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

Toshitaka Funaki, Tsutomu Yasuhara, Satoshi Kugawa, Yohei Yamazaki, Emi Sugano, Yoshimi Nagakura, Katsuhiko Yoshida, Kunihiko Fukuchi. SCCmec typing of PVL-positive community-acquired Staphylococcus aureus (CA-MRSA) at a Japanese hospital.

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 SCCmec was determined by detection of each SCCmec-specific region, class complex, and ccr. SCCmec 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 SCCmec type III-specific region, CZ049, was amplified in 2 type V strains, 4 type VII strains, and 4 unclassified strains. In 4 unclassified
strains, CZ049 and ccr5 were detected, but neither the SCCmec-specific region nor class complex was detected.

The PVL-positive rate was lower than that in Western countries. The SCCmec 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 (SCCmec) (Udo and Al-Sweih, 2017). In addition to meca, the SCCmec region contains site-specific cassette chromosome recombinases (ccr) that are responsible for the integration of SCCmec into the S. aureus genome. In general, SCCmec types have been reported to differ between CA-MRSA and HA-MRSA. SCCmec types I, II, and III are common in HA-MRSA, whereas CA-MRSA harbors SCCmec types IV, V, VI, VII, and VIII (IWG-SCC, 2009; David and Daum, 2010). Additionally, some reports have demonstrated that SCCmec 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
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 SCCmec 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 MgCl₂, 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 ºC, 30 sec at 60 ºC, and 60 sec at 72 ºC for 30 cycles, followed by a final elongation at 72 ºC for 5 min using ABI9700 (Funaki et al., 2017).

2.3. SCCmec typing

The type of SCCmec was determined by a combination of the SCCmec-specific region, the type of ccr gene, and the class of mec gene complex, which is composed of mecA, mecR1, and mecI. Seven SCCmec 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.
| Gene        | Nucleotide sequence                          | Size (bp) | Ref               |
|-------------|-----------------------------------------------|-----------|-------------------|
| SCCmec type I | F: GCTTTAAGAGTGTCGTTACAGG<br>R: GTCTCTCATAGTAGTGAAGTCC | 613       | Zhang et al. (2005) |
| SCCmec type II | F: CGTTGAAGATGATGAAGCG<br>R: CGAAATCTATGGTTAATGGACC | 398       | Zhang et al. (2005) |
| SCCmec type III | F: CCATATTGTGATCAGATG<br>R: CTTTAGGTGTGCAAACAGATCG | 280       | Zhang et al. (2005) |
| SCCmec type Iva | F: GCCTTTATGGAGAAAACCG<br>R: CTATCTCTGAAAAAGGCTCG | 776       | Zhang et al. (2005) |
| SCCmec type IVb | F: TCTGGAAATTACTTCAGCTGC<br>R: AAACAAATATGCTCTTCCC | 493       | Zhang et al. (2005) |
| SCCmec type IVc | F: ACAAAATTTTGTATATCGGAGGC<br>R: TTGGFATGAGGTATGCTTG | 200       | Zhang et al. (2005) |
| SCCmec type IVd | F: CTTCAAAATACGGACCCCAATACA<br>R: TGCTCCAGTAAATGTGCTAAAG | 881       | Zhang et al. (2005) |
| SCCmec type IVg | F: GCAAGCTTATGCGGCATT<br>R: GATCGTTCGTGTTTGTGTGC | 378       | Zhang et al. (2005) |
| SCCmec type IVh | F: TTCTCTGTTTTTTTCGGAAGG<br>R: CAAACACGTATATGGCTCG | 664       | Zhang et al. (2005) |
| SCCmec type IVi | CB18F1: CCAAGAAATTAATGTCGTCG<br>CBB18R3: AGGCTTTAACAAGTGGAGAAGC | 1099      | This study |
| SCCmec type IVj | C18F1: ATCTGTTGACCTTTTGCAACC<br>C18R2: CGCTCTTAATGAATCTTCC | 331       | This study |
| SCCmec type V | F: GAACTTTTGTACTTTAAATGACGC<br>R: TGAAAGTTGCTACCCCTTGACACC | 325       | Zhang et al. (2005) |
| SCCmec type VII | F: CAGAGGCTCTACTACATCCT<br>R: TGTCTGCTATAACCTTCCACA | 304       | Higuchi et al., (2008) |
| mecA         | F: GTGAAAGATATACCAAGTGGATT<br>R: ATGGCCTATAGATTGAAAGGAT | 147       | Zhang et al. (2005) |
| Class A      | F: CCCTTTTATACAAACTCCTG<br>R: ATATCATCTGCAGAATGGG | 146       | Zhang et al. (2005) |
| Class B      | F: TATTTTTGGGTTTCTACCTCG<br>R: CTCCACGTAAATTCTCATTACACC | 1305      | Zhang et al. (2005) |
| Class C1     | IS431F: ACATTAGATATTGTTGTCGT<br>mecRIR1: GTCTCCAGTGAATTCATCATT | 239       | Katayama et al., (2001) |
| Class C2     | IS431RI(F):TGAGGTTATTCAGA<br>TATTTCGATGT<br>mecAR1(R): TATACCAACAACCGACAAC | 832       | Katayama et al., (2001) |
| ccr1         | ccrAB-22F: AACCCTATATCATCAATGAGTCAGT<br>ccrAB-22TR: ATCCCTTGATAAATAGGCTTCT<br>ccrAB-22CR: ATGAGGCCTTATATGCGCCTTCT | 695       | Zhang et al., (2005) |
| ccr2         | ccrABx3F: TAAAGGCAATCATGACAAACACT<br>ccrAB-22TR: | 937       | Zhang et al., (2005) |

(continued on next page)
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 \( \leq 8 \mu g/ml \) and imipenem \( \leq 4 \mu g/ml \) 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. SCCmec type

The results of PCR are summarized in Table 2. We determined the SCCmec 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 SCCmec type, and corresponding \( ccr \) and class types are shown in Supplementary Table 2 (IWJ-SCC, 2009).

Thirty-three strains were classified as SCCmec type IV \( (71.7\%) \), and the SCCmec type IVa-specific region was identified in 23 strains. Of these 23 strains, class B
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 SCCmec type IVc. One strain each was identified as SCCmec type IVg and IVh. For the remaining 5 strains, although class B and ccr2 were positive, none of the SCCmec types, IVa, b, c, d, g, h, i, or j, were detected; therefore, they were categorized as IVNT in this study. The SCCmec 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 SCCmec 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 SCCmec 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. SCCmec analysis revealed that 33 strains (71.7%) were SCCmec 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 SCCmec type III-specific region, CZ049, was detected in 10 strains.
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 it 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 SCCmec type IV, V, VI, VII, and VIII (IWC-SCC, 2009; David and Daum, 2010). SCCmec 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). SCCmec type V is rare in Europe and the USA, but SCCmec type V is detected more frequently than SCCmec type IV in Taiwan (Wang et al., 2015) and Uganda (Asiimwe et al., 2017). In the present study, the prevalence of SCCmec 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 SCCmec 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 IVNT strains were detected other than IVa. These 5 IVNT strains may have novel SCCmec type and class complex structures.

Detection of the type III-specific region, CZ049 (Zhang et al., 2005), in 2 SCCmec type V strains (Ito et al., 2004), 4 SCCmec 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 SCCmec type IV with varying structures, the involvement of several SCCmec 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.
Emi Sugano, Yoshimi Nagakura, Tsutomu Yasuhara, Satoshi Kugawa, Yohei Yamazaki, and Katsuhiko Yoshida: Performed the experiments.

**Funding statement**

This work was supported by JSPS KAKENHI grant number 24590708 and 17K09021.

**Competing interest statement**

The authors declare no conflict of interest.

**Additional information**

Supplementary content related to this article has been published online at https://doi.org/10.1016/j.heliyon.2019.e01415.

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