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EPIDEMIOLOGY

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Spread of *seb*-Positive Methicillin-Resistant *Staphylococcus aureus* SCC*mec* Type II-ST764 Among Elderly Japanese in Nonacute Care Settings

AU2 ▶

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We investigated the prevalence and molecular epidemiology of methicillin-resistant *Staphylococcus aureus* (MRSA) among 356 residents of nine long-term care facilities (LTCFs) in Japan during 2015 and 2017. In total, 800 specimens were tested and 39 MRSA isolates were recovered from 31 (8.71%) residents. PCR-based open reading frame typing (POT) and pulsed-field gel electrophoresis typing were performed for the 39 MRSA isolates; five of them showing identical pulsotypes, and POT scores were excluded in further analysis. Staphylococcal cassette chromosome *mec* (SCC*mec*) typing, multilocus sequence typing, and toxin gene detection were performed for one representative MRSA isolate per resident. Among the 34 unrelated MRSA isolates, 15 (44.1%) and 19 (55.9%) were of SCC*mec* types II and IV, respectively, and belonged to seven sequence types (STs). Among the 15 SCC*mec* II isolates, 11 (73.3%), 3, and 1 belonged to ST764 (clonal complex [CC]5), ST5 (CC5), and ST630 (CC8), respectively. Among the 19 SCC*mec* IV isolates, 13 (68.4%), 3, 2, and 1 belonged to ST1 (CC1), ST474 (CC1), ST8 (CC8), and ST380 (CC8), respectively. Among the 14 CC5 lineage-SCC*mec* II isolates, one ST5 isolate and 7 of the 11 ST764 isolates (63.6%) carried *seb* gene, and 14 (87.5%) of 16 CC1 lineage-SCC*mec* IV isolates had *sea* gene ($p < 0.05$). The results indicate that the *seb*-positive SCC*mec* type II-ST764 clone has spread in Japanese LTCF environments. As LTCF residents have multiple comorbidities and increased susceptibility to infections, it is necessary to monitor MRSA colonization in LTCFs through periodic screening to prevent dissemination.

Keywords: methicillin-resistant *Staphylococcus aureus*, SCC*mec* type, toxin gene, long-term care facility, ST764

Introduction

AU4 ▶

METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) is one of the most important pathogens causing health care-associated and community-associated infections worldwide. MRSA can colonize humans and cause a wide spectrum of infections, including bacteremia, pneumonia, and endocarditis[1]; as it can resist dry and stressful environments, and can spread in hospitals and nursing homes. A decrease in the MRSA rate has been documented by several studies in the United States, Europe, and Asia since the early-to-mid 2000s.[2–4] Although a similar downward trend has been observed in Japan, the prevalence of MRSA among multidrug-resistant pathogens isolated in clinical settings in Japan remains high. Therefore, it is still very important to control MRSA carriage and dissemination in various communities, including inpatient populations, to prevent further increase of nosocomial MRSA infections in clinical settings. Accordingly, Japan Nosocomial Infections Surveillance (JANIS) system of the Ministry of Health, Labour and Welfare has specified that MRSA needs to be particularly monitored.[5]

Focus has increased on residents living in long-term care facilities (LTCFs), including nursing homes. These people often have multiple comorbidities and functional disabilities that impair their protection ability to infections. LTCF residents are frequently treated empirically with broad-spectrum antimicrobial agents such as the third-generation cephalosporins and fluoroquinolones,[6,7] which intensify

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the selection pressure for drug-resistant bacteria, resulting in colonization. Furthermore, sharing of the living environment and equipment for physical therapy and rehabilitation, and frequent cross-contact with health care providers can potentially cause unintentional and unrecognized person-to-person transmission of antimicrobial-resistant microorganisms and promote MRSA colonization of LTCF residents. Indeed, higher rates of MRSA carriage among LTCF residents compared with that in the general population (~1.5%) have been reported in the United Kingdom and the United States,[8,9] suggesting that LTCFs could be viewed as incubators of multidrug-resistant microorganisms.

With the increase in the number of LTCFs in Japan, it is important to collect information on MRSA carriage by their residents, as it would help in preventing the spread of nosocomial infections within the facility, as well as their transfer between different health care institutions. Furthermore, as the elderly population continues to grow, the number of LTCFs has become insufficient and many senior citizens are cared for by medical professionals at home,[10–12] often receiving broad-spectrum antimicrobials; since these senior citizens are frequently transferred to and from clinical settings, they may develop similar problems with MRSA as LTCF residents. However, the data on MRSA carriage among the elderly living at home or in LTCFs in Japan are limited because of the lack of comprehensive microbiological screening and molecular epidemiological analysis of the circulating MRSA. To address this issue, we investigated the state of prevalence of MRSA in LTCFs in Japan and characterized the genetic profiles of the isolates.

Materials and Methods

Sample collection and isolation of bacteria

This study was conducted with the approval of the Ethics Committee for epidemiological studies of the Nagoya University Graduate School of Medicine (approval No. 2015-0304). Pharyngeal and nasal swabs and urine and stool samples were collected between November 2015 and March 2017 from residents of nine LTCFs located in five prefectures of Japan: Aichi, Gifu, Ishikawa, Toyama, and Gunma. Among the nine facilities that participated in this investigation, there were seven nursing homes with a capacity of 40–150 beds. In the other two facilities, elderly individuals were cared for by their families at home and periodically (every 2 weeks) visited by physicians, as they had some underlying diseases. In total, 356 participants were included after random selection using a random number table; informed consent for the study was obtained after oral explanation and presentation of the documentation regarding the purpose, protocol, and ethical aspects. For all participants, a history of any antibiotic treatment and/or hospitalization within 3 months preceding the study was obtained to evaluate risk factors for MRSA colonization.

Pharyngeal and nasal mucus samples were collected using BD ESwab (Nippon Becton Dickinson Company, Ltd., Tokyo, Japan), fecal specimens were obtained using fecal-swab (Beckman Coulter, Inc., Tokyo, Japan), and midstream urine was collected into urine sample containers. All specimens were sent to Nagoya University at 4°C within 24 hr after collection.

For initial screening, samples were directly plated on CHROMagar™ MRSA medium (Kanto Chemical Co., Inc., Tokyo, Japan) and incubated at 35°C for 24 hr. Single colonies were grown on Luria–Bertani (LB) agar (Becton, Dickinson and Company, Sparks, MD) and isolates identified using the VITEK MS system (SYSMEX bioMérieux Co., Ltd., Tokyo, Japan).

Genotyping of MRSA isolates

Total DNA was extracted using the Cica Geneus DNA Extraction Reagent (Kanto Chemical Co., Inc.). Detection of the *mecA* gene and staphylococcal cassette chromosome *mec* (SCC*mec*) typing were performed as previously described.[13] Molecular typing of MRSA isolates was carried out by PCR-based open reading frame typing (POT) (Cica Geneus Staph POT KIT; Kanto Chemical Co., Inc.) developed by Suzuki *et al.*,[14] using the Cica Geneus Staph POT Kit (Kanto Chemical Co., Inc.). Briefly, two rounds of multiplex PCR were performed according to the manufacturer's instructions using the extracted DNA and primers specific for 22 target genetic elements: 13 from integrated prophages (POT2-2 to -7, POT3-1 to -7), one from a genomic island (POT2-8), two from genomic islets (POT1-5, POT6), one from a transposon (POT2-1), and five from SCC*mec* elements (POT1-1 to -4, and -7) (Supplementary Table S1; Supplementary Data are available online at www.liebertpub.com/mdr).[14] Scores for POT1, 2, and 3 were calculated based on the presence or absence of PCR-amplified products in a binary manner. The POT1 score was calculated from the typing results of SCC*mec* elements and genomic islets and provided estimation of clonal complexes (CCs) and identification of SCC*mec* types II and IV, whereas POT2 and three scores were calculated mainly from the results of prophage-derived ORFs and this allows MRSA discrimination at the strain level.

In addition, we performed pulsed-field gel electrophoresis (PFGE) after digestion with *Sma*I to analyze the clonality of MRSA isolates[15]; strain H9812 of *Salmonella enterica* serotype Braenderup was used as a control. A dendrogram showing genetic relatedness among the isolates was constructed using the Fingerprinting II software (Bio-Rad Laboratories, Tokyo, Japan) according to the Dice similarity index,[14] and isolates from each sample were considered to be clonally related if their pulsotypes showed ≥85% similarity.[16]

To confirm sequence types (STs), MRSA isolates were subjected to multilocus sequence typing (MLST) using seven housekeeping genes (*arcC*, *aroE*, *glpF*, *gmk*, *pta*, *tpi*, and *yqiL*).[17] PCR was carried out with Qiagen Taq polymerase (QIAGEN K.K., Tokyo, Japan) using specific primers (https://pubmlst.org/saureus/info/primers.shtml) and chromosomal DNA as a template at the following conditions: annealing at 55°C for 1 min and extension at 72°C for 30 sec, 30 cycles. STs were assigned using the *S. aureus* MLST database (https://pubmlst.org/saureus/).

Detection of toxin genes

MRSA isolates were screened for the presence of the toxic shock syndrome toxin-1 (TSST-1) gene *tst*, exfoliative toxin genes *eta* and *etb*, staphylococcal enterotoxin genes *sea*, *seb*, and *sec*, and Panton–Valentine leukocidin gene *pvl* as previously described.[18,19]

Antimicrobial susceptibility testing

Antimicrobial susceptibility was examined using the agar dilution method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (document M7-A10),[20] and minimum inhibitory concentrations (MICs) were determined and interpreted using CLSI breakpoints.[21] One type of β-lactam and five types of anti-MRSA antimicrobials were used: vancomycin, linezolid, daptomycin (Wako Pure Chemical Industries, Osaka, Japan), cefoxitin, teicoplanin (Sigma-Aldrich Japan, Tokyo, Japan), and arbekacin (Sequoia Research Products Ltd., Berkshire, United Kingdom). *S. aureus* ATCC 25293 was used as the control strain.

Statistical analysis

MRSA rates according to age, gender, antimicrobial treatment, and previous hospitalization were compared by continuity-adjusted χ^2 test using SPSS software version 20.0 for Windows (SPSS, Inc., Chicago, IL). The association of toxin genes with SCC*mec* types, STs, and CCs was evaluated by Fisher's exact test, and p values <0.05 were considered to indicate statistically significant differences.

Results

Prevalence of MRSA isolates

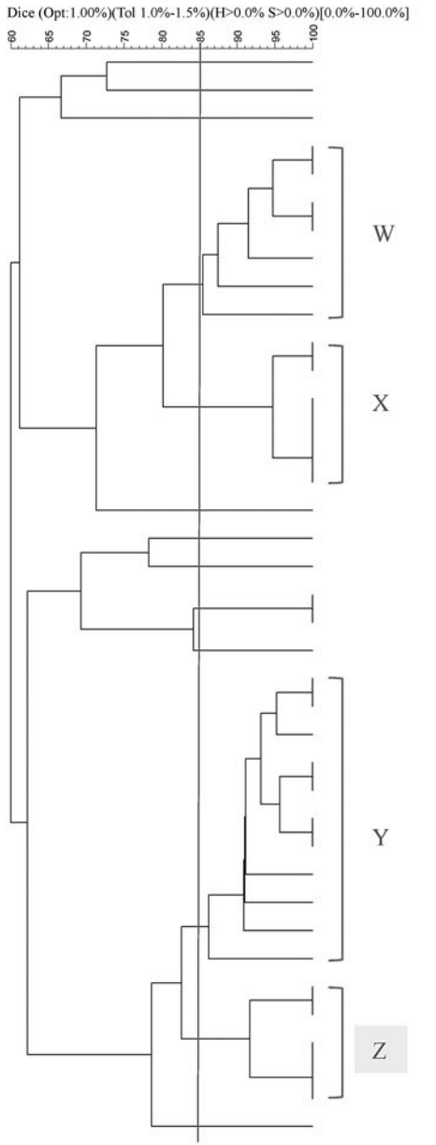
Among the 356 participating residents, there were 96 men and 260 women with an average age of 86.1 years (range, 57–100 years) and 86.5 years (range, 58–104 years), respectively. Seventy-three participants (20.5%) had a history of receiving antimicrobials and 53 (14.9%) were hospitalized within the 3 months preceding the study. A total of 800 samples, including 278 pharyngeal, 30 nasal, and 258 fecal swabs and 234 urine specimens, were obtained from the 356 participants. Among the 800 samples, 39 (23 pharyngeal, 2 nasal, and 10 fecal swabs and 4 urine specimens) obtained from 31 residents yielded colonies of one type on CHROMagar™ MRSA medium (Table 1) without apparent difference in color and/or morphology; these isolates were identified as *S. aureus* by VITEK MS and all of them carried the *mecA* gene.

MRSA isolates were detected in all of the nine participating LTCFs at varying rates: from 1.7% to 10.0% (Table 1). Among the 39 MRSA isolates, 23 were detected in only one kind of specimen obtained from 23 residents and the remaining 16 were detected in two specimens from 8 residents: pharyngeal and fecal swabs (3 residents), pharyngeal and nasal swabs (2 residents), pharyngeal swab and urine (2 residents), and feces and urine (1 resident) (Fig. 1). In total, 31 residents (31/356, 8.7%), including 7 men (7/96, 7.3%) and 24 women (24/260, 9.2%), were colonized by MRSA isolates; among them, 11 (11/31, 35.5%) had a history of receiving antimicrobials and 5 (5/31, 16.1%) had been hospitalized during the 3 months preceding the study. Residents who received previous antimicrobial treatment carried MRSA more frequently than those who did not ($p<0.05$), whereas previous hospitalization, age, and gender were not significantly associated with the MRSA colonization rate.

TABLE 1. DETECTION RATE OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* ISOLATES IN FOUR KINDS OF SPECIMENS RECOVERED FROM NINE LONG-TERM CARE FACILITIES

	Facilities and residents screened, n *(number of samples detected MRSA/number of samples [%])*									
Specimens	*A*[a] *66*	*B*[a] *74*	*C*[a] *65*	*D* *34*	*E* *14*	*F*[a] *45*	*G* *26*	*H* *21*	*I* *11*	*Total* *356*
Pharyngeal swab ($n=278$)	2/30 (6.7)	5/68 (7.4)	5/65 (7.7)	1/1 (100)	2/14 (14.3)	5/42 (11.9)	2/26 (7.6)	1/21 (4.8)	0/11 (0.0)	23/278 (8.3)
Nasal swab ($n=30$)	2/30 (6.7)	0/0 (0.0)	0/0 (0.0)	0/0 (0.0)	0/0 (0.0)	0/0 (0.0)	0/0 (0.0)	0/0 (0.0)	0/0 (0.0)	2/30 (6.7)
Urine ($n=234$)	0/29 (0.0)	1/69 (1.4)	0/25 (0.0)	0/30 (0.0)	0/5 (0.0)	3/37 (0.8)	0/21 (0.0)	0/8 (0.0)	0/10 (0.0)	4/234 (1.7)
Feces ($n=258$)	2/36 (5.6)	6/72 (8.3)	1/38 (2.6)	0/28 (0.0)	0/1 (0.0)	0/31 (0.0)	0/20 (0.0)	0/21 (0.0)	1/11 (9.1)	10/258 (3.9)
Total ($n=800$)	6/125 (4.8)	12/209 (5.7)	6/128 (4.7)	1/59 (1.7)	2/20 (10.0)	8/110 (7.3)	2/67 (3.0)	1/50 (2.0)	1/32 (3.1)	39/800 (4.9)
STs	ST5 (2)	ST1 (7[a])	ST380 (1)	ST630 (1)	ST474 (2)	ST1 (6)	ST1 (1)	ST764 (1)	ST8 (1)	ST1 (14)
	ST764 (4[a])	ST5 (1)	ST764 (5[a])			ST474 (1)	ST764 (1)			ST5 (3)
		ST8 (2[a])				ST764 (1)				ST8 (3)
		ST764 (2)								ST380 (1)
										ST474 (3)
										ST630 (1)
										ST746 (14)

[a]MRSA isolates were recovered from plural samples in eight residents of three facilities: pharyngeal swab and feces ($n=2$/facility-B and $n=1$/facility-C); pharyngeal swab and nasal swab ($n=2$/facility-A); nasal swab and urine ($n=2$/facility-F); and feces and urine ($n=1$/facility-B). The number of MRSA isolates included isolates harboring the same genetic backgrounds.
MRSA, methicillin-resistant *Staphylococcus aureus*.



Strain ID		LTCF	Specimen	H	A	SCC*mec*	POT	ST	CC	*sea*	*seb*	*sec*	*tst*
A117-3		A	feces	+	+	II	93-191-40	5	5	−	−	+	+
H42-1		H	pharyngeal	−	+	II	93-129-101	764	5	−	−	−	−
A13-3		A	feces	−	−	II	93-190-47	5	5	+	−	+	+
C125-1	*	C	pharyngeal	−	−	II	93-159-103	764	5	−	+	−	−
C125-3	*	C	feces	−	−	II	93-159-103	764	5	−	+	−	−
C104-1		C	pharyngeal	−	−	II	93-159-103	764	5	−	+	−	−
C109-1		C	pharyngeal	−	−	II	93-159-103	764	5	−	+	−	−
G41-1		G	pharyngeal	+	−	II	93-137-117	764	5	−	+	−	−
C25-1		C	pharyngeal	−	+	II	93-159-117	764	5	−	+	−	−
F24-1		F	pharyngeal	−	+	II	93-149-109	764	5	−	−	−	−
B3-2		B	urine	−	−	II	93-153-127	764	5	−	+	−	−
B3-3		B	feces	−	−	II	93-153-63	764	5	−	+	−	−
A22-1	*	A	pharyngeal	−	−	II	93-191-47	764	5	−	−	−	−
A22-5	*	A	nasal	−	−	II	93-191-47	764	5	−	−	−	−
A44-1	*	A	pharyngeal	−	−	II	93-191-34	764	5	−	−	−	−
A44-5	*	A	nasal	−	−	II	93-191-34	764	5	−	−	−	−
B49-1		B	pharyngeal	+	−	II	93-146-104	5	5	−	+	+	+
C119-1		C	pharyngeal	−	+	IV	106-159-120	380	8	−	−	−	−
D299-1		D	pharyngeal	−	+	II	95-19-47	630	8	−	−	−	−
B44-1	*	B	pharyngeal	−	−	IV	106-9-117	8	8	−	−	+	+
B44-3	*	B	feces	−	−	IV	106-9-117	8	8	−	−	+	+
I103-3		I	feces	−	+	IV	106-137-85	8	8	−	−	−	−
F24-2		F	urine	−	+	IV	106-191-32	1	1	−	−	−	−
F5-1		F	pharyngeal	−	−	IV	106-191-37	1	1	−	−	−	−
B11-3		B	feces	−	−	IV	106-183-32	1	1	+	−	−	−
F38-1		F	pharyngeal	−	−	IV	106-163-13	1	1	+	−	−	−
F75-2		F	urine	−	−	IV	106-151-40	1	1	+	−	−	−
B58-1		B	pharyngeal	+	−	IV	106-183-32	1	1	+	−	−	−
E11-1		E	pharyngeal	−	+	IV	106-55-37	474	1	+	−	−	−
E101-1		E	pharyngeal	+	+	IV	106-55-37	474	1	+	−	−	−
F79-2		F	urine	−	−	IV	106-183-37	1	1	+	−	−	−
F82-1		F	pharyngeal	−	−	IV	106-55-37	474	1	+	−	−	−
G88-1		G	pharyngeal	−	−	IV	106-183-37	1	1	+	−	−	−
B35-1	*	B	pharyngeal	−	+	IV	106-183-34	1	1	+	−	−	−
B35-3	*	B	feces	−	+	IV	106-183-34	1	1	+	−	−	−
B10-1		B	pharyngeal	−	−	IV	106-183-34	1	1	+	−	−	−
B62-3		B	feces	−	+	IV	106-183-34	1	1	+	−	−	−
B66-3		B	feces	−	−	IV	106-183-34	1	1	+	−	−	−
F79-1		F	pharyngeal	−	−	IV	106-151-45	1	1	+	−	−	−

FIG. 1. Dendrogram of PFGE patterns in 39 MRSA isolates. *Asterisks* (*) indicate that the PFGE pattern and three POT scores were identical between two isolates obtained from five residents (strain IDs A22-1 and -5, A44-1 and -5, B35-1 and -3, B44-1 and -3, and C125-1 and -3). Strains B3-2 and -3 (from urine and feces) were identical in the PFGE pattern but not in three POT scores (93-153-127 and 93-153-63, respectively). ''H'' and ''A'' columns indicate hospitalization and antibiotic treatment, respectively, within the 3 months preceding the study. *sea*, *seb*, and *sec*, staphylococcal enterotoxin genes; *tst*, toxic shock syndrome toxin-1 (TSST-1) gene; PFGE, pulsed-field gel electrophoresis; POT, PCR-based open reading frame typing.

*SCC*mec *typing, clonality analysis, and MLST of MRSA isolates*

SCC*mec* typing of 39 MRSA isolates revealed that 18 of them harbored SCC*mec* type II (18/39, 46.2%) and 21 had SCC*mec* type IV (21/39, 53.8%). Genetic profiles of the MRSA isolates were further evaluated by POT and PFGE. The 18 isolates with SCC*mec* II showed POT1 scores of 93 ($n=17$) or 95 ($n=1$) and all the 21 isolates with SCC*mec* IV showed a POT1 score of 106. PFGE revealed 26 pulsotypes with patterns completely different between SCC*mec* II isolates with the POT1 score 93 and SCC*mec* IV isolates with the POT1 score 106, except for one isolate (strain ID C119-1) (Fig. 1). Twenty-nine MRSA isolates were assigned to four distinct pulsotypes (W–Z), and the remaining 10 isolates all belonged to different pulsotypes. Strains carrying SCC*mec* II (W), SCC*mec* II (X), and SCC*mec* IV (Y) were isolated from 7, 6, and 11 individuals, respectively (Fig. 1 and Table 2), residing in LTCFs located in geographically different regions. T2 ▶

Among the MRSA isolates detected in multiple specimens, five pairs had identical PFGE patterns and three POT scores (strain IDs A22-1 and -5, A44-1 and -5, B35-1 and -3, B44-1 and -3, and C125-1 and -3; Fig. 1), indicating that the genetic backgrounds of the two isolates from the same individuals were very similar. Therefore, five MRSA isolates (strain IDs A22-5, A44-5, B35-3, B44-3, and C125-3) were excluded in further analysis. One representative MRSA isolate per resident was further characterized by MLST, toxin gene prevalence, and antibiotic susceptibility. Among the remaining 34 nonduplicated isolates, 15 (15/34, 44.1%) harbored SCC*mec* II and 19 (19/34, 55.9%) harbored SCC*mec* IV.

MLST analysis performed for the 34 MRSA isolates revealed seven STs, including ST1 (13/34, 38.2%) and ST764 (11/34, 32.4%) (Fig. 1). One to four different STs were detected in a single facility, but there was no correlation between ST diversity and the number of residents per LTCF (Table 1 and Fig. 2). ST1 was predominant in two facilities F2 ▶ (B and F), and ST764 was found in six facilities (A, B, C, F, and G), which were geographically distant (Fig. 2). Among the 34 MRSA isolates, 15 harboring SCC*mec* type II belonged to ST764 ($n=11$; CC5), ST5 ($n=3$; CC5), and ST630 ($n=1$; CC8). Two of the three ST5-SCC*mec* II isolates were recovered from LTCF residents who had a history of hospitalization during 3 months preceding the study (H column in Fig. 1). The 19 isolates harboring SCC*mec* IV belonged to ST1 ($n=13$; CC1), ST474 ($n=3$; CC1), ST8 ($n=2$; CC8), and ST380 ($n=1$; CC8) (Table 3). ◀T3

*Correlation between toxin genes, SCC*mec *type, and STs*

Next, we analyzed the prevalence of seven toxin genes among the 34 nonduplicated MRSA isolates. The *sea*, *seb*, and *sec* genes were carried by 15 (15/34, 44.1%), 8 (8/34, 23.5%), and 4 (4/34, 11.8%) isolates, respectively; all 4 isolates carrying the *sec* gene also had the *tst* gene. The frequency of the *seb* gene was high among ST5 and ST764 isolates harboring SCC*mec* II (8/15, 53.3%) and that of the *sea* gene was significantly high among ST1 and ST474 isolates harboring SCC*mec* IV (14/19, 73.7%) ($p<0.05$) (Table 3). In addition, the combination of *tst* and *sec* genes was found in ST5 isolates harboring SCC*mec* II and in ST8 isolates harboring SCC*mec* IV. However, none of the isolates carried *eta*, *etb*, or *pvl* genes.

Antimicrobial susceptibility profiles

All MRSA isolates from LTCF residents were resistant to cefoxitin, but susceptible to anti-MRSA agents, including vancomycin, linezolid, teicoplanin, and arbekacin, with the exception of one isolate, which showed resistance to daptomycin (MIC, 2 μg/mL) (Table 4). Although breakpoints of ◀T4 the European Committee on Antimicrobial Susceptibility Testing (EUCAST) are much lower than those of CLSI, all the 34 MRSA isolates were susceptible to vancomycin and linezolid based on the EUCAST criteria.

Table 2. Prevalence of 39 Methicillin-Resistant *Staphylococcus aureus* Isolates in Nine Long-Term Care Facilities in Japan

Code of LTCFs	*Residents screened,* n	*Specimens,* n	*Residents colonized MRSA,* n *(%)*	*MRSA isolates,* n *(%)*	*MRSA strain*[a,b] *(no. of residents colonized: ST-SCCmec type)*
A	66	125	4 (6.1)	6 (4.8)[b]	X (4: ST764-SCC*mec* II)
B	74	209	9 (12.2)	12 (5.7)[b]	X (2: ST764-SCC*mec* II), Y (2: ST1-SCC*mec* IV), Z (5: ST1-SCC*mec* IV)
C	65	128	5 (7.7)	6 (4.7)[b]	W (5: ST764-SCC*mec* II)
D	34	59	1 (2.9)	1 (1.7)	
E	14	20	2 (14.3)	2 (10.0)	Y (2: ST474-SCC*mec* IV)
F	45	110	6 (13.3)	8 (7.3)	W (1: ST764-SCC*mec* II), Y (5: ST1- SCC*mec* IV and 1: ST474-SCC*mec* IV)
G	26	67	2 (7.7)	2 (3.0)	W (1: ST764-SCC*mec* II), Y (1: ST1-SCC*mec* IV)
H	21	50	1 (4.8)	1 (2.0)	
I	11	32	1 (9.1)	1 (3.1)	
Total	356	800	31 (8.7)	39 (4.9)	

[a]MRSA isolates with ≥85% similarity results in pulsed-field gel electrophoresis analysis were assigned to a distinct strain (W-Z).
[b]Pulsed-field gel electrophoresis pattern and three PCR-based open reading frame typing scores were identical between two specimens recovered from five residents (sample ID A22-1 and -5; A44-1 and -5; B44-1 and -3; B35-1 and -3; B44-1 and -3; and C125-1 and -3 in Fig. 1). Therefore, the total number of MRSA isolates ($n=39$) included isolates harboring the same genetic backgrounds.
LTCF, long-term care facility.

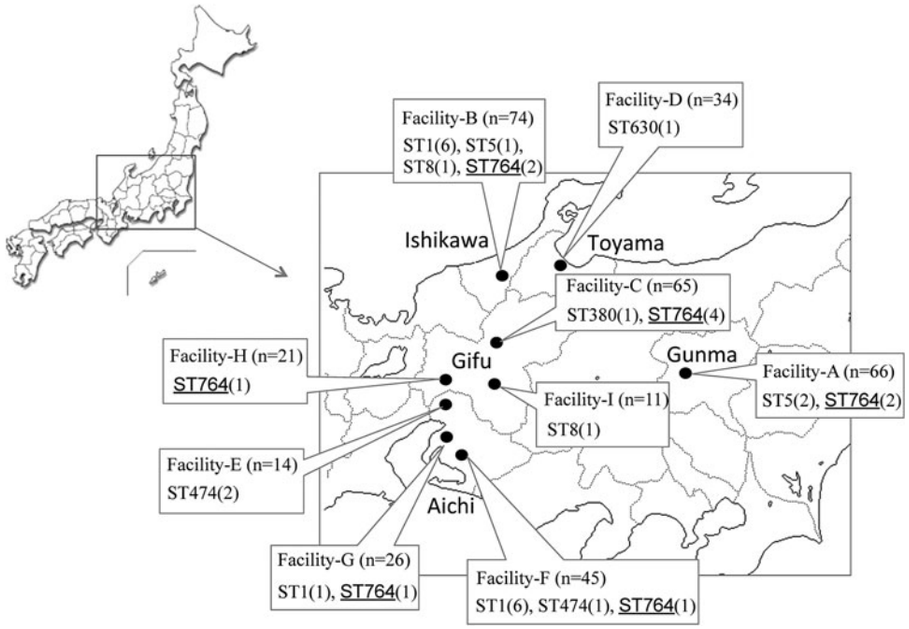


FIG. 2. Geographical locations of the nine investigated LTCFs and ST distribution among 34 nonduplicated MRSA isolates. *Black circles* mark LTCF locations. Facility ID, total number of residents screened (n), MRSA STs, and the number of isolates belonging to each ST are indicated. LTCF, long-term care facility; MRSA, methicillin-resistant *Staphylococcus aureus.*

Comparison of antimicrobial sensitivity between MRSA with SCC*mec* type II and type IV revealed that MICs of daptomycin, vancomycin, and linezolid were similar for the two groups, whereas the MIC of arbekacin was slightly higher for isolates with SCC*mec* type II (Table 4).

Discussion

Over the past several years, an increasing number of studies have focused on the prevalence and spread of antimicrobial-resistant bacteria, including MRSA, among LTCF residents worldwide.[6,7,22] Current evidence suggests that people carrying MRSA develop more invasive infections than those carrying methicillin-susceptible *S. aureus*.[23] The colonized individuals may serve as reservoirs of MRSA and provide routes for the introduction of virulent strains into health care facilities, including community-associated MRSA (CA-MRSA) producing toxins such as Panton–Valentine leukocidin or alpha-toxin.[24] Thus, MRSA colonization is a serious problem for infection control within LTCFs, and it is very important to evaluate MRSA prevalence in the elderly. However, information regarding the spread of MRSA among LTCFs in Japan remains quite limited; therefore, in this study, we assessed MRSA carriage by LTCF residents in five prefectures of Japan and performed molecular characterization of the isolates.

Table 3. Relationship Between Toxin Genes, SCC*mec* Types, CCs, and Sequence Types in Nonduplicated 34 Methicillin-Resistant *Staphylococcus aureus* Isolates

	Number of isolates (n *[%]*)[b]							p *value*[c]		
	SCCmec type II (15)			*SCCmec type IV (19)*						
	CC5 (14)		*CC8 (1)*	*CC1 (16)*		*CC8 (3)*				
Toxin genes[a]	*ST5* (n = *3)*	*ST764* (n = *11)*	*ST630* (n = *1)*	*ST1* (n = *13)*	*ST474* (n = *3)*	*ST8* (n = *2)*	*ST380* (n = *1)*	*SCCmec type II vs. IV*	*SCCmec type II ST5 vs. ST764*	*SCCmec type IV CC1 vs. CC8*
sea	1 (33.3)	0	0	11 (84.6)	3 (100)	0	0	<0.05	0.21	<0.05
seb	1 (33.3)	7 (63.6)	0	0	0	0	0	<0.05	0.54	1
sec	3 (100)	0	0	0	0	1 (50)	0	0.30	<0.05	0.16
tst	3 (100)	0	0	0	0	1 (50)	0	0.30	<0.05	0.16

[a]Staphylococcal enterotoxin genes, *sea*, *seb*, and *sec*; toxic shock syndrome toxin-1 (TSST-1) gene, *tst*.
[b]Pulsed-field gel electrophoresis pattern and three PCR-based open reading frame typing scores were identical between two specimens recovered from four residents harboring SCC*mec* type II MRSA isolates (sample ID A22-1 and -5; A44-1 and -5; B44-1 and -3; and C125-1 and -3) and one resident harboring SCC*mec* type IV MRSA isolates (sample ID B35-1 and -3). Of 39 MRSA isolates, five isolates harboring similar genetic backgrounds were eliminated. As a result, the prevalence of toxin genes among 34 nonduplicated MRSA isolates was evaluated.
[c]$p < 0.05$ was considered to indicate statistically significant differences.
CC, clonal complex.

AU7 ▶ Table 4. Antimicrobial Susceptibility Profiles of Antimicrobial Agents in 34 Methicillin-Resistant *Staphylococcus aureus* Isolates

Antimicrobial agents and disinfectants	*SCCmec type*	*Number of isolates at each MIC (μg/mL) of antimicrobials and disinfectants*										*Breakpoint*[a]		*Number of resistant strains* (n *[%]*)
		≤0.125	*0.25*	*0.5*	*1*	*2*	*4*	*8*	*16*	*32≤*	MIC_{50}	MIC_{90}	*CLSI*	*EUCAST*	
CFX	II	0	0	0	0	0	0	**15**	0	0	8	8	≤8	—	15 (100)
	IV	0	0	0	0	0	0	**19**	0	0	8	8			19 (100)
DAP	II	0	0	**13**	**1**	**1**	0	0	0	0	0.5	0.5	—	<1	1 (5.6)
	IV	0	0	**18**	**1**	0	0	0	0	0	0.5	0.5			0
VCM	II	0	0	**10**	**5**	0	0	0	0	0	0.5	1	≤16	<2	0
	IV	0	0	**13**	**6**	0	0	0	0	0	0.5	1			0
TEIC	II	0	**1**	**1**	**9**	**3**	**1**	0	0	0	1	2	≤32	<2	0
	IV	0	0	**3**	**11**	**5**	0	0	0	0	1	2			0
LZD	II	0	0	**13**	**2**	0	0	0	0	0	0.5	1	≤8	<4	0
	IV	0	0	**11**	**8**	0	0	0	0	0	0.5	1			0
ABK	II	0	**1**	**5**	**9**	0	0	0	0	0	1	1	—	—	0
	IV	0	**2**	**17**	0	0	0	0	0	0	0.5	0.5			0

AU8 ▶ [a]Susceptibilities of MRSA isolates to antimicrobial agents were categorized in accordance with CLSI criteria. When maximum inhibitory concentrations (MICs) of daptomycin were ≤1 μg/mL, the isolates were categorized as susceptible. With respect to ABK, as there was no mention of breakpoint, we used the breakpoint of gentamycin as a substitute. When MICs of arbekacin were ≤2 μg/mL, the isolates were categorized as resistant.

OXA, oxacillin; CFX, cefoxitin; DAP, daptomycin; VCM, vancomycin; TEIC, teicoplanin; LZD, linezolid; ABK, arbekacin

Our results revealed that the MRSA colonization rate was 8.71% (31/356), which is lower than that observed in a 7-year study of nursing homes in the Kinki region of Japan (11.1% ± 4.0%),[7] but significantly higher than that among healthy Japanese (0.72%).[25] Our findings are consistent with reports on MRSA prevalence in a Japanese hospital after active surveillance (7.5%)[26] and in the emergency department on admission (6.3%).[27] However, the rate of MRSA colonization in LTCFs in Sweden, the Netherlands, and Germany was lower (0–6.5%), whereas that in France, the United Kingdom, and the United States was much higher (22.0–37.6%)[22] compared with our data. Thus, there are considerable variations in the prevalence of MRSA in LTCFs among countries and regions, which may be attributed to the differences in the number of health care staff and residents per LTCF, clinical state of patients, and infection control programs.

Previous hospitalization has been confirmed as a predisposing factor for MRSA carriage both among healthy people and LTCF residents[7,25]; however, in our study, this parameter was not identified as a possible risk factor. At the same time, we revealed significant differences in MRSA colonization depending on previous treatment with antimicrobials. These data are consistent with the findings of Hogardt *et al.*,[22] indicating that medical history and antimicrobial therapy during the last 3 months were significantly associated with MRSA colonization. Similar results were reported by Nucleo *et al.*[6] who showed that the use of antimicrobials, especially fluoroquinolones, penicillins, and cephalosporins, in the preceding 3 months was a possible risk factor for MRSA colonization among LTCF residents. Cumulatively, these data suggest that antibiotic treatment may provide selective pressure promoting MRSA persistence and that it may be necessary to reconsider empirical therapy with broad-spectrum antimicrobials for LTCF residents. In this study, we could not identify particular antimicrobials used to treat LTCF residents, and the number of participants was insufficient for comprehensive evaluation of risk factors. Therefore, further investigations based on larger populations and considering additional risk factors should be conducted to clarify the relationship between the use of antimicrobials and MRSA carriage in LTCFs.

MRSA isolates recovered from LTCF residents in our study were classified as SCC*mec* type II (15/34, 44.1%) and SCC*mec* type IV (19/34, 55.9%). The proportion of isolates belonging to SCC*mec* type II (44.1%) was similar to those observed in 68 hospitals in 2016 (38% and 47% in eastern and western Japan, respectively).[19] In the present study, two of three ST5-SCC*mec* II isolates were recovered from LTCF residents who had been hospitalized during the preceding 3 months, suggesting their probable transmission from hospitals to LTCFs. We also observed high prevalence of SCC*mec* II isolates belonging to ST764 (11/15, 73.3%), a single-locus variant of ST5, which exceeded those reported in previous studies for clinical isolates in Japan (38/72, 52.8% and 34/55, 61.8%).[19,28] ST764 was first identified in Japan as a hybrid variant of hospital-associated (HA) MRSA belonging to ST5 (New York/Japan clone) and CA-MRSA with acquired virulence genes, including *seb* but not *pvl*, *tst*, and *sec*.[29,30] In recent studies, Osaka *et al.*[19] found that the evolutionary change from ST5 to its single allele variant ST764 might have occurred within the HA-MRSA New York/Japan clone in Japan, whereas Challagundla *et al.*[31] described the spatial and temporal population structure of CC5 and showed that the New York/Japan clone was polyphyletic. Our finding supports the hypothesis that the evolutionary changes, which may be ongoing in ST5, differentiated the ST5 into polyphyletic clones consisting of CC5. Hybrid ST764 clones were found in geographically distinct LTCFs, indicating their rapid dissemination in LTCF settings, which has not been previously recognized. The high prevalence of ST764 observed in our present study

suggests that ST764 MRSA isolates may have spread in the community as well as in health care institutions. The ST764 isolates harbor arginine catabolic mobile element (ACME) II, including the 6.2-kb *arc* cluster. It was shown that CA-MRSA USA300 harbored ACMEI carrying *arcA* and *opp-3* regions and it is thought that this element enhances MRSA colonization and survival on the skin.[29] Although the role of ACMEII, which lacks the *opp* region of ACMEI, remains less understood, the acquisition of the *arc* cluster from CA-MRSA may contribute to the long-term colonization of LTCF residents by the bacteria and their persistence in the LTCF environment with lower antimicrobial selective pressure. In addition, ST764 isolates have been reported as a cause of MRSA pneumonia.[28] Therefore, careful long-term follow-up of LTCF residents colonized by MRSA ST764 may be necessary. Further investigation would be required for better understanding of the mode of spread and clinical significance of the ST764 lineage.

Toxins, including TSST-1 and enterotoxins, are important virulence factors of MRSA, which act as superantigens and promote massive release of proinflammatory cytokines. Our study showed that the prevalence of toxin genes significantly differed depending on the ST. The *tst* and *sec* genes were found in isolates belonging to ST5 (CC5), whereas the *seb* gene was predominantly detected in those belonging to ST764 (CC5), and the *sea* gene was associated with ST1 and ST474 (both CC1). These findings are consistent with the results of previous studies performed in hospitals.[19,28] The detected enterotoxin genes are located on mobile genetic elements such as *S. aureus* pathogenicity island (SaPI) and prophages of the *Siphoviridae* family,[32] suggesting a possibility of enterotoxin gene transfer among various MRSA isolates. Challagundla *et al.*[31] have revealed the prevalence of virulence factors and phages in CC5 including the New York/Japan clone. Therefore, further investigations are necessary to clarify the presence of SaPI and prophages in isolates belonging to the ST764, which would have been evolutionarily derived from ST5.

This study has some limitations. First, the number of examined residents was small because of difficulties in convincing elderly people and obtaining their informed consent for such investigation. Second, the clinical data were not sufficient for comprehensive evaluation of risk factors. Third, only one sample of each type was collected from each resident during the study period, which may have resulted in the low isolation rate of MRSA. Further investigations based on larger resident groups and encompassing additional risk factors (*e.g.*, type of antimicrobial agents used and time of application, and patients' pathological conditions) should be conducted to confirm the actual MRSA prevalence and clarify the correlation between antibiotic use and the type of carried MRSA in LTCFs.

In conclusion, we found that 31 (8.71%) of the 356 LTCF residents carried MRSA. The use of antimicrobial agents within 3 months preceding the investigation appeared to be a risk factor for MRSA colonization, emphasizing the necessity to reconsider the empirical application of broad-spectrum antimicrobials among LTCF residents. Although it was not easy to perform a systematic study enrolling a large number of elderly residents, we found that the hybrid ST764 clone constituted a significant proportion of SCC*mec* type II isolates (11/15, 73.3%). MRSA of ST764 was isolated in geographically separated LTCFs, suggesting that the *seb*-positive ST764 clone might have spread through LTCF environments in Japan. Most MRSA isolates harbored *sea* or *seb* toxin genes, which strongly correlated with affiliation to CC1 and CC5, respectively. As LTCF residents have multiple comorbidities and functional impairments that increase their susceptibility to infection, their long-term carriage of MRSA harboring toxin genes might lead to serious diseases. Therefore, it is necessary to monitor MRSA colonization in LTCFs through continuous or periodic screening and implement measures to prevent MRSA dissemination.

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Ethical Approval

This study was approved by the Ethics Committee for epidemiological studies at the Nagoya University Graduate School of Medicine (approval No. 2015-0304) in November 2015. All participants provided written informed consent.

Disclosure Statement

All authors have read and approved this article; there are no conflicts of interest to declare.

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Supplementary Data

Supplementary Table 1. Target Genes Used for Pot Typing

POT number	*Target gene*	*Product size (bp)*
Reaction mixture 1		
POT1-1	*mecA* gene	530
POT1-2	*mec* gene complex class B	449
POT1-3	SCC*mec* type IIa-specific	355
POT2-1	Tn*554*	304
POT2-2	Prophage-1	271
POT2-3	Prophage-2	228
POT2-4	Prophage-3	197
POT2-5	Prophage-4	161
POT2-6	Prophage-5	131
POT2-7	Prophage-6	104
POT2-8	Genomic island	81
Reaction mixture 2		
POT1-4	Cassette chromosome recombinase A2	477
POT1-5	Genomic islet	388
POT1-6	Genomic islet	320
POT1-7	*mec* gene complex class A	273
POT3-1	Prophage-7	243
POT3-2	Prophage-8	197
POT3-3	Prophage-9	171
POT3-4	Prophage-10	140
POT3-5	Prophage-11	115
POT3-6	Prophage-12	95
POT3-7	Prophage-13	78

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Supplementary Reference

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AU1: Please note that gene symbols in any article should be formatted as per the gene nomenclature. Thus, please make sure that gene symbols, if any in this article, are italicized.
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