The authors declare no conflicts of interest.

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