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SCC*mec* typing of PVL-positive community-acquired *Staphylococcus aureus* (CA-MRSA) at a Japanese hospital

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Abstract

The epidemiology of Panton-Valentine leukocidin (PVL)-positive MRSA in community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) was examined. Three hundred and forty-two CA-MRSA strains that were susceptible to imipenem and cefazolin were isolated from 1107 samples (intravenous catheter, blood, sputum, urine, skin, wound, and pharynx) from outpatients at Showa University Hospital in Japan between September 2009 and March 2017. The PVL gene was detected in 46 of 342 CA-MRSA strains, accounting for 13.5%. The type of SCC*mec* was determined by detection of each SCC*mec*-specific region, class complex, and *ccr*. SCC*mec* type IV comprised 33 strains, type V comprised 5 strains, type VII comprised 4 strains, and the unclassified type comprised 4 strains. Among the type IV strains, subtype IVa was dominant, comprising 23 of 33 strains, and the remaining 10 strains were of varying subtypes. The SCC*mec* type III-specific region, CZ049, was amplified in 2 type V strains, 4 type VII strains, and 4 unclassified strains. In 4 unclassified

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strains, CZ049 and *ccr5* were detected, but neither the SCC*mec*-specific region nor class complex was detected.

The PVL-positive rate was lower than that in Western countries. The SCC*mec* types of PVL-positive CA-MRSA strains were found to vary, indicating a diverse spreading route.

Keywords: Epidemiology, Infectious disease, Microbiology

1. Introduction

The emergence and spread of community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) are serious public health problems worldwide. In contrast to healthcare-associated MRSA (HA-MRSA) infections, for which there is a predisposing risk factor or condition, CA-MRSA infections can occur in healthy individuals, suggesting that CA-MRSA strains have enhanced virulence compared with traditional HA-MRSA strains (DeLeo et al., 2010). *S. aureus* is a prevalent human pathogen that causes numerous infectious diseases from mild skin and soft-tissue infections to severe systemic infections such as sepsis and necrotizing pneumonia (David and Daum, 2010; DeLeo et al., 2010).

The resistance of MRSA to β-lactam antibiotics is associated with penicillin-binding protein 2′ encoded by the *mecA* gene, which is located on a mobile genetic element called staphylococcal cassette chromosome *mec* (SCC*mec*) (Udo and Al-Sweih, 2017). In addition to *mecA*, the SCC*mec* region contains site-specific cassette chromosome recombinases (*ccr*) that are responsible for the integration of SCC*mec* into the *S. aureus* genome. In general, SCC*mec* types have been reported to differ between CA-MRSA and HA-MRSA. SCC*mec* types I, II, and III are common in HA-MRSA, whereas CA-MRSA harbors SCC*mec* types IV, V, VI, VII, and VIII (IWG-SCC, 2009; David and Daum, 2010). Additionally, some reports have demonstrated that SCC*mec* type IV strains are susceptible to imipenem and cefazolin (Motoshima et al., 2010; Yamaguchi et al., 2012).

An important cytotoxin produced by *S. aureus* is Panton-Valentine leukocidin (PVL), which is encoded by two genes, *lukS*-PV and *lukF*-PV (Bhatta et al., 2016). In Europe and the USA, PVL is harbored by the majority of CA-MRSA strains and is rarely present in hospital isolates. Therefore, PVL is recognized as a marker of CA-MRSA (DeLeo et al., 2010). PVL gene-positive CA-MRSA strains cause severe suppurative infections such as skin abscess formation, pleural effusion, and necrotizing pneumonia (Campbell et al., 2008; David and Daum, 2010). The most abundant CA-MRSA strains in Europe were reported to be distinct from those in North America, Oceania, and other parts of the world (Tristan et al., 2007), and the PVL-positive rate in CA-MRSA strains varies by country and area (David and Daum, 2010). The rate of PVL gene-positive CA-MRSA in Japan was reported to

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be low compared with Western countries, and CA-MRSA strains isolated in Japan were reported to be genetically diverse (Yamaguch et al., 2012; Yamamoto et al., 2004). Therefore, comparing the molecular epidemiology of strains with that of strains from other countries provides comprehensive information about the spread of CA-MRSA. In this study, we analyzed the SCC*mec* structure of PVL gene-positive CA-MRSA strains isolated from outpatients at Showa University Hospital between September 2009 and March 2017.

2. Materials and methods

2.1. Bacterial isolates and antimicrobial susceptibility tests

MRSA strains from 1107 samples (blood, sputum, skin, wound, bile, and pharynx) from outpatients were collected at Showa University Hospital in Japan between September 2009 and March 2017. The hospital is located in the Southern part of Tokyo. It has 1000 beds and an average of 44,500 to 53,000 outpatients per month.

Identification and antimicrobial susceptibility tests were performed by Microscan WalkAway using the Pos Comb 3.1J panel (Siemens Healthcare Diagnostics. Deerfield, IL). Susceptibility intermediate and resistant (SIR) categories of penicillin G, oxacillin, ampicillin, cefazolin, cefotiam, imipenem, gentamicin, erythromycin, clindamycin, minocycline, vancomycin, levofloxacin, teicoplanin, and linezolid were classified according to the Clinical Laboratory Standards Institute guidelines (CLSI, 2008).

2.2. DNA extraction and PCR

Bacterial DNA was extracted using Sepagene (EIDIA Co. Ltd. Tokyo, Japan) and 10 ng was used as the template for PCR. The PCR assays were performed in a 25 μl mixture containing 1.5 mM $MgCl_2$, 1 mM dNTP, 1 unit Roche taq polymerase, and each forward and reverse primer listed in Table 1 at a concentration of 2 mM (Berglund et al., 2009; Higuchi et al., 2008; Katayama et al., 2001; Kondo et al., 2007; Zhang et al., 2005). PCR cycling comprised 30 sec at 95 °C, 30 sec at 60 °C, and 60 sec at 72 °C for 30 cycles, followed by a final elongation at 72 °C for 5 min using ABI9700 (Funaki et al., 2017).

2.3. SCC*mec* typing

The type of SCC*mec* was determined by a combination of the SCC*mec*-specific region, the type of *ccr* gene, and the class of *mec* gene complex, which is composed of *mecA*, *mecR1*, and *mecI*. Seven SCC*mec* Types, I, II, III, IV, V, VI, and VII; five *ccr* genes, *ccr1*, *ccr2, ccr3, ccr4, and ccr5*; and four class complexes, A, B, C1, and C2 were examined by PCR.

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Table 1. PCR primers used in this study.

Gene	Nucleotide sequence	Size (bp)	Ref
SCC*mec* type I	F: GCTTTAAAGAGTGTCGTTACAGG R: GTTCTCTCATAGTATGACGTCC	613	Zhang et al. (2005)
SCC*mec* type II	F: CGTTGAAGATGATGAAGCG R: CGAAATCAATGGTTAATGGACC	398	Zhang et al., (2005)
SCC*mec* type III	F: CCATATTGTGTACGATGCG R: CCTTAGTTGTCGTAACAGATCG	280	Zhang et al., (2005)
SCC*mec* type Iva	F: GCCTTATTCGAAGAAACCG R: CTACTCTCTGAAAAGCGTCG	776	Zhang et al., (2005)
SCC*mec* type IVb	F: TCTGGAATTACTTCAGCTGC R: AAACAATATTGCTCTCCCTC	493	Zhang et al., (2005)
SCC*mec* type IVc	F: ACAATATTTGTATTATCGGAGAGC R: TTGGTATGAGGTATTGCTGG	200	Zhang et al., (2005)
SCC*mec* type IVd	F: CTCAAAATACGGACCCCAATACA R: TGCTCCAGTAATTGCTAAAG	881	Zhang et al., (2005)
SCC*mec* type IVg	F: GCAAGCTGTTATCGGCATTT R: GATCGTTCGTGTTTGTGTGC	378	Zhang et al., (2005)
SCC*mec* type IVh	F: TTCCTCGTTTTTTCTGAACG R: CAAACACTGATATTGTGTCG	664	Zhang et al., (2005)
SCC*mec* type IVi	CB18F1: CCAAGAAATTAATGTCGTCG CB18R3: AGGCTTCAACGATTTGAGAAC	1099	This study
SCC*mec* type IVj	C18F1: ATCTGTTGACTTTGTCAACC C18R2: CGCTCTTAATTGAATTCTTCC	331	This study
SCC*mec* type V	F: GAACATTGTTACTTAAATGAGCG R: TGAAAGTTGTACCCTTGACACC	325	Zhang et al., (2005)
SCC*mec* type VII	F: CAGAGGCTCATCTACATCCT R: TGTTCTGCTATACCTTCCACA	304	Higuchi et al., (2008)
mecA	F: GTGAAGATATACCAAGTGATT R: ATGCGCTATAGATTGAAAGGAT	147	Zhang et al., (2005)
Class A	F: CCCTTTTTATACAATCTCGTT R: ATATCATCTGCAGAATGGG	146	Zhang et al., (2005)
Class B	F: TATTTTTGGGTTTCACTCGG R: CTCCACGTTAATTCCATTAATACC	1305	Zhang et al., (2005)
Class C1	IS431F: ACATTAGATATTTGGTTGCGT *mec*RIR1: GTCTCCACGTTAATTCCATT	239	Katayama et al., (2001)
Class C2	IS431R1(F):TGAGGTTATTCAGA TATTTCGATGT *mec*AR1(R): TATACCAAACCCGACAAC	832	Katayama et al., (2001)
ccr1	*ccr*AB-α2F: AACCTATATCATCAATCAGTACGT *ccr*ABβ2TR: ATTGCCTTGATAATAGCCTTCT *ccr*ABβ2CR: ATTGCCTTGATAATAGCCCTCT	695	Zhang et al., (2005)
ccr2	*ccr*ABα3F: TAAAGGCATCAATGCACAAACACT *ccr*ABβ2TR:	937	Zhang et al., (2005)

(*continued on next page*)

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Table 1. (*Continued*)

Gene	Nucleotide sequence	Size (bp)	Ref
	ATTGCCTTGATAATAGCCTTCT *ccr*ABβ2CR: ATTGCCTTGATAATAGCCCTCT		
ccr3	*ccr*ABα4F: AGCTCAAAAGCAAGCAATAGAAT *ccr*ABβ2TR: ATTGCCTTGATAATAGCCTTCT *ccr*ABβ2CR: ATTGCCTTGATAATAGCCCTCT	1791	Zhang et al., (2005)
ccr4	*ccr*A4B4F: GTATCAATGCACCAGAACTT *ccr*A4B4R: TTGCGACTCTCTTGACGTTT	1287	Kondo et al., (2007)
ccr5	*ccr*CF: ATGAATTCAAAGAGCATGGC *ccr*CR: GATTTAGAATTGTCGTGATTGC	336	Zhang et al., (2005)

2.4. Ethics statement

This study was approved by the research ethics committee of Showa University School of Health Sciences (Approval No. 371).

3. Results

3.1. Isolation of CA-MRSA

CA-MRSA was identified in 342 strains from 342 patients classified as sensitive to cefazolin $\leq$8 μg/ml and imipenem $\leq$4 μg/ml among 1107 outpatient samples.

The PVL gene was detected in 46 of the 342 strains. Among 46 strains, 40 strains were isolated from severe suppurative lesions. The MIC values of the 46 strains are shown in Supplementary Table 1. All MRSA isolates were susceptible to vancomycin, teicoplanin, linezolid, minocycline, and arbekacin, but some isolates were resistant to gentamicin (9/46 = 19.5%), erythromycin (38/46 = 82.6%), clindamycin (16/46 = 34.7%), and levofloxacin (21/46 = 45.6%).

3.2. SCC*mec* type

The results of PCR are summarized in Table 2. We determined the SCC*mec* type in the strains from two or three PCR products using the specific region, *ccr* type, and class type following the report by Zhang (Zhang et al., 2005). The SCC*mec* type, and corresponding *ccr* and class types are shown in Supplementary Table 2 (IWJ-SCC, 2009).

Thirty-three strains were classified as SCC*mec* type IV (71.7%), and the SCC*mec* type IVa-specific region was identified in 23 strains. Of these 23 strains, class B

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Table 2. Identified SCC*mec* type, and detection of the specific region, *ccr* type, and class type.

SCC*mec* type	Subtype	Specific region	Class type	*ccr* type	Number of Strains*
IV	IVa	IVa	B	2	15 (No. 2, 7, 8, 10, 12, 14, 15, 18, 22, 23, 27, 29, 34, 35, 41)
	IVa	IVa	B	ND	8 (No. 24, 25, 31, 32, 37, 39, 40, 44)
	IVc	IVc	B	2	3 (No.19, 20, 36)
	IVg	IVg	B	2	1 (No. 16)
	IVh	IVh	B	2	1 (No. 45)
	IV_{NT}	ND	B	2	5 (No. 9, 26, 33, 43, 46)
V		V	C2	5	3 (No. 28, 38, 42)
		V+(III)	C2	5	1 (No. 21)
		V+(III)	ND	5	1 (No. 11)
VII		VII+(III)	ND	5	4 (No.3, 4, 13, 17)
Unclassified		(III)	ND	5	4 (No.1, 5, 6, 30)
					46

(III): products by type III-specific primers.
ND: not determined.
IV_{NT}: non-typeable.
* Numbers in parenthesis correspond to Supplementary Table 1.

was detected in all strains and *ccr2* was detected in 15 strains, but no *ccr* was found in the remaining 8 strains. Three strains were identified as SCC*mec* type IVc. One strain each was identified as SCC*mec* type IVg and IVh. For the remaining 5 strains, although class B and *ccr2* were positive, none of the SCC*mec* types, IVa, b, c, d, g, h, i, or j, were detected; therefore, they were categorized as IV_{NT} in this study. The SCC*mec* type V-specific region was detected in 5 strains (10.9%). *ccr5* was detected in all 5 strains and class C2 was detected in 4 of the 5 strains, but the class complex was not determined in 1 strain. Four strains were classified as SCC*mec* type VII because of possession of the VII-specific region and *ccr5*, although the class complex was not determined. For the remaining 4 strains, *ccr5* and the SCC*mec* type III-specific region, CZ049, were detected; therefore, they were considered to be an unclassified type. CZ049 was also detected in 2 type V strains and all 4 type VII strains.

4. Discussion

Of the MRSA strains isolated from 1107 outpatient samples, 342 strains were identified as CA-MRSA. The PVL gene was detected in 48 strains (13.4%) of CA-MRSA. SCC*mec* analysis revealed that 33 strains (71.7%) were SCC*mec* type IV, 5 strains (10.9%) were type V, 4 strains (8.7%) were type VII, and 4 strains (8.7%) were an unclassified type. Among the 13 type V or VII strains, the SCC*mec* type III-specific region, CZ049, was detected in 10 strains.

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The PVL-positive rate in CA-MRSA strains reported in Japan was low, being 2.3% (4/171) in 2008−2009 (Yanagihara et al., 2012) and 16.6% (3/18) in 2013 (Kono et al., 2013), whereas is was high in other countries such as Colombia (92% in 2006−2007) (Portillo et al., 2013), India (48% in 2013) (Vysakh and Jeya, 2013), and Saudi Arabia (76% in 2016) (Eed et al., 2016). The PVL-positive rate in the present study was 13.4% (46/342), which was lower than that in other countries.

The PVL gene has been epidemiologically linked to prevalent CA-MRSA strains harboring SCC*mec* type IV, V, VI, VII, and VIII (IWC-SCC, 2009; David and Daum, 2010). SCC*mec* type IV is associated with the major CA-MRSA strains, including USA type 300 and European clone ST80 (David and Daum, 2010; Stegger et al., 2014), and has been reported in Japan (Yanagihara et al., 2012). SCC*mec* type V is rare in Europe and the USA, but SCC*mec* type V is detected more frequently than SCC*mec* type IV in Taiwan (Wang et al., 2015) and Uganda (Asiimwe et al., 2017). In the present study, the prevalence of SCC*mec* types IV, V, and VII was 71.7%, 10.9%, and 8.7%, respectively, indicating that the routes of spreading of PVL-positive MRSA were diverse.

Among the SCC*mec* type IV subtypes, type IVa has been reported as the most common type. Type IVc has been frequently found in European MRSA isolates (Berglund et al., 2009). Type IVb has been found in USA but rarely in Japan (Berglund et al., 2009). In Japan, frequent isolation of types IVc and IVd was reported in the early 1980s (IVa 1/52 = 1%, IVc 37/52 = 38.1%, IVd 10/52 = 10.3%, IVn 4/52 = 4.1%) (Ma et al., 2006). In the present study, IVc, IVg, and IVh, and 5 IV_{NT} strains were detected other than IVa. These 5 IV_{NT} strains may have novel SCC*mec* type and class complex structures.

Detection of the type III-specific region, CZ049 (Zhang et al., 2005), in 2 SCC*mec* type V strains (Ito et al., 2004), 4 SCC*mec* type VII strains, and 4 unclassified strains may reflect recombination.

The positive rate of the PVL gene in CA-MRSA was lower at our hospital than that in other countries. Although the most prevalent PVL-positive CA-MRSA strains were SCC*mec* type IV with varying structures, the involvement of several SCC*mec* types was demonstrated.

Declarations

Author contribution statement

Toshitaka Funaki: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Kunihiko Fukuchi: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

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Emi Sugano, Yoshimi Nagakura, Tsutomu Yasuhara, Satoshi Kugawa, Yohei Yamazaki, and Katsuhiko Yoshida: Performed the experiments.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

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