Original Article

Clinical Aspects of Infection with Methicillin-Resistant *Staphylococcus aureus* USA300 Strain, Generally Regarded as Community-Acquired, in Japan

Itaru Nakamura¹, Tetsuo Yamaguchi^{1,2*}, Yuri Miura^{1,2}, Hiroyuki Shimizu¹, Shinji Fukushima¹, Yasutaka Mizuno¹, and Tetsuya Matsumoto^{1,2}

¹Department of Infection Control and Prevention, Tokyo Medical University Hospital, Tokyo 160-0023; and

²Department of Microbiology, Tokyo Medical University, Tokyo 160-8402, Japan

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SUMMARY: The characteristics of community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) infection in Japan have not yet been completely established compared with those in Europe and the United States. CA-MRSA infections with the USA300 clone are very rare in Japan. In this study, we describe 4 cases of CA-MRSA infections, particularly the USA300 clone. Case 1 involved a 21-year-old man without any remarkable medical history or risk factors of CA-MRSA who suffered from a rapidly progressive infection in his left arm. Case 2 involved a 34-year-old man and Case 3 a 22-year-old man who presented with recurrent and refractory furuncles. Both men were members of a combat sports gym where other members also had skin infections. Case 4 involved a 60-year-old man with lumbar canal stenosis who suffered from surgical site infection 7 days after lumbar laminectomy and posterolateral fusion. Only 5 cases of USA300 infections were reported in Japan from 2007 to 2009, and 4 cases were detected at Tokyo Medical University Hospital from 2010 to 2011. The diversity of the routes of infection in these cases may indicate the possible spread of the USA300 clone in Japan.

INTRODUCTION

A new strain of methicillin-resistant *Staphylococcus aureus* (MRSA) has emerged in community setting since the 1990s, causing infections among young and healthy individuals. The strain was named community-acquired MRSA (CA-MRSA) and since then, other CA-MRSA strains have emerged worldwide.

CA-MRSA has primarily been described as a cause of skin and soft tissue infections (SSTIs), but some CA-MRSA strains have been implicated in severe infections, including necrotizing skin infections, necrotizing pneumonia, bacteremia, and infective endocarditis (1-3).

In the USA, almost all MRSA infections in community settings were due to CA-MRSA, primarily the USA300 clone, which appears to have replaced pre-existing MRSA clones (4). CA-MRSA has become the predominant cause of SSTIs in people seeking medical care, particularly in emergency departments (5).

The USA300 clone is positive for Panton-Valentine leukocidin (PVL) and carries the type IV staphylococcal cassette chromosome *mec* (SCC*mec*). However, most CA-MRSA isolates in Japan are PVL-negative, and there is little information regarding the characteristics of CA-MRSA infections caused by the USA300 clone.

To the best of our knowledge, only a few cases of

USA300 infection have been reported in Japan. In order to further clarify the characteristics of the USA300 clone in Japan, we describe 4 cases with a review of the previous cases.

MATERIALS AND METHODS

We collected a total of 809 MRSA isolates from the clinical specimens of 329 patients treated at Tokyo Medical University Hospital (TMUH) from 2010 to 2011. We searched the medical records of patients definitely infected with the USA300 clone as verified by pulsed-field gel electrophoresis (PFGE) among MRSA isolates that were relatively susceptible to β -lactams, yet clinically refractory to the appropriate treatment during the same period. The medical records of patients were retrospectively reviewed for age, sex, underlying diseases, previous medications, manifestation, predisposing factors, treatments, route of infection, and treatment outcomes.

Antimicrobial susceptibility and minimum inhibitory concentrations (MICs) were determined using the MicroScan Walk Away plus system® (Siemens Healthcare Diagnostics, Deerfield, Ill., USA). Antimicrobial agents included oxacillin (MIPIC), ampicillin (ABPC), cefazolin (CEZ), cefotiam (CTM), imipenem/cilastatin (IPM/CS), arbekacin (ABK), erythromycin (EM), clindamycin (CLDM), minocycline (MINO), levofloxacin (LVFX), sulfamethoxazole/trimethoprim (ST), vancomycin (VCM), and linezolid (LZD). Inducible CLDM resistance was tested by broth microdilution using a single well containing a combination of EM and CLDM.

*Corresponding author: Mailing address: Department of Microbiology, Tokyo Medical University, 6-1-1 Shinjuku, Shinjuku-ku, Tokyo 160-8402, Japan. Tel: +81-3-3351-6141 ext. 241, Fax: +81-3-3351-6160, E-mail: t_yama@tokyo-med.ac.jp

Molecular characterization of MRSA isolates was performed. Multilocus sequence and SCC*mec* typing were performed as previously described (6,7). The presence of PVL genes and the arginine catabolic mobile element (ACME) was confirmed by the identification of the *lukS-PV* and *lukF-PV*, and the *arcA* genes by polymerase chain reaction (8,9). PFGE was performed as previously described (10) using the restriction enzyme SmaI and USA300-0114 as a control, which was kindly provided by Professor Keiichi Hiramatsu (Juntendo University, Tokyo, Japan). The lambda ladder (BioRad Laboratories, Tokyo, Japan) was used to determine the sizes.

RESULTS

Case 1: A 21-year-old healthy man with no medical history visited the Department of Plastic Surgery of TMUH. He had not traveled overseas, participated in contact sports except tennis, used intravenous drugs, or engaged in sex with men. Three days prior to the visit, he found an itchy coin-sized lesion on the external side of his left elbow, similar to a mosquito bite. He had no history of trauma. The lesion gradually became more swollen. He consulted a primary care dermatologist and was administered with the antibiotic cefcapene pivoxil hydrochloride hydrate (CFPN, 300 mg/day). However, post-treatment, the lesion spread between the elbow and wrist, accompanied by pain, redness and swelling. Following a second consultation, the primary care dermatologist referred the patient to TMUH. On his visit, his circulation was stable and his body temperature was 37.8°C. Redness and swelling further spread from the middle of the patient's left upper arm to his palm. The primary lesion formed an abscess that discharged pus from the center (Fig. 1). He had elevated white blood cell counts and liver enzyme levels. The results of a pus culture analysis revealed the presence of MRSA, and the results of antibiotic sensitivity tests are summarized in Table 1. The plastic surgeon at TMUH immediately diagnosed an abscess forming under the skin and performed emergency debridement to prevent necrotizing fasciitis. Ampicillin sulbactam (ABPC/ST, 12 g/day IV) and CLDM (1800 mg/day IV) were administered on the basis of the advice of an infectious disease specialist. Following debridement and antibiotic treatment, the wound began to heal. The patient was discharged from the hospital after 6 days. Antibiotics were changed from intravenous ABPC/ST and CLDM to oral CLDM based on the results of the antibiotic sensitivity test.

Case 2: A 34-year-old man visited the dermatology unit of TMUH with a 3-day skin lesion. He was a member of a combat sports gym, in which purulent skin lesions were endemic among the members. He suffered from a furuncle lesion on the left side of his jaw. Oral ST (320 mg/day) was administered as an empiric therapy after consulting with an infectious disease specialist in our department. MRSA was detected from the pus culture. The lesion did not heal after 3 days of ST treatment, indicating a very low dosage, which was increased from 320 mg/day to 480 mg/day. The patient was completely cured after 13 days. However, 11 days after his last visit to TMUH, a new lesion appeared on the right knee: thus recurrence of MRSA infection was suspect-

Fig. 1. (Color online) A lesion forming an abscess with discharge of pus from the center (arrow).

Table 1. Results of the antibiotic sensitivity test for the cases examined

	Case 1	Case 2	Case 3	Case 4 (Blood)	Case 4 (Wound)
	MIC	MIC	MIC	MIC	MIC
MPIPC	>2	>2	>2	>2	>2
ABPC	>8	>8	>8	>8	>8
CEZ	<8	<8	<8	<8	<8
CTM	<8	<8	<8	<8	<8
IPM/CS	<1	<1	<1	<1	<1
ABK	<1	<1	<1	<1	<1
EM	<0.25	>4	>4	<0.25	<0.25
CLDM	<0.5	<0.5	<0.5	<0.5	<0.5
MINO	<2	<2	<2	<2	<2
LVFX	>4	>4	>4	>4	>4
ST	<1	<1	<1	<1	<1
VCM	1	1	1	1	1
LZD	<2	<2	<2	<2	<2

MIC, minimum inhibitory concentration (μg/mL); MPIPC, oxacillin; ABPC, ampicillin; CEZ, cefazolin; CTM, cefotiam; IPM/CS, imipenem/cilastatin; ABK, arbekacin; EM, erythromycin; CLDM, clindamycin; MINO, minocycline; LVFX, levofloxacin; ST, sulfamethoxazole/trimethoprim; VCM, vancomycin; LZD, linezolid.

ed. Once again, MRSA was detected from the pus of this furuncle and flora of the nasal cavity. An alternative antibiotic, MINO (200 mg/day PO), was administered for 21 days. After MINO treatment, the lesion improved and his nasal swab tested negative for MRSA.

Case 3: One week after the first visit of the patient in Case 2, 6 members from his combat sports gym who developed furuncles consulted the Department of Dermatology of TMUH. MRSA was isolated from the sporadic furuncles in the buccal region and scalp of a 22-year-old man (Case 3), but no pathogens were isolated from the other individuals. One month before the visit, Case 3 developed a furuncle on his left elbow. He consulted a general practitioner and was prescribed MINO, and the lesion was immediately cured. On his latest visit, he was administered oral ST (480 mg/day) for 14 days. However, the degree of healing was insufficient and thus ST was changed to oral MINO (200 mg/day). MINO was administered for 7 days, but the improvement remained insufficient. As an additional treatment, the patient received a bed bath with 5.0% povidone iodine immediately prior to training. The lesion gradually began to heal. His last visit to TMUH

was 5 weeks after his initial visit.

Cultures from the patients in Cases 2 and 3 tested positive for MRSA. The results of the antibiotic sensitivity test are shown in Table 1.

Case 4: A 60-year-old man was admitted to TMUH for lumbar canal stenosis operation, lumbar laminectomy and posterolateral fusion. He previously underwent 2 surgeries (1 and 4 years ago) for lumbar canal stenosis. After the last admission, he received no medication or medical care for 1 year. Seven days after the operation, serous exudates formed on the surgical wound accompanied by pain. The cultured exudate tested positive for MRSA. Results of the antibiotic sensitivity test are shown in Table 1. Eight days after the operation, a curettage procedure was performed on the infected wound, and continuous perfusion was achieved. Twenty-eight days after the operation, vertebral screws were removed. VCM treatment (1.0 g/day) was started after evaluating the postoperative culture results. Based on the results of therapeutic drug monitoring for VCM, we adjusted the trough concentration to 15–20 µg/mL. Twenty-three days after the operation, VCM was replaced with LZD (1200 mg/day) because the patient suffered from a low-grade fever during VCM treatment. After changing to LZD treatment, he developed a co-in-

fection of *Pseudomonas aeruginosa* at the surgical site on postoperative day 35. We therefore added LVFX (500 mg/day) to the LZD treatment. The patient was treated with anti-MRSA antibiotics for a total of 64 days.

Molecular characterization: The 4 isolates shared an identical PFGE pattern with the USA300-0114 clone (Fig. 2). Similar to the USA300-0114 clone, these 4 isolates belonged to the ST8 and were positive for PVL and ACME. Although the SCCmec types of isolates from cases 1 and 4 were identified as type IVa, those of Cases 2 and 3 could not be identified as SCCmec types I to V because no ccr gene complex was identified. It indicates that there was a slight divergence between these strains and the USA300 clone.

DISCUSSION

CA-MRSA infections have been reported among prisoners, intravenous drug users, athletes, military trainees, people with tattoos, healthcare workers (HCWs) and men who have sex with other men (MSM) (11–13). CA-MRSA clones emerged in the 1980s and started globally spreading in the late 1990s.

The USA300 clone is a highly transmissible and virulent clone of CA-MRSA. For instance, in the USA, most SSTIs in patients in emergency departments are caused by the USA300 clone, and this is usually associated with inadequate initial antibiotic therapy (5).

The USA300 clone is rapidly becoming a major clone in the USA not only in communities but also in hospitals, replacing the pre-existing MRSA clones (14,15). Previously, the predominant hospital-acquired MRSA strain in the USA was USA100, but currently, USA300, which was previously considered as the archetypal community strain, accounts for 28% of infections (15). CA-MRSA strains are presently a major cause of MRSA bacteremia in some hospitals in the USA.

On the other hand, the characteristics of CA-MRSA strains are distinct in Japan. Most isolates suspected as CA-MRSA were PVL-negative and did not contain the

Fig. 2. Pulsed-field gel electrophoresis analysis of methicillin-resistant *Staphylococcus aureus* isolated from Case 1 (lane 1), Case 2 (lane 2), Case 3 (lane 3), and Case 4 (lane 4), in comparison to the USA300-0114 (lane 5). Strains from our patients (Cases 1–4) show the same band patterns as the USA300-0114.

Table 2. Reported cases of USA300 infection in Japan

Study group (Reference)	Year	Age of patient	Manifestation	Route of infection
Shibuya (18)	2007	3 mo	Subcutaneous abscess	Unknown ¹⁾
Higuchi (17)	2008	11 mo	Cellulitis	Suspected intra-familial
Higashiyama (19)	2008	25 y	Epidural abscess	Unknown ¹⁾
Nagao (20)	2008	23–47 y (HCWs ²⁾), 33 y (patient)	Subcutaneous abscess	Nosocomial
Mine (21)	2009	25–31 y (HCWs), 14–65 y (patients)	Subcutaneous abscess	Nosocomial
This study Case 1	2011	21 y	Severe skin and soft tissue infection	Unknown
Cases 2 and 3	2011	34 y, 22 y	Furunculosis	Combat sports
Case 4	2010	60 y	Surgical site infection	Nosocomial

¹⁾: Suspected to be imported cases from the United States.

²⁾: HCWs, healthcare workers.
mo, months; y, years.

USA300 clone (16). To the best of our knowledge, only 5 cases of USA300 infection have been reported in Japan from 2007 to 2009 (17–21). According to these Japanese reports, CA-MRSA infection with the USA300 clone is presumed to be very rare. These reports are highly significant from the perspective of clarifying the clinical presentation of CA-MRSA in Japan and to serve as a warning against its potential to rapidly spread. The first case was a 3-month-old girl who was born in the USA and subsequently moved to Japan (18). Another case was an American woman who came from the USA to the US navy stationed in Japan (19). These cases were presumed to be imported cases of the USA300 clone. Two other reports described outbreaks among HCWs and patients in hospitals (20,21). The remaining case was suspected to be an intra-familial infection (17). Hence, only 3 cases were unique to Japan, and 2 cases were imported. In this article, we report 4 cases of USA300 infection. A summary of the cases of USA300 infection reported in Japan are shown in Table 2. Including our present cases, a total of 8 USA300 infections have occurred in Japan. A careful evaluation of previous reports regarding the MRSA clone USA300 revealed that infections by this clone may be underestimated in the literature because USA300 is a genotype as shown by PFGE, which requires substantial labor and control strains.

In the present report, Cases 2 and 3 were athletes. Being an athlete is one of the risk factors already reported for CA-MRSA infection in Japan, suggesting that this also applies to USA300 infections.

However, one important point to consider is that Case 1 was not associated with any risk factors and had no history of overseas travel. The diversity of the routes of infection in these cases may indicate the possibility of the widespread distribution of the USA300 clone throughout Japan.

Case 1 had severe progressive SSTI. In Japan, this patient would not normally be considered for MRSA antibiotic treatment because he had no MRSA risk factors and no history of overseas travel. In addition, the present prevalence of CA-MRSA and the USA 300 clone in Japan is not considered sufficiently high to require emergent care.

Case 4 had surgical site infection by the USA300 clone. In addition to the 2 previously reported probable nosocomial infection cases, the fact that the USA300 clone was detected among hospitalized patients may indicate that CA-MRSA strains are present in hospital environments in Japan.

Of the 8 cases of USA300 infection reported in Japan since 2007, 4 were detected at TMUH from 2010 to 2011. This may indicate potential spread of the USA300 clone throughout Japan. If the USA300 clone continues to spread, this may be associated with inadequate initial antibiotic therapy. In accordance with previously reported cases, our cases also mainly experienced SSTIs. Therefore, clinicians, including those in Japan, should recognize the possibility of CA-MRSA infection, particularly in severe SSTI cases. Moreover, clinicians should be able to recognize USA300 infection and its spread, and thereby consider this possibility when selecting an empiric therapy for SSTIs.

CA-MRSA isolates are characterized by relative sus-

ceptibility to β -lactams, and USA300 is generally resistant to quinolones (20). Our cases indicated susceptibility to β -lactams, CLDM, ST, and MINO, and resistance to quinolones. These results may conform to those of USA300 isolates and therefore be suggestive.

Results of the susceptibility tests to CLDM, ST, and MINO were all “sensitive.” In addition, the susceptibility test results of the inducible CLDM resistance test for all isolates were “sensitive.” In the present cases, antibiotic treatments with CLDM, ST, and MINO were administered and considered to be effective. CLDM treatment was favorable in Case 1, thus CLDM was assumed to be a candidate for treatment against USA300. Although ST treatment failed in Case 2, increasing the dosage may be effective for clinical improvement. An adequate dose of ST may include, for example, more than 5 mg/kg of trimethoprim. However, when increasing the dosage of ST, side effects should be closely monitored. MINO was also suggested to be a fair option because of the excellent results of the susceptibility test and good tolerability. The possibility of reinfection through contact sports in Cases 2 and 3 was considered, rather than failure of ST and MINO treatments. Regarding 5.0% povidone iodine, its effectiveness is still poorly understood. However, in our cases, antibiotic effect against reinfection by dermal contact and the effect of adjunctive treatment were considered. A 5.0% povidone iodine bath may be an option for treatment-resistant skin infections, both as a precaution for transmission and as therapy.

We described the characteristics of USA300 infection in 4 cases. Our report includes some new aspects regarding USA300 infection. However, there is a need to conduct a survey to determine the epidemic status of USA300 infections in Japan. It is imperative for clinicians to have good knowledge of the prevalence of CA-MRSA, particularly the USA300 clone and to be able to select an optimal anti-MRSA treatment.

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Conflict of interest None to declare.

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