

Short Communication

Epidemiological and Microbiological Analysis of Community-Associated Methicillin-Resistant *Staphylococcus aureus* Strains Isolated from a Japanese Hospital

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SUMMARY: Reports of community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) infections have recently increased in Japan; however, these studies contain limited information on their epidemiology. We performed a single-center study in the Tokyo Medical University Hospital located in Shinjuku, a central area of Tokyo, Japan. From 2,099 MRSA isolates obtained during July 2007 to March 2009, we selected 44 MRSA isolates with a MIC of $< 2 \mu\text{g}/\text{mL}$ for imipenem. Among 44 isolates, 28 strains had type IV or type V SCCmec, and we classified them as CA-MRSA. We identified only 1 Panton-Valentine leukocidin (PVL)-positive MRSA strain, which belonged to SCCmec type V. The PVL-positive CA-MRSA strain was isolated from a patient with multiple subcutaneous abscesses. The patient had returned to Japan from India; thus, the strain may have been contracted from outside of Japan. Thirteen (46.4%) and 15 strains (53.6%) were isolated from outpatients and inpatients, respectively. The major sites of infection included the respiratory tract (8 strains, 28.6%), skin/soft tissue (4 strains, 14.3%), and nasal cavity (4 strains, 14.3%). It is important to note that the most common site of CA-MRSA infection in inpatients was the respiratory tract; respiratory infections with CA-MRSA frequently cause severe infectious diseases.

Methicillin-resistant *Staphylococcus aureus* (MRSA) emerged in the 1960s as a cause of infection among patients exposed to bacteria in healthcare settings. However, community-associated MRSA (CA-MRSA) was recognized in the mid-1990s (1) and has emerged worldwide as a life-threatening pathogen. CA-MRSA causes skin/soft tissue infections, but it has also been associated with sepsis and necrotizing pneumonia (2). In contrast to healthcare-associated MRSA (HA-MRSA) infections, for which there is a predisposing risk factor or illness, CA-MRSA infections can occur in healthy individuals, suggesting that these bacterial strains have greater virulence than traditional HA-MRSA strains. Reports of CA-MRSA infections have recently increased in Japan; however, previous studies have contained limited information on their epidemiology.

CA-MRSA strains contain a methicillin resistance element, known as staphylococcal chromosome cassette mec (SCCmec) type IV or V (3), whereas traditional HA-MRSA strains contain a larger SCCmec element type I-III. In contrast to most HA-MRSA isolates, CA-MRSA isolates are susceptible to non- β -lactam antibiotics and therefore, are not multidrug resistant.

In this study, we collected MRSA isolates with a MIC

of $< 2 \mu\text{g}/\text{mL}$ for imipenem (IPM) because some references suggest that SCCmec type IV strains are susceptible to IPM (4). The isolates were tested for SCCmec type (5) and were classified as CA-MRSA if the strains had type IV or type V SCCmec. Epidemiological data of CA-MRSA are very useful for clinicians; we investigated genotypic characteristics of MRSA strains isolated in our hospital and analyzed clinical data collected by reviewing medical records to determine the epidemiology of CA-MRSA among university hospitals in Japan.

The MIC of each isolate was determined using a broth microdilution assay, according to Clinical and Laboratory Standard Institute reference methods (6). Antimicrobial susceptibility of each strain was tested using ready-made dry plates (DP32) manufactured by the Eiken Chemical Co. (Tokyo, Japan). Plates contained 18 antimicrobial agents, including oxacillin (MIPIC), ampicillin (ABPC), cefoxitin (CFX), cefazolin (CEZ), cefmetazole (CMZ), flomoxef (FMOX), IPM, gentamicin (GM), arbekacin (ABK), minomycin (MINO), erythromycin (EM), clindamycin (CLDM), fosfomycin (FOM), sulfamethoxazole/trimethoprim (ST), levofloxacin (LVFX), vancomycin (VCM), teicoplanin (TEIC), and linezolid (LZD). Inoculum was adjusted to yield a cell density of 5×10^5 CFU/mL. Plates were incubated for approximately 24 h at 35°C and examined by visual observation.

Bacterial DNA was extracted using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) with lysostaphin (Wako, Osaka, Japan). Genomic DNA was used as a template for PCR amplification. SCCmec types (I to

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V) of MRSA were analyzed using a PCR assay as previously described and reference strains (7). For *SCCmec* type II and *SCCmec* type IV, subtypes were further analyzed using a PCR assay (5). Multilocus sequence typing (MLST) was performed using 7 housekeeping genes as previously described (8). An allelic profile was obtained from the MLST website (<http://WWW.mlst.net/>). Staphylococcal virulence genes were detected using a PCR assay with previously reported primers. Targeted genes included the Panton-Valentine leukocidin (PVL) gene (9), 3 exfoliative toxin genes (*eta*, *etb*, and *etd*) (10,11), and toxic shock syndrome toxin-1 gene (*tst-1*) (9).

Because some *SCCmec* types are rare in Japan, the following strains were used as positive controls in this study: NCTC10442 (*SCCmec* type I), N315 (*SCCmec* type IIa), JCSC3063 (*SCCmec* type IIb), 85/2082 (*SCCmec* type III), JCSC4744 (*SCCmec* type IVa), JCSC2172 (*SCCmec* type IVb), JCSC4788 (*SCCmec* type IVc), JCSC4469 (*SCCmec* type IVd), JCSC4796 (*SCCmec* type IVg), and WIS (*SCCmec* type V). These strains were provided by Professor Keiichi Hiramatsu (Juntendo University, Tokyo, Japan). ATCC49775 was used as a positive control for the PVL genes.

The 44 strains were selected from 2,099 MRSA strains isolated between July 2007 and March 2009 in the microbiology laboratory of the Tokyo Medical University Hospital in Japan and were classified as *SCCmec* type I (2, 4.5%), type II (12, 27.3%), type IV (19, 43.2%), and type V (9, 20.5%). Two isolates (4.5%) were designated as nontypeable. For *SCCmec* type II, 4 subtypes were analyzed. Among 12 *SCCmec* type II strains, only 1 strain was *SCCmec* type IIa, 4 were *SCCmec* type IIb, and 7 were nontypeable (IIn). *SCCmec* type IIa strain belonged to sequence type (ST) 5, with the allelic profile 1-1-1-1-22-1-1, and was isolated from a 57-year-old female inpatient. Among the remaining 11 isolates, 8 were isolated from outpatients, and 3 were from pediatric inpatients. For *SCCmec* type IV, 8 types were analyzed and classified into 5 subtypes. Nineteen strains were classified as *SCCmec* type IVa (6, 31.6%), type IVc (1, 5.3%), type IVg (3, 15.8%), type IVj (1,

5.3%), and nontypable (8, 42.1%). *SCCmec* type IVa strains were the same type as the USA300 clone; however, in this study, 6 strains of *SCCmec* type IVa showed different pulsed-field patterns than USA300 (Fig. 1), and they were negative for PVL genes.

We analyzed virulence genes and antimicrobial susceptibilities of these strains. Results of virulence gene detection are shown in Table 1. Of all isolates, the PVL gene was detected in only 1 strain, the ET gene in 3 strains, and the TSST-1 gene in 13 strains. A PVL-positive CA-MRSA strain was *SCCmec* type V and was isolated from multiple subcutaneous abscesses of a patient. Because the response to treatment was negative, this patient needed to be hospitalized. This strain belonged to ST772, with the allelic profile 1-1-1-1-22-1-1, which is a single-locus variant of ST1. The strain was isolated from a patient who had returned from India.

Resistance to antimicrobial agents of the 44 strains is

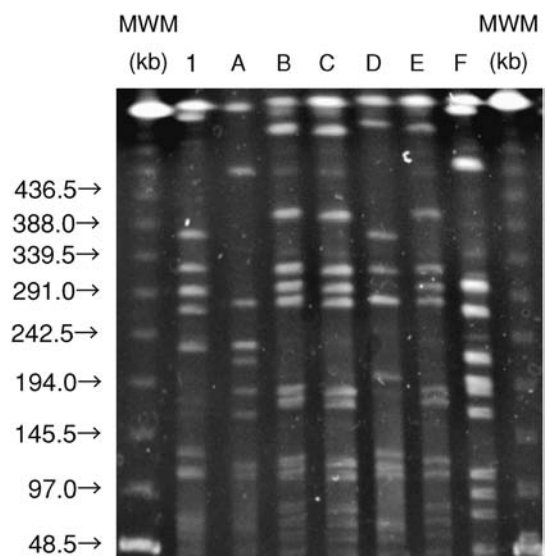


Fig. 1. PFGE patterns of *SmaI*-digested DNA of *SCCmec* type IVa strains. Lane 1, USA300-0114. Lane A-F, *SCCmec* type IVa strains in this study.

Table 1. Virulence gene and resistance to antimicrobial agent

<i>SCCmec</i> type	I	II	III	IV	V	NT	Total
	2	12	0	19	9	2	
Virulence gene							
<i>lukpvSF</i>	0 (0)	0 (0)	0 (0)	0 (0)	1 (11.1)	0 (0)	1 (2.3)
<i>eta</i>	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>etb</i>	0 (0)	1 (8.3)	0 (0)	1 (5.3)	1 (11.1)	0 (0)	3 (6.8)
<i>etd</i>	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>tst-1</i>	0 (0)	2 (16.7)	0 (0)	9 (47.4)	2 (22.2)	0 (0)	13 (29.5)
Resistant to							
- CEZ (>16 µg/mL)	0 (0)	1 (8)	0 (0)	1 (5)	0 (0)	1 (50)	3 (7)
- IPM (>8 µg/mL)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
- LVFX (>4 µg/mL)	2 (100)	2 (17)	0 (0)	8 (42)	3 (33)	0 (0)	15 (34)
- GM (>8 µg/mL)	2 (100)	8 (67)	0 (0)	6 (32)	7 (78)	1 (50)	24 (55)
- CLDM (>2 µg/mL)	0 (0)	12 (100)	0 (0)	0 (0)	0 (0)	2 (100)	14 (32)
- EM (>4 µg/mL)	2 (100)	12 (100)	0 (0)	2 (11)	5 (56)	2 (100)	23 (52)

SCCmec, staphylococcal cassette chromosome *mec*; NT, nontypeable; CEZ, cefazolin; IPM, imipenem; LVFX, levofloxacin; GM, gentamicin; CLDM, clindamycin; EM, erythromycin.

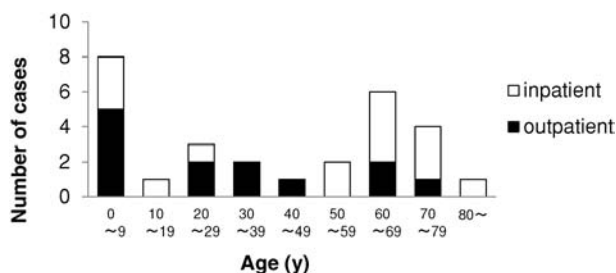


Fig. 2. Age distribution (SCCmec type IV and type V strains). Among CA-MRSA type strains, 15 strains (53.6%) were isolated from inpatients. In inpatients, most strains were isolated from senior citizens. In outpatients, most strains were isolated from children.

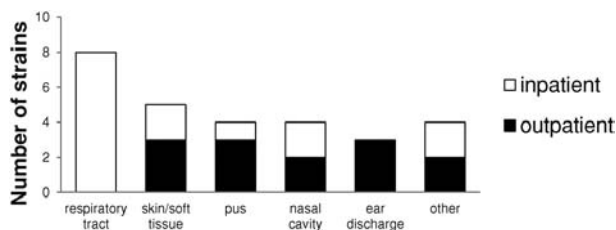


Fig. 3. Site of infection (SCCmec type IV and type V strains). Major sites of infection were the respiratory tract and skin/soft tissue. All specimens from the respiratory tract were isolated from inpatients. Among 8 strains from the respiratory tract, 7 strains were SCCmec type IV and 1 strain was SCCmec type V.

shown in Table 1. MICs for the IPM of all strains (44 strains) were $\leq 1 \mu\text{g}/\text{mL}$. Among SCCmec type IV and type V isolates, MICs for CLDM and EM were significantly lower than those of SCCmec type II strains. In particular, no isolates with an MIC $\leq 0.25 \mu\text{g}/\text{mL}$ for CLDM were identified in type II strains, whereas all type IV and type V strains had an MIC of $\leq 0.25 \mu\text{g}/\text{mL}$ ($P < 0.01$). Among type IV and type V strains, 11 strains (39.3%) and 13 strains (46.4%) showed resistance to LVFX and GM, respectively.

Age distribution and infection site in patients with isolated CA-MRSA are shown Fig. 2 and 3, respectively. In outpatients, many CA-MRSA strains were isolated from skin/soft tissue infections. In inpatients, strains were most frequently isolated from the upper respiratory tract. Most of these patients appeared to be carriers of CA-MRSA strains, but 3 patients were diagnosed as pneumonia based on the CA-MRSA strain.

CA-MRSA strains, such as USA300, are spreading, according to an epidemiological study conducted in the USA in 2004, which suggested that 78% of *S. aureus* isolated from skin/soft tissue infections were MRSA. Surprisingly, 97% of the analyzed MRSA were the same as clones analyzed in the USA300 (12). It is thought that PVL is a major virulence factor of USA300 (13–15), but few cases of severe infections caused by PVL-positive CA-MRSA have been reported in Japan. The first death case, due to a PVL-positive CA-MRSA in Japan, was a 16-month-old child with no medical history (16). The patient was diagnosed with pneumonia and appropriate treatment was given, but the patient died 10 days after admission. The strain isolated from this case was ST30 and SCCmec type IVa and was positive for the PVL gene. There have also been reports of infections caused

by the USA300 clone in Japan (17–19).

Reports have suggested an increase in the proportion of CA-MRSA isolates in a hospital in USA (20); therefore, we analyzed the CA-MRSA strains isolated from a university hospital in Japan. Many CA-MRSA strains are susceptible to IPM. Therefore, the screening criterion of CA-MRSA that we adopted in this study was IPM susceptibility of strains. However, since there may have been some IPM-resistant CA-MRSA strains, it was likely that there were more CA-MRSA strains among the 2,099 MRSA isolates.

In this study, we identified only 1 PVL-positive MRSA, which belonged to ST772 and SCCmec type V. PVL-positive ST772 strains have been found in Malaysia, Bangladesh, India, England, and Italy (21–24) but have not been isolated in Japan. Because the ST772 strain was isolated in many clinical isolates in India, the PVL positive strain in this study may have been introduced from India (22,25).

This study suggested that most CA-MRSA strains in Japan do not contain the PVL gene. The pathogenicity of PVL-negative CA-MRSA strains appears to be weak. Similarly to PVL-positive CA-MRSA, PVL-negative CA-MRSA strains cause skin/soft tissue infections in healthy individuals, but these strains rarely cause severe infections. However, many PVL-negative CA-MRSA strains were isolated from upper respiratory tract samples of inpatients in our study. Although the CA-MRSA strains were PVL-negative, these strains can cause severe infections, such as pneumonia, in immunocompromised hosts. More CA-MRSA strains were detected among inpatients than among outpatients. However, among all samples in our hospitals, most MRSA strains were isolated from inpatients. Therefore, the ratio of CA-MRSA among all MRSA isolates from inpatients was very low.

Since we selected MRSA strains based on antimicrobial sensitivity in this study, most SCCmec type II strains were type IIb and IIc, which are not multiresistant to antimicrobial agents. Only 1 isolate belonged to SCCmec type IIa and ST5; thus, we concluded that this strain was a New York/Japan clone. The SCCmec type IIb and type IIc strains were isolated from pediatric outpatients or inpatients. We treated these strains as HA-MRSA in this study; however, SCCmec type IIb MRSA strains are sometimes isolated from within the community (26,27).

PVL-positive CA-MRSA infections are rare in Japan, but PVL-negative CA-MRSA strains cause not only skin/soft tissue infections but also severe infections in immunocompromised hosts. Thus, care should be taken when treating immunocompromised patients that have nosocomial infections. PVL-positive CA-MRSA strains can be imported and may spread throughout Japan. Therefore, it is important to carefully monitor the occurrence of these strains.

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Conflict of interest None to declare.

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