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SHORT COMMUNICATION

Molecular characterization of healthcare and community-associated methicillin-resistant *Staphylococcus aureus* using phage open-reading frame typing

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ABSTRACT

The polymerase chain reaction-based open reading frame typing (POT) method is a simple and rapid method for the strain-level discrimination of methicillin-resistant *Staphylococcus aureus* (MRSA). We investigated the molecular characteristics of *S. aureus* strains by multilocus sequencing typing (MLST) and POT and the profiles of antibiotic resistance and virulence genes of MRSA isolates in a single center of Tokyo, Japan. Five types by MLST and 19 types by POT were detected in the 25 MRSA isolates. ST5 and a POT1 score of 93 were associated with healthcare-associated MRSA, whereas ST8 and a POT1 score of 106 were associated with community-associated MRSA. Each strain evaluated by POT score was completely associated with similar profiles of antibiotic resistance and virulence genes. These data showed that the POT system was a powerful molecular tool for the epidemiological characterization of MRSA isolates, which correlated with the profiles of antibiotic resistance and virulence genes.

Keywords: Methicillin-resistant *Staphylococcus aureus*; Polymerase chain reaction; Open reading frames; Sequence analysis; Drug resistance; Leucocidin

INTRODUCTION

In the past few decades, the epidemiology of methicillin-resistant *Staphylococcus aureus* (MRSA) infection has changed from a hospital to a community setting. Since the 1980's, the spread of MRSA infections has been accompanied by healthcare-associated setting, but decreasing trend has been observed since 2008 in Japan (1). By contrast, community-associated infections have been rising the frequency since 1990's around the world (2, 3). Recently, various molecular typing methods, such as pulse-field gel electrophoresis (PFGE), multilocus sequencing typing (MLST), *Staphylococcus* protein A (*spa*) typing, and *Staphylococcus* chromosomal cassette mec (SCC*mec*) typing, have led to an understanding of the molecular epidemiology of MRSA across different geographical regions and populations (2, 3). Among these methods, PFGE has been used for the understanding of the epidemiology of bacterial strains. However, limitations of PFGE are labor intensive, expensive, time consuming, and difficulty of interpretation between each band.

The polymerase chain reaction (PCR)-based open reading frame (ORF) typing (POT) method involv-

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ing two multiplex PCR reactions with a set of 22 PCR primer pairs. This is a simple and rapid method for the strain-level discrimination of MRSA (1, 4-9), particularly during MRSA nosocomial outbreaks (6-8). In this study, we investigated the molecular characteristics by MLST and POT and the profiles of antibiotic resistance and virulence genes of MRSA isolates in a single center of Tokyo, Japan.

MATERIALS AND METHODS

This was a laboratory-based surveillance study in our hospital. Twenty-five MRSA isolates collected in our hospital between June 2017 -April 2000 were stored at -80°C, and were used for analysis. Among them, 19 healthcare-associated MRSA (HA-MRSA) isolates were derived from blood culture, whereas 6 community-associated MRSA (CA-MRSA) isolates were isolated from a cutaneous abscess or wound in an outpatient setting.

Genomic DNA was extracted using achromopeptidase (Wako Chemical Co. Ltd, Osaka, Japan) in combination with InstaGene Matrix (Bio-Rad Laboratories, Hercules, CA, USA). All PCR assays were performed using each DNA extraction from isolate.

The *mecA* and Panton–Valentine leucocidin *(pvl, lukS/F-PV)* genes were examined using multiplex PCR assay using specific primers as previously described (10, 11). The PCR reaction conditions for amplification of DNA were as follows: initial denaturation at 94°C for 1 min, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 30 s.

MLST was performed by PCR and Sanger sequencing using the primers of 7 housekeeping genes (*arcC, aroE, glpF, gmk, pta, tpi*, and *yqiL*). The *Staphylococcus aureus* MLST database (https://pubmlst.org/saureus/) was used to assign the sequence types (STs) as previously described (12).

Genotyping of MRSA isolates was also performed by the commercially available POT system using the Cica Geneus Staph POT Kit (Kanto Chemical, Tokyo, Japan), which was originally reported (4). In brief, two multiplex PCR reactions with a set of 22 PCR primer pairs (5 from the SCC*mec* elements, 2 from genomic islets, 1 from a transposon, 13 from integrated prophage, and 1 from genomic island) were scored in the order of their PCR amplicon size, with either '1' for present or '0' for absent in a binary manner according to manufacturer's instructions. These scores were converted to decimal numbers, where the results of each binary in the code were multiplied by 2n (n = 6-0) and added (Fig. 1).

Multiplex PCR methods for the detection of 8 antibiotic resistance genes other than *mecA* [*aacA-aphD, tetK, tetM, erm* (A), *erm* (C), *vat* (A), *vat* (B), and *vat* (C)] and 8 virulence genes other than *pvl* (*sea, seb, sec, sed, see, tst, etaA*, and *etaB*) were also performed as previously described (13, 14).

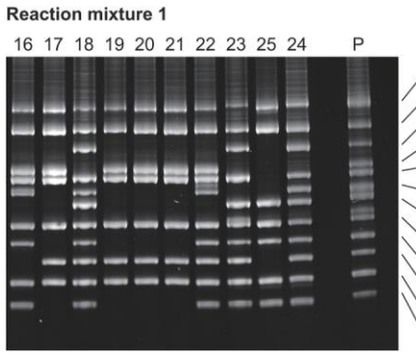
Ethical approval. The Institute of Medical Science, The University of Tokyo, considered that this study was not applicable to "Ethical Guidelines for Medical and Health Research Involving Human Subjects", because no patient data were included in this study. This judgment has been confirmed by the institutional review board of our institute.

RESULTS

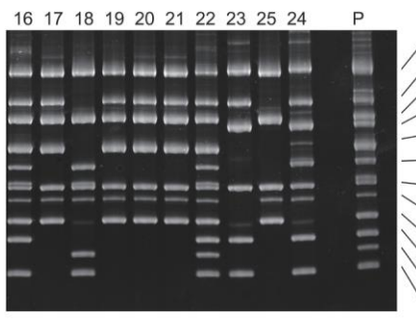
The results of MLST, POT, and detection of antibiotic resistance genes and virulence genes are shown in Table 1. The *mecA* gene was detected in all isolates. Five types by MLST and 19 types by POT were detected in the 25 MRSA isolates. ST5 (15 isolates, 60%) was the predominant type by MLST, followed by 6 isolates (24%) with ST8 and 2 isolates (8%) with ST764. A POT1 score of 93 (15 isolates, 60%) was the most frequent followed by 6 isolates (24%) with 106. ST5 and a POT1 score of 93 were strongly associated with HA-MRSA, whereas ST8 and a POT1 score of 106 were strongly associated with CA-MRSA. The most prevalent antibiotic resistance gene was *ermA* (88%), followed by *tetM* (64%) and *aacA-aphD* (60%). Among 15 isolates with ST5 and a POT1 score of 93, 13 (86%) isolates carried both the *sec* and *tst* genes. The *pvl* gene was detected in 4 isolates, all of which were ST8 and a POT1 score of 106. Two isolates carrying the *pvl* had the same POT score of 106-77-113.

DISCUSSION

MRSA strains can be characterized using several molecular typing methods, such as PFGE, MLST, *spa* typing, SCC*mec* typing, POT, and whole genome sequencing (WGS). Among these methods, WGS is the



Base pairs	Target domain	POT module	POT No	16	17	18	19	20	21	22	23	25	24
601	*femA*			1	1	1	1	1	1	1	1	1	1
530	*mecA*	64	POT1-1	1	1	1	1	1	1	1	1	1	1
449	*mec* gene complex class B	32	POT1-2	0	0	1	0	0	0	0	1	0	1
355	SSC*mec* type IIa	16	POT1-3	1	1	0	1	1	1	1	0	0	0
304	Tn*554*	128	POT2-1	1	1	1	1	1	1	1	1	0	1
271	Prophage	64	POT2-2	1	0	1	0	0	0	1	0	0	1
228	Prophage	32	POT2-3	0	0	1	0	0	0	0	1	1	1
197	Prophage	16	POT2-4	1	1	1	1	1	1	1	1	1	1
161	Prophage	8	POT2-5	1	0	1	0	0	0	1	1	1	1
131	Prophage	4	POT2-6	0	1	1	1	1	1	1	1	0	1
104	Prophage	2	POT2-7	1	1	1	1	1	1	1	1	1	1
81	Genomic island	1	POT2-8	1	0	1	0	0	0	1	1	1	1



Base pairs	Target domain	POT module	POT No	16	17	18	19	20	21	22	23	25	24
601	*femA*			1	1	1	1	1	1	1	1	1	1
477	Cassette chromosome recombinase A2	8	POT1-4	1	1	0	1	1	1	1	1	0	1
388	Genomic Islet	4	POT1-5	1	1	1	1	1	1	1	0	1	0
320	Genomic Islet	2	POT1-6	0	0	0	0	0	0	0	1	0	1
273	*mec* gene complex class A	1	POT1-7	1	1	0	1	1	1	1	0	0	0
243	Prophage	64	POT3-1	1	0	1	0	0	0	1	0	0	1
197	Prophage	32	POT3-2	1	1	1	1	1	1	1	1	1	1
171	Prophage	16	POT3-3	1	1	1	1	1	1	1	0	1	1
140	Prophage	8	POT3-4	1	1	0	1	1	1	1	0	1	0
115	Prophage	4	POT3-5	1	0	0	0	0	0	1	1	0	1
95	Prophage	2	POT3-6	0	0	1	0	0	0	1	0	0	0
78	Prophage	1	POT3-7	1	0	1	0	0	0	1	1	0	1
	POT1			93	93	100	93	93	93	93	106	68	106
	POT2			219	150	255	150	150	150	223	191	59	255
	POT3			125	56	115	56	56	56	127	37	56	117

Fig. 1. Electrophoresis patterns and calculation method of POT point of MRSA isolates.

most precise and discriminatory, but it is expensive and requires a bioinformatics analysis. The POT system, recently developed by Suzuki et al. has shown a discriminatory power higher than MLST and *spa* typing (9) but comparable to PFGE (4). Indeed, in our study of 25 MRSA isolates, 5 types by MLST and 19 types by POT were detected. Moreover, as expected, each strain evaluated by POT score (106-77-113, 93-190-35, 93-190-39, or 93-150-56) was completely associated with similar profiles of antibiotic resistance and virulence genes. Therefore, the POT system is a simple and rapid method for the strain-level discrimination of MRSA in outbreaks and the epidemiological characterization of MRSA.

The epidemiology of MRSA has been characterized by the serial emergence of regionally predominant strain types (1, 2). In Japan, the ST5 SCC*mec* type II strain, which belongs to the New York/Japan clone, was the previously dominant HA-MRSA clone, whereas ST8 SCC*mec* type IV strain was the previously dominant CA-MRSA clone (15). However, the differences have begun to homogenize between HA-MRSA and CA-MRSA. According to the POT score in our study, a POT1 score of 93, which was associated with HA-MRSA, corresponded to the ST5 SCC*mec* type II lineage, including the New York/Japan clone (9). Among 15 isolates with a POT1 score of 93, 13 isolates (86%) were characterized by carrying the *sec* and *tst* combination. However, a POT 1 score of 106, which was associated with CA-MRSA, corresponded to the ST8 SCC*mec* type IV lineage (9). Among 4 isolates carrying *pvl*, two had the same POT score of 106-77-113, which is typically shown by many USA300 strains (5, 9). Indeed, the POT1 score was calculated from the results of SCC*mec* elements and genomic islets associated with ST typing. POT2 and 3 scores were calculated mainly from the results of prophage-derived ORFs, which could estimate strain-level discrimination of MRSA (3, 4). These data demonstrated that the POT system is a useful tool for the epidemiological evaluation for MRSA strains over time.

Our study identified one new MLST sequence type, ST5597, which was isolated in 2009 from a CA-MR-

Table 1. Molecular characteristics of 25 MRSA isolates.

Number	Year	CA/HA-MRSA	*mecA*	*pvl*	ST	POT1	POT2	POT3	Antibiotic resistance genes	Virulence genes
1	2003	CA-MRSA	+	-	5	93	182	37	*ermA, tetM*	*sec, tst*
2	2009	CA-MRSA	+	-	5597	104	137	80	*aacA-aphD, ermA, tetM*	None
3	2014	CA-MRSA	+	+	8	106	77	113	None	None
4	2016	CA-MRSA	+	-	8	106	137	81	*aacA-aphD, ermA, tetM*	None
5	2017	CA-MRSA	+	+	8	106	77	113	None	None
6	2014	CA-MRSA	+	+	8	106	127	53	*aacA-aphD, ermA*	*sea*
7	2000	HA-MRSA	+	-	5	93	223	57	*aacA-aphD, ermA, tetM*	*sec, tst*
8	2001	HA-MRSA	+	-	5	93	191	63	*aacA-aphD, ermA*	*sec, tst*
9	2001	HA-MRSA	+	-	5	93	190	35	*ermA, tetM*	*sec, tst*
10	2001	HA-MRSA	+	-	5	93	190	63	*aacA-aphD, ermA*	*sec, tst*
11	2002	HA-MRSA	+	-	5	93	190	35	*ermA, tetM*	*sec, tst*
12	2002	HA-MRSA	+	-	5	93	190	39	*ermA, tetM*	*sec, tst*
13	2002	HA-MRSA	+	-	5	93	190	37	*ermA, tetM*	*sec, tst*
14	2004	HA-MRSA	+	-	5	93	190	39	*ermA, tetM*	*sec, tst*
15	2007	HA-MRSA	+	-	8	98	248	71	*aacA-aphD, ermA, tetM*	None
16	2008	HA-MRSA	+	-	764	93	219	125	*aacA-aphD, ermA, tetM*	*seb*
17	2009	HA-MRSA	+	-	5	93	150	56	*aacA-aphD, ermA, tetM*	*sec, tst*
18	2009	HA-MRSA	+	-	5	100	255	115	*ermA*	*seb*
19	2009	HA-MRSA	+	-	5	93	150	56	*aacA-aphD, ermA, tetM*	*sec, tst*
20	2009	HA-MRSA	+	-	5	93	150	56	*aacA-aphD, ermA, tetM*	*sec, tst*
21	2011	HA-MRSA	+	-	5	93	150	56	*aacA-aphD, ermA, tetM*	*sec, tst*
22	2014	HA-MRSA	+	-	764	93	223	127	*aacA-aphD, ermA, tetM*	*seb*
23	2014	HA-MRSA	+	-	1	106	191	37	*aacA-aphD, ermA*	None
24	2014	HA-MRSA	+	+	8	106	255	117	*aacA-aphD, ermA*	*sea*
25	2015	HA-MRSA	+	-	5	68	59	56	*ermC*	None

SA-infected wound and was deposited in the PubMLST. Interestingly, ST5597 (POT score of 104-137-80) was similar to ST8 (POT score of 106-137-81) by MLST, except for one point mutation in the *yqiL* gene, and had the same profiles of antibiotic resistance genes (*ermA, tetM,* and *aacA-aphD*). These findings suggested a clonal shift from ST8 to newly identified ST5597. Therefore, POT score might also be used to evaluate new MRSA strains.

In summary, although the limitation of this study was an insufficient number of MRSA isolates, but the POT system was found as a powerful molecular tool for the epidemiological characterization of MRSA isolates, correlating with the profiles of antibiotic resistance and virulence genes.

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