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# Clinical characteristics and factors related to infection with SCCmec type II and IV Methicillin-resistant *Staphylococcus aureus* in a Japanese secondary care facility: a single-center retrospective study

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## ABSTRACT

**Objectives:** Differences in virulence genes, including *psm-mec*, which is a phenol-soluble modulins-*mec* (PSM-*mec*) encoding gene, of predominant staphylococcal cassette chromosome *mec* (SCCmec) types II and IV Methicillin-resistant *Staphylococcus aureus* (MRSA) may contribute to the virulence and clinical features of MRSA in Japan. We aimed to clarify the clinical characteristics and risk factors of infection among SCCmec types II and IV MRSA isolates from a Japanese secondary acute care hospital.

**Methods:** We analysed 58 SCCmec type II and 83 SCCmec type IV MRSA isolates collected from blood, central venous catheter tips, deep or superficial tissues, and sputum.

**Results:** SCCmec type II MRSA risk factors for progression to infection were *seb*, enterotoxin gene cluster, *psm-mec* mutation, and vancomycin minimum inhibitory concentrations (MIC) of 1 or 2 mg/L as virulence factors (adjusted odds ratio [aOR] = 11.8; 95% confidence interval [CI]: 2.49–77.7; *P* = 0.004); solid tumour was a host factor (aOR = 25.9; 95% CI: 3.66–300; *P* = 0.003). SCCmec type IV MRSA risk factors were *sea*, *cna*, and vancomycin MIC of 1 or 2 mg/L as virulence factors (aOR = 3.14; 95% CI: 1.06–10.6; *P* = 0.049) and intravascular indwelling catheter as host factors (aOR = 3.78; 95% CI: 1.03–14.5; *P* = 0.045). Compared with SCCmec type II, SCCmec type IV MRSA resulted in more frequent bloodstream infections and higher Sequential Organ Failure Assessment scores.

**Conclusion:** We found that factors related to virulence genes and bacteriological and host characteristics are associated with SCCmec types II and IV MRSA infection and severity. These risk factors may be useful criteria for designing infection control programs.

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## 1. Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) remains one of the most recognized multidrug-resistant pathogens causing healthcare-associated (HA) infections. Since the 1990s, increasing numbers of reports have been published regarding community-associated MRSA (CA-MRSA) infections in patients with no history of medical exposure.

HA-MRSA strains have been associated with pneumonia, bacteraemia, and invasive infections in healthcare settings, with patients tending to be elderly or with comorbidities [1]. Although CA-MRSA strains mainly cause skin and soft tissue infections (SSTIs) in healthy individuals, including children and adolescents, they are sometimes associated with severe conditions such as necrotizing pneumonia and sepsis [2]. CA-MRSA strains are also more susceptible to non- $\beta$ -lactam antibiotics than HA-MRSA strains [3–5]. Additionally, MRSA can colonize the skin, nasal flora, and oropharynx.

Regarding its molecular characteristics, HA-MRSA typically harbors staphylococcal cassette chromosome *mec* (SCCmec) types I, II, and III, whereas CA-MRSA typically harbors SCCmec types IV and

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V. In Japan, SCCmec type II MRSA, the representative HA-MRSA, is predominant; however, SCCmec type IV, the representative CA-MRSA, is increasing in hospitalized patients [5].

One possible difference in virulence between SCCmec types II and IV MRSA is whether the strain carries the *psm-mec*, which is a phenol-soluble modulins-mec (PSM-mec) encoding gene. Because *psm-mec* is located in the class A *mec* complex of SCCmec, SCCmec type IV MRSA, which carries the class B *mec* complex, lacks *psm-mec*. SCCmec type II MRSA with intact *psm-mec* reportedly suppresses the expression of PSM $\alpha$ , a cytolytic toxin of *S. aureus* involved in colony spreading, compared with MRSA that have mutation in or the absence of *psm-mec* [6]. However, little information exists on *psm-mec* in clinical isolates.

MRSA can also produce toxins such as enterotoxins or toxic shock syndrome toxin-1 (TSST-1). These are superantigens that trigger T-cell activation and proliferation without the need for antigen processing, and they lead to the massive release of proinflammatory cytokines [7]. Staphylococcal enterotoxins A and B encoded by *sea* and *seb* genes and TSST-1 encoded by the *tst* gene are associated with septic shock, severe inflammation, and toxic shock syndrome, respectively. Previous studies have shown that MRSA harboring the *sea*, *seb*, or *tst* gene correlate with the severity of infection [8,9], suggesting that patterns of virulence genes harbored by MRSA may also be important in predicting clinical characteristics and severity of infection.

In Japan, most SCCmec type IV MRSA strains differ from the USA300 clone, the main SCCmec type IV strain, as well as from CA-MRSA detected in the United States and Europe. The USA300 clone harbors virulence genes including the Panton-Valentine leukocidin (PVL) genes (*lukF-PV/lukS-PV*) and arginine catabolic mobile element (ACME) [2,10,11]. PVL is a two-component pore-forming toxin encoded by two-transcribed genes, *lukF-PV* and *lukS-PV*, which are related to virulence, and ACME, which is related to colonization and spread [2,12]. The prognoses for SCCmec type IV MRSA infection harboring PVL-encoding genes vary, despite reports of rapid progression and fatalities [13,14]. SCCmec type IV MRSA infection is considered to have a better prognosis than that of SCCmec type II MRSA; however, a controlled study found no differences [15]. Although the number of cases with USA300 clone are reported to be increasing in Japan [16], SCCmec type IV MRSA harboring PVL-encoding genes has rarely been reported [4]. Little information exists on the clinical characteristics and virulence genes in SCCmec type IV MRSA in Japan, except that it harbors PVL-encoding genes. Additionally, SCCmec type IV MRSA differs from traditionally defined MRSA and has been isolated from hospitalized patients, indicating that it may be a HA pathogen [17].

Regarding the prevalence of SCCmec types II and IV MRSA and differences in their clinical features in Japanese healthcare settings, we previously found that more than half of MRSA strains in a tertiary referral hospital carried SCCmec type IV, the proportion of MRSA-associated pneumonia was significantly higher for SCCmec type II MRSA than for SCCmec type IV, and distributions of the MRSA virulence gene differed significantly. Furthermore, the SCCmec type II MRSA strain that causes MRSA pneumonia is thought to belong to sequence type (ST) 764 [3].

To extend our previous findings, we conducted the present study to clarify the clinical characteristics and risk factors related to infection among inpatients with SCCmec types II and IV MRSA isolates in a secondary acute care hospital in Japan.

## 2. Materials and methods

### 2.1. Study design and collection of clinical isolates

In this retrospective, single-center, observational study, we isolated 284 MRSA samples from inpatients in all departments of

Saiseikai Futsukaichi Hospital between March 2019 and December 2020. Isolates were obtained from blood, central venous catheter tips, deep tissues (organs, body cavities), superficial tissues (epidermis to soft tissues), and sputum (spontaneous or tracheal aspirated sputum).

No MRSA outbreaks occurred during the observational period. The exclusion criteria were patients with detected MRSA who had been transferred to another hospital within 24 hours, paediatric patients younger than 15 years, and patients who requested not to participate.

We allocated one sample per patient if MRSA was isolated from multiple samples from the same patient, using the first sample collected. Multiple samples collected on the same day were randomly allocated. SCCmec types II and IV MRSA, which are considered the predominant types in Japan, were included in the analysis; SCCmec types I, III, and V were excluded (Fig. 1).

The ethics committees of Kyushu University Hospital (2020-48) and Saiseikai Futsukaichi Hospital (294) approved the study. No informed consent was required because of the study's retrospective design.

Clinical information was collected from the records of patients with MRSA isolates and included age, sex, infection or colonization status, intensive care unit admission, clinical department, intravascular indwelling catheter (percutaneous central venous, peripherally inserted central, and implanted central venous catheters), Charlson comorbidity index, surgical site infection (SSI), and Sequential Organ Failure Assessment (SOFA) score [18,19]. MRSA isolates were classified as HA-MRSA or CA-MRSA based on patient background, and bacteriological information on MRSA was collected for antimicrobial susceptibility. MRSA was classified by SCCmec type, and the virulence genes in SCCmec types II and IV MRSA were analysed.

The primary endpoint was to identify risk factors useful for predicting progression to infection in patients with SCCmec types II or IV MRSA. The secondary endpoint was to identify the clinical characteristics of SCCmec types II and IV MRSA isolates, including antimicrobial susceptibility, prior infected organs, proportions of progression to infection, bloodstream infection (BSI), SSI, SOFA score, and 30-day mortality rate.

### 2.2. Study definitions

CA-MRSA was defined as MRSA isolated from a patient within 48 hours of hospitalization, excluding patients undergoing haemodialysis, those with a history of surgery, long-term care facility residence, or hospitalization in the previous year, and those with permanent indwelling catheter or percutaneous device or previous isolation of MRSA [1]. Infection was defined as a clinical disease requiring antimicrobial treatment. Colonization was defined as the isolation of MRSA from uninfected sites.

Infectious diseases were classified as (1) bacteraemia of unidentified origin (BUO), (2) catheter-related bloodstream infection (CRBSI), (3) bone and joint infection (BJI), (4) SSTIs, (5) pneumonia and lung abscess, or (6) intra-abdominal infection (IAI). The disease diagnoses were made per Mermel et al. for CRBSI and SSI [20], Nagaoka et al. for pneumonia and lung abscesses [21], Stevens et al. for SSTIs [22], and Horan et al. for BJI and IAI [23].

### 2.3. Antimicrobial susceptibility testing and detection methods for SCCmec type, *psm-mec*, and virulence genes of isolated MRSA

According to the Clinical and Laboratory Standards Institute M100S22, antimicrobial susceptibility was tested using the following equipment. A dryplate 192 (Eiken Chemical Co., Ltd., Tokyo, Japan) was used to measure the minimum inhibitory concentration (MIC). An Inoculator  $\Sigma$  (Eiken Chemical Co., Ltd.) was used

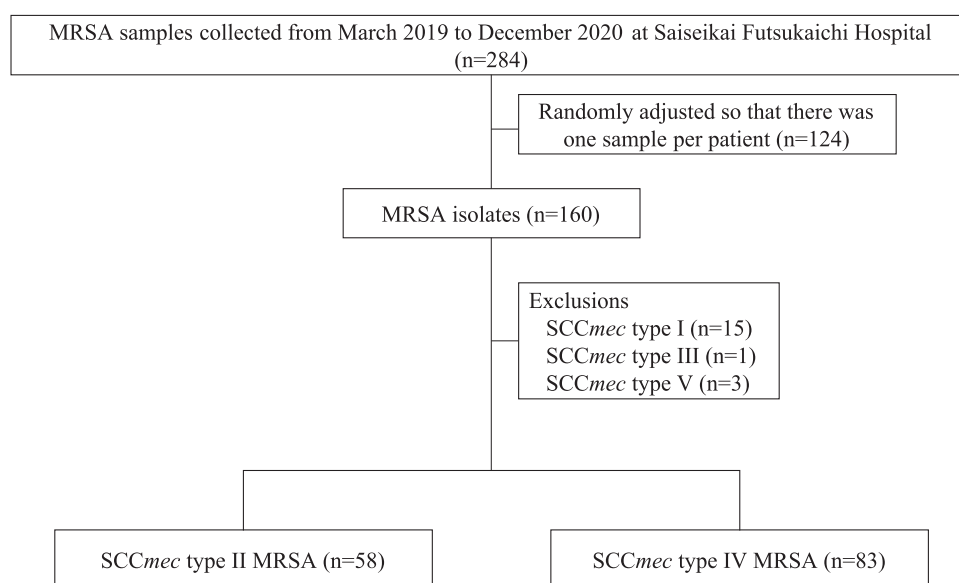


Fig. 1. Study flowchart. SCCmec, staphylococcal cassette chromosome mec; MRSA, Methicillin-resistant *Staphylococcus aureus*.

to dispense the bacterial solution, and a DPS 192 (Eiken Chemical Co., Ltd.) was used to read the plate. If MRSA was susceptible to vancomycin (VCM), susceptibility testing was measured at MIC  $\leq 0.5$  mg/L, MIC = 1 mg/L, and MIC = 2 mg/L. Real-time PCR was used to identify the SCCmec type of the MRSA strain [24] and virulence genes [10,13,25–27]. *Psm-mec* mutation and their absence were analysed via direct sequencing of 400 bp of the *psm-mec* region [6]. *Psm-mec* mutation is presented as nucleotide numbers and substitutions from the transcription start site. T>C indicates that thymine was substituted for cytosine. SCCmec types II and IV MRSA were analysed for genes encoding toxic shock toxins (*tst*) and staphylococcal enterotoxins (*sea*, *seb*, enterotoxin gene cluster [*egc*] containing *sem*, *seo*). SCCmec type II MRSA strains were analysed for the absence and mutation (–7T>C) of *psm-mec*. SCCmec type IV MRSA strains were analysed for PVL-encoding genes; ACME containing the prominent gene *arcA*; microbial surface components recognizing adhesive matrix molecule encoding genes including *fmbB*, *cna*, and *sasG* [28]; and *spj* encoding the cell wall–anchored surface protein with the LPXTG motif [26].

## 2.4. Statistical analysis

Statistical significance was defined as  $P < 0.05$ . Data were analysed using R v.3.6.1 (R Core Team 2020; R Foundation for Statistical Computing, Vienna, Austria).

Because all eligible patients were enrolled, the sample size was not calculated.  $P$ -values were not adjusted for multiplicity because this study was exploratory.  $P$ -values other than the primary endpoint are presented as nominal values. Regarding summary statistics, continuous data are presented as the median and interquartile range, and categorical variables are presented as proportion. Continuous variables were compared using the Wilcoxon rank-sum test. Categorical variables were compared using Yates's  $\chi^2$  test, Fisher's exact test, and logistic regression analysis. Cox proportional hazards models were used to estimate the hazard ratio (HR) for the 30-day mortality rate of MRSA infection, censoring deaths not caused by MRSA infection and patients lost to follow-up. Proportional hazard assumption was confirmed using the Schoenfeld residual.

We attempted to identify risk factors related to progression to infection among patients with SCCmec types II or IV MRSA isolates. In the logistic regression analysis, patients were classified accord-

ing to age <75 years or  $\geq 75$  years (defined as late elderly). Variables regarding MRSA pathogenetic factors were classified according to virulence gene combinations, VCM MIC of 0.5 mg/L for the MIC low group, and VCM MIC of 1 or 2 mg/L for the MIC high group. Diseases included in the Charlson comorbidity index and intravascular indwelling catheter were used as variables for the host factors. Variables in the multivariate logistic regression analysis were selected as clinically relevant according to the results of univariate analysis ( $P < 0.1$ ). It was not necessary to consider interactions among host factors in the analyses stratified by age and virulence. The models were evaluated using receiver operating characteristic (ROC) analysis.

## 3. Results

### 3.1. Collected strains and clinical backgrounds

Of the 284 samples collected before allocation, 32 (11%) were from blood, 7 (2.5%) from central venous catheter tips, 16 (5.6%) from deep tissues, 37 (13%) from superficial tissues, and 192 (68%) from sputum. After allocating one sample per patient, we obtained 160 MRSA strains. None of these patients met the initial exclusion criteria. After excluding samples with SCCmec types I, III, and V MRSA, 58 SCCmec type II and 83 SCCmec type IV MRSA isolates were analysed (Fig. 1).

Table 1 shows the clinical backgrounds of the patients with SCCmec types II and IV MRSA isolates. The proportion of patients with CA-MRSA was low, with no significant differences between those with SCCmec types II and IV MRSA (nominal  $P = 0.52$ ). Additionally, the clinical background was not significantly different between the groups, other than for CA-MRSA.

### 3.2. Antimicrobial susceptibility of SCCmec types II and IV MRSA

Fig. 2 shows a comparison of antimicrobial susceptibilities between the SCCmec types II and IV MRSA isolates. Except for erythromycin, clindamycin/erythromycin, and levofloxacin, SCCmec type IV MRSA had significantly higher susceptibility to non- $\beta$ -lactams than SCCmec type II MRSA. The SCCmec type IV MRSA isolates had a higher VCM MIC than that of SCCmec type II MRSA, but this difference was not statistically significant.

**Table 1**

Clinical backgrounds of patients with MRSA isolates harboring SCCmec types II or IV

	Overall N = 141 <sup>a</sup>	Type of SCCmec		nominal P value
		II N = 58 <sup>a</sup>	IV N = 83 <sup>a</sup>	
Male	88 (62)	37 (64)	51 (61)	0.92 <sup>b</sup>
Age, y	82.0 (74.0–89.0)	83.0 (74.0–89.0)	82.0 (75.0–88.5)	0.79 <sup>c</sup>
CA-MRSA	15 (11)	5 (8.6)	10 (12)	0.71 <sup>b</sup>
HA-MRSA	126 (89)	53 (91)	73 (78)	
Department				0.72 <sup>b</sup>
Internal medicine	96 (68)	38 (66)	58 (70)	
Surgery	45 (32)	20 (34)	25 (30)	
Intensive care unit	28 (20)	12 (21)	16 (19)	> 0.99 <sup>b</sup>
Intravascular indwelling catheter	30 (21)	16 (28)	14 (17)	0.19 <sup>b</sup>
Charlson comorbidity index	2.00 (1.00–4.00)	2.00 (1.00–4.75)	2.00 (1.00–3.50)	0.24 <sup>c</sup>
Prior myocardial infarction	8 (5.7)	1 (1.7)	7 (8.4)	0.14 <sup>d</sup>
Congestive heart failure	30 (21)	11 (19)	19 (23)	0.73 <sup>b</sup>
Peripheral vascular disease	8 (5.7)	2 (3.4)	6 (7.2)	0.47 <sup>d</sup>
Cerebrovascular disease	42 (30)	16 (28)	26 (31)	0.77 <sup>b</sup>
Dementia	45 (32)	22 (38)	23 (28)	0.27 <sup>b</sup>
Chronic pulmonary disease	12 (8.5)	7 (12)	5 (6.0)	0.23 <sup>d</sup>
Rheumatologic disease	16 (11)	7 (12)	9 (11)	>0.99 <sup>b</sup>
Peptic ulcer disease	12 (8.5)	7 (12)	5 (6.0)	0.23 <sup>d</sup>
Diabetes	28 (20)	10 (17)	18 (22)	0.66 <sup>b</sup>
Diabetes with chronic complications	6 (4.3)	2 (3.4)	4 (4.8)	>0.99 <sup>d</sup>
Moderate to severe renal disease	11 (7.8)	3 (5.2)	8 (9.6)	0.53 <sup>d</sup>
Cerebrovascular (hemiplegia) event	13 (9.2)	7 (12)	6 (7.2)	0.50 <sup>b</sup>
Leukaemia	2 (1.4)	2 (3.4)	0	0.17 <sup>d</sup>
Lymphoma	2 (1.4)	1 (1.7)	1 (1.2)	>0.99 <sup>d</sup>
Solid tumour	28 (20)	14 (24)	14 (17)	0.40 <sup>b</sup>
Solid tumour without metastases	14 (9.9)	5 (8.6)	9 (11)	0.78 <sup>d</sup>
Metastatic solid tumour	14 (9.9)	9 (16)	5 (6.0)	0.09 <sup>d</sup>
Mild liver disease	2 (1.4)	1 (1.7)	1 (1.2)	>0.99 <sup>d</sup>
Moderate or severe liver disease	2 (1.4)	1 (1.7)	1 (1.2)	>0.99 <sup>d</sup>
Acquired immunodeficiency syndrome	0	0	0	

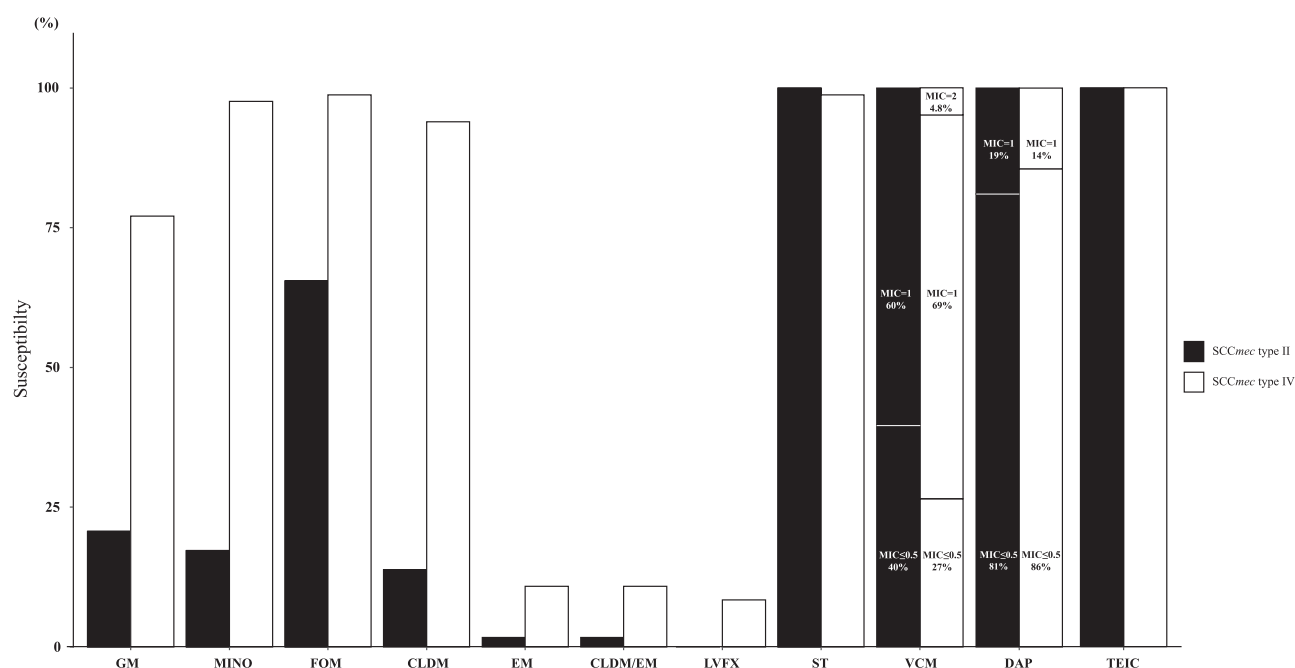
MRSA, Methicillin-resistant *Staphylococcus aureus*; SCCmec, staphylococcal cassette chromosome mec; CA-MRSA, community-associated Methicillin-resistant *Staphylococcus aureus*; HA-MRSA, healthcare-associated Methicillin-resistant *Staphylococcus aureus*.

<sup>a</sup> Statistics; number (%); median (interquartile range).

<sup>b</sup> Yates's  $\chi^2$  test performed.

<sup>c</sup> Wilcoxon rank-sum test performed.

<sup>d</sup> Fisher's exact test performed.



**Fig. 2.** Comparison of SCCmec types II and IV MRSA susceptibility to antimicrobial agents. SCCmec, staphylococcal cassette chromosome mec; GM, gentamicin; MINO, minocycline; FOM, fosfomycin; CLDM, clindamycin; EM, erythromycin; LVFX, levofloxacin; ST, sulfamethoxazole and trimethoprim; VCM, vancomycin; DAP, daptomycin; TEIC, teicoplanin; MIC, minimum inhibitory concentration.

**Table 2**  
Distribution of virulence genes of SCCmec types II or IV MRSA isolates

Virulence gene	Type of SCCmec					
	II			IV		
	Overall N = 58 <sup>a</sup>	Infection N = 24 <sup>a</sup>	Colonization N = 34 <sup>a</sup>	Overall N = 83 <sup>a</sup>	Infection N = 24 <sup>a</sup>	Colonization N = 59 <sup>a</sup>
<b>Virulence gene</b>						
<i>sea</i>	4 (6.9)	0	4 (12)	63 (76)	20 (83)	43 (73)
<i>seb</i>	47 (81)	22 (92)	25 (74)	0	0	0
<i>egc</i> ( <i>sem</i> and <i>seo</i> )	55 (95)	24 (100)	31 (91)	2 (2.4)	0	2 (3.4)
<i>tst</i>	11 (19)	2 (8.3)	9 (26)	3 (3.6)	1 (4.2)	2 (3.4)
<i>promoter</i> (-7T>C)						
C	44 (76)	23 (96)	21 (62)			
T	14 (24)	1 (4.2)	13 (38)			
PVL-encoding genes ( <i>lukS</i> -PV and <i>lukF</i> -PV)				0	0	0
<i>arcA</i>				0	0	0
<i>cna</i>				66 (80)	20 (83)	46 (78)
<i>sasG</i>				0	0	0
<i>spj</i>				3 (3.6)	1 (4.2)	2 (3.4)
<i>fnbB</i>				0	0	0
<b>Virulence gene combinations</b>						
<i>seb</i> , <i>egc</i> , and <i>psm-mec</i> -7T>C	41 (71)	21 (88)	20 (59)			
<i>seb</i> , <i>egc</i> , <i>tst</i> , and <i>psm-mec</i> -7T>C	1 (1.7)	1 (4.2)	0			
<i>egc</i> and <i>psm-mec</i> -7T>C	2 (3.4)	1 (4.2)	1 (2.9)			
<i>egc</i> and <i>tst</i>	3 (5.2)	1 (4.2)	2 (5.9)			
<i>sea</i> , <i>egc</i> , and <i>tst</i>	4 (6.9)	0	4 (12)			
<i>seb</i> , <i>egc</i> , and <i>tst</i>	3 (5.2)	0	3 (8.8)			
<i>seb</i>	2 (3.4)	0	2 (5.9)			
<i>egc</i>	1 (1.7)	0	1 (2.9)	2 (2.4)	0	2 (3.4)
None	1 (1.7)	0	1 (2.9)	11 (13)	3 (13)	8 (14)
<i>sea</i> and <i>cna</i>				63 (76)	20 (83)	43 (73)
<i>cna</i>				3 (3.6)	0	3 (5.1)
<i>spj</i> and <i>tst</i>				2 (2.4)	1 (4.2)	1 (1.7)
<i>spj</i>				1 (1.2)	0	1 (1.7)
<i>tst</i>				1 (1.2)	0	1 (1.7)

NOTE: *Psm-mec* mutation is presented as nucleotide numbers and substitutions from the transcription start site. T>C indicates that thymine was substituted for cytosine. *Psm-mec* is gene encoding phenol-soluble modulins (PSM-mec).

*fnbB*, *cna*, and *sasG* are genes encoding microbial surface components recognizing adhesive matrix molecule. *spj* is gene encoding cell-wall-anchored surface protein with the LPXTG motif.

SCCmec, staphylococcal cassette chromosome mec; MRSA, Methicillin-resistant *Staphylococcus aureus*; *egc*, enterotoxin gene cluster; PVL, Panton valentine leukocidin.

<sup>a</sup> Statistics; number (%).

### 3.3. Distribution of virulence genes in SCCmec types II and IV MRSA isolates

Table 2 shows the distribution of virulence genes and their combinations in SCCmec types II and IV MRSA. The SCCmec type II MRSA isolates mainly harbored *seb*, *egc*, and *psm-mec* -7T>C. The proportion of *psm-mec* -7T>C was significantly higher in SCCmec type II MRSA isolates harboring *seb* than in those not harboring *seb* (89% vs. 18%, nominal  $P < 0.01$ ). The results of virulence gene combinations results demonstrated that *psm-mec* -7T>C was present in 96% of SCCmec type II MRSA infections. The SCCmec type IV MRSA isolates mainly harbored *sea* and *cna* but not PVL-encoding genes, *arcA*, *sasG*, or *fnbB*.

### 3.4. Clinical characteristics of SCCmec types II and IV MRSA

Fig. 3 shows a comparison between the proportions of prior infected organs, progression to infection, BSI, SSI, SOFA scores, and 30-day mortality rate in patients with SCCmec types II and IV MRSA isolates.

The proportion of prior infected organs did not differ significantly between the two groups. Patients with SCCmec type II MRSA tended to have IAI, and those with SCCmec type IV MRSA tended to have BUO or CRBSI (Fig. 3A). The proportions of patients with progression to infection and SSI tended to be higher, but not significantly, for SCCmec type II MRSA than for SCCmec type IV MRSA (Fig. 3B and 3C). The proportions with BSI or with SOFA scores

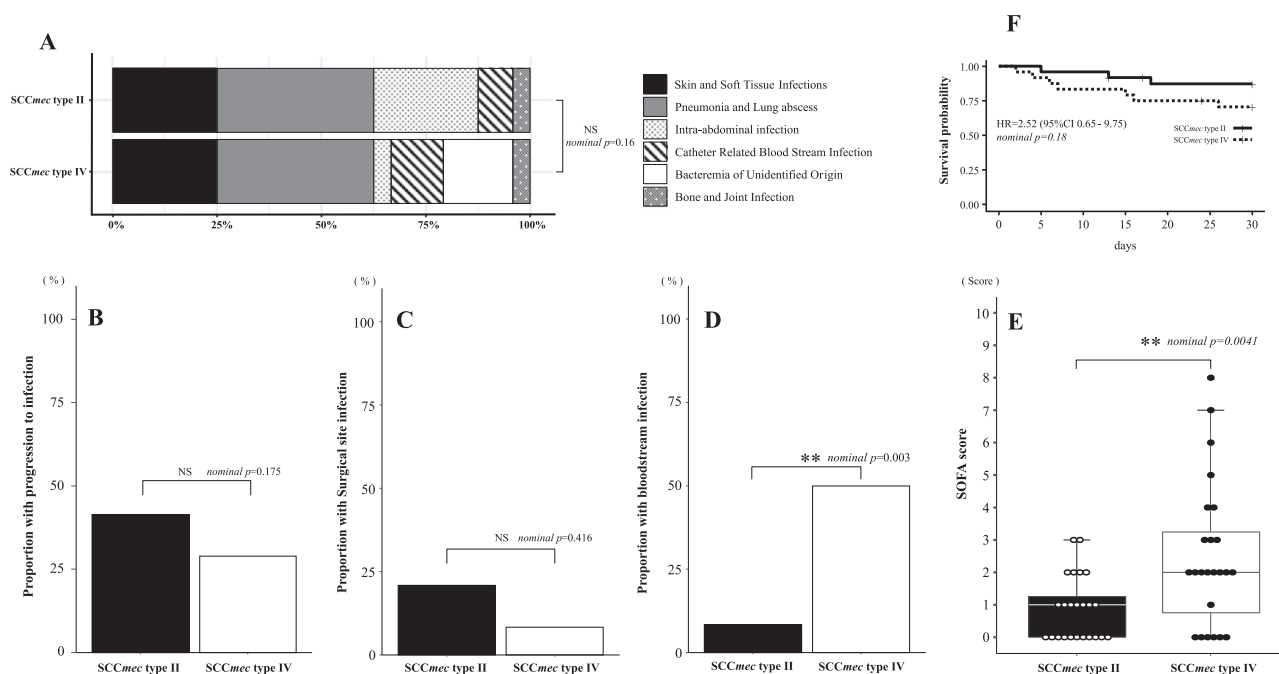
>2 points were significantly higher in SCCmec type IV MRSA than in SCCmec type II MRSA (Fig. 3D and 3E). SCCmec type IV MRSA tended to have a higher HR for 30-day mortality rate than SCCmec type II MRSA, but this was not significant (HR = 2.52; 95% confidence interval [CI]: 0.65–9.75;  $P = 0.18$ ; Fig. 3F). The proportional hazard assumption was confirmed by the Schoenfeld residual results ( $P = 0.75$ ).

### 3.5. Risk factors for progression to infection

Tables 3 and 4 show the univariate and multivariate logistic regression analyses of risk factors for progression to infection in SCCmec types II and IV MRSA isolates. SCCmec type II infection was associated with the virulence gene combinations *seb*, *egc*, and *psm-mec* -7T>C; SCCmec type IV MRSA infection was associated with *sea* and *cna* (Table 2). The virulence gene combinations of SCCmec types II and IV were then classified according to VCM MIC.

For patients with SCCmec type II MRSA isolates, we selected variables associated with progression to infection including age  $\geq 75$  years; *seb*, *egc*, *psm-mec* -7T>C and VCM MIC high; intravascular indwelling catheter; and solid tumour. Multivariate logistic regression analysis revealed that *seb*, *egc*, *psm-mec* -7T>C, and VCM MIC high were virulence factors (adjusted odds ratio [aOR] = 11.8; 95% CI: 2.49–77.7;  $P = 0.004$ ), and solid tumour was a host factor (aOR = 25.9; 95% CI: 3.66–300;  $P = 0.003$ ) (Table 3). ROC analysis of the multivariate logistic regression for SCCmec type





**Fig. 3.** Clinical characteristics of patients infected with SCCmec types II or IV MRSA. (A) Comparison of the proportions of prior infected organs. (B) Comparison of the proportions of progression to infection. (C) Comparison of the proportions of surgical site infections. (D) Comparison of the proportions of bloodstream infections. (E) Comparison of the SOFA scores of SCCmec types II and IV MRSA. (F) Comparison of the 30-day mortality rate of SCCmec types II and IV MRSA. SCCmec, staphylococcal cassette chromosome *mec*; MRSA, Methicillin-resistant *Staphylococcus aureus*; SOFA score, Sequential Organ Failure Assessment score.

**Table 3**

Univariate and multivariate logistic regression analysis of risk factors related to the progression to infection of the patients with SCCmec type II MRSA isolates

	Overall N = 58 <sup>a</sup>	Infection N = 24 <sup>a</sup>	Colonization N = 34 <sup>a</sup>	Univariate analysis		Multivariate analysis	
				OR (95% CI)	P-value	aOR (95% CI)	P-value
Age ≥75 y	41 (71)	13 (54)	28 (82)	0.25 (0.07–0.81)	0.024	0.19 (0.03–1.01)	0.06
<b>Virulence factors</b>							
<i>seb</i> , <i>egc</i> , <i>psm-mec</i> -7T>C, and VCM MIC high	22 (38)	16 (67)	6 (18)	9.33 (2.89–34.2)	<0.001	11.8 (2.49–77.7)	0.004
<i>seb</i> , <i>egc</i> , <i>psm-mec</i> -7T>C, and VCM MIC low	20 (34)	6 (25)	14 (41)	0.48 (0.14–1.46)	0.2		
<b>Host factors</b>							
Intravascular indwelling catheter	16 (28)	11 (46)	5 (15)	4.91 (1.48–18.4)	0.012	4.6 (0.75–35.5)	0.11
Solid tumour	14 (24)	12 (50)	2 (5.9)	16 (3.69–113)	<0.001	25.9 (3.66–300)	0.003
Solid tumour without metastases	5 (8.6)	4 (17)	1 (2.9)	6.6 (0.90–134)	0.1		
Metastatic solid tumour	9 (16)	8 (33)	1 (2.9)	16.5 (2.70–320)	0.011		

NOTE: *Psm-mec* mutation is presented as nucleotide numbers and substitutions from the transcription start site. T>C indicates that thymine was substituted for cytosine. VCM MIC 0.5 mg/L was classified as MIC low group and MIC 1 or 2 mg/L as MIC high group. SCCmec, staphylococcal cassette chromosome *mec*; MRSA, Methicillin-resistant *Staphylococcus aureus*; *egc*, enterotoxin gene cluster; *psm-mec*, phenol-soluble modulins *mec*; VCM, vancomycin; MIC, minimum inhibitory concentration; Odds ratio, OR; adjusted Odds ratio, aOR; confidence interval, CI.

<sup>a</sup> Statistics; number (%).

**Table 4**

Univariate and multivariate logistic regression analysis of risk factors related to the progression to infection of the patients with SCCmec type IV MRSA isolates

	Overall N = 83 <sup>a</sup>	Infection N = 24 <sup>a</sup>	Colonization N = 59 <sup>a</sup>	Univariate analysis		Multivariate analysis	
				OR (95% CI)	P-value	aOR (95% CI)	P-value
Age ≥75 y	63 (76)	15 (62)	48 (81)	0.38 (0.13–1.11)	0.074	0.54 (0.16–1.81)	0.3
<b>Virulence factors</b>							
<i>sea</i> , <i>cna</i> , and VCM MIC high	48 (58)	18 (75)	30 (51)	2.9 (1.05–8.94)	0.048	3.14 (1.06–10.6)	0.049
<i>sea</i> , <i>cna</i> , and VCM MIC low	15 (18)	2 (8.3)	13 (22)	0.32 (0.05–1.30)	0.2		
<b>Host factors</b>							
Intravascular indwelling catheter	14 (17)	7 (29)	7 (12)	3.06 (0.93–10.2)	0.064	3.78 (1.03–14.5)	0.045
Diabetes with chronic complications	4 (4.8)	3 (12)	1 (1.7)	8.29 (1.00–173)	0.074		
Moderate to severe renal disease	8 (9.6)	5 (21)	3 (5.1)	4.91 (1.10–25.8)	0.041	4.88 (0.91–30.3)	0.068

NOTE: *cna* is one of the genes encoding microbial surface components recognizing adhesive matrix molecule. VCM MIC 0.5 mg/L was classified as MIC low group and MIC 1 or 2 mg/L as MIC high group. SCCmec, staphylococcal cassette chromosome *mec*; MRSA, Methicillin-resistant *Staphylococcus aureus*; VCM, vancomycin; MIC, minimum inhibitory concentration; Odds ratio, OR; adjusted Odds ratio, aOR; confidence interval, CI.

<sup>a</sup> Statistics; number (%).

II MRSA infection showed 75.0% sensitivity, 91.2% specificity, and an area under the ROC curve (AUC) of 0.89 (95% CI: 0.80–0.98).

For patients with SCCmec type IV MRSA, we selected variables associated with progression to infection including age  $\geq 75$  years; *sea*, *cna*, and VCM MIC high; moderate to severe renal disease; and intravascular indwelling catheter. Because three of the four patients with diabetes and chronic complications had coexisting moderate to severe renal disease, we selected moderate to severe renal disease as the variable.

Multivariate logistic regression analysis revealed that *sea*, *cna*, and VCM MIC high were virulence factors (aOR = 3.14; 95% CI: 1.06–10.6;  $P = 0.049$ ) and that intravascular indwelling catheter was a host factor (aOR = 3.78; 95% CI: 1.03–14.5;  $P = 0.045$ ) (Table 4). ROC analysis of the multivariate logistic regression for SCCmec type IV MRSA infection showed 58.3% sensitivity, 74.6% specificity, and an AUC of 0.75 (95% CI: 0.66–0.85).

#### 4. Discussion

SCCmec type IV MRSA isolates were more common in elderly hospitalized patients, and most of the SCCmec types II and IV MRSA isolates were HA-MRSA. Thus, the SCCmec type could not be determined using traditional definitions. The antimicrobial susceptibility patterns of MRSA isolated herein tended to be similar to those of previous reports, with SCCmec type IV MRSA being more susceptible to non- $\beta$ -lactam antibiotics such as gentamicin, minocycline, fosfomycin, and clindamycin than SCCmec type II MRSA [3]. This may be clinically useful because the SCCmec type can be roughly inferred from these non- $\beta$ -lactam antimicrobial susceptibility patterns.

In the present study, patients with SCCmec types II and IV MRSA isolates had prior pneumonia and lung abscess, SSTIs, and BSI such as BUO or CRBSI. Our results were consistent with those of the 2020 Japan Nosocomial Infections Surveillance. SCCmec type II MRSA tended to cause more infections and SSI than SCCmec type IV MRSA, but these infections were less severe. Conversely, SCCmec type IV MRSA tended to have a lower proportion of infection than SCCmec type II MRSA, but with BSI that could be severe. SCCmec type IV MRSA infection, which tends to cause BSI, might have had a higher mortality rate than that of SCCmec type II, but this analysis lacked statistical power because of the insufficient number of enrolled patients.

The SCCmec type II MRSA isolates in our secondary acute care hospital mainly harbored *seb* and *egc*. These MRSA isolates had a tendency to cause pneumonia and lung abscess, SSTIs, or IAI. These results were similar to those of our previous report that SCCmec type II MRSA with pneumonia harbored *seb* and *egc* in a tertiary care institution.

The frequency of *psm-mec* mutation in SCCmec type II MRSA was higher than that in previous reports, and its absence was comparable to that of previous studies. For example, Chikara et al. and Aoyagi et al. reported 37.1% *psm-mec* mutation and 0% absence and 19.2% *psm-mec* mutation and 1.9% absence, respectively [6,29]. These differing results are probably owing to differences in the collection period and the institutions or regions in which the samples were collected. Additionally, *psm-mec* mutation was significantly more frequent in SCCmec type II MRSA harboring *seb* than in those isolates lacking *seb* (Table 2).

It has been reported that transcription products of *psm-mec* suppress colony spreading and the production of PSMs, which has cytolytic and proinflammatory activity, while promoting biofilm formation [6,30]. Thus, the *psm-mec* has been suggested to play a potentially important role in immune evasion and disease [6,31]. Previous studies have shown that SCCmec type II MRSA with *psm-mec* mutation increases the expression of PSMs, suggesting the association between *psm-mec* mutation or absence and in-

creased virulence. Aoyagi et al. reported that SCCmec type II MRSA with *psm-mec* mutation showed increased production of PSM $\alpha$ 3 and suppression of biofilm formation compared with strains that had intact *psm-mec* in patients with MRSA bacteraemia. Our results also showed that SCCmec type II MRSA with *psm-mec* mutation tended to cause more infections than colonization.

Because SCCmec type IV MRSA lacks *psm-mec*, PSMs production are not suppressed [6,29]. It has been reported that SCCmec type IV MRSA isolated from patients with bacteraemia showed a higher production of PSMs than SCCmec type II MRSA [32], which may influence the differences in clinical presentation between SCCmec types II and IV MRSA. The SCCmec type IV MRSA isolated herein mainly harbored *sea* and *cna*, as well as low levels of *egc*, but not PVL-encoding genes or *arcA*. The *egc* is generally considered to be associated with mucosal colonization [33,34] and is reported to suppress the excessive release of Th<sub>1</sub> cytokines by inducing an early mild Th<sub>2</sub> response to counteract the Th<sub>1</sub> response [35]. SCCmec type IV MRSA infection in the present study involved a higher proportion of BSI and SOFA scores than those of SCCmec type II MRSA (Fig. 3D and 3E). A recent study showed that SCCmec type IV MRSA harboring *cna* had a tendency to cause BSI by forming plasma-biofilm [36]. Thus, *sea* as superantigen, *cna* as adhesion factor, and low levels of *egc* suppressing Th<sub>1</sub> response might be associated with the clinical features and disease severity of SCCmec type IV MRSA infection.

In this study, we identified independent risk factors useful for predicting progression to infection in patients with SCCmec types II or IV MRSA isolates. The *psm-mec* mutation plays an important role in risk related to infection factors for SCCmec type II MRSA [6]. The finding that solid tumour is a host factor for SCCmec type II MRSA infection is related to the immune dysfunction of T cells [37]. Conversely, *cna*, which mediates adhesion to host cells, tissues, and artifacts, have important roles as risk factors for SCCmec type IV MRSA infection [38]. Therefore, intravascular indwelling catheters may promote BSI development via SCCmec type IV MRSA harboring *cna*. Additionally, an elevated VCM MIC was a common risk factor for SCCmec types II and IV MRSA infections, most likely because the cell wall thickening helps MRSA to survive in the host cells [39,40].

This study had several limitations. First, we identified the genes of the isolated strains but could not identify the clonal complex. Second, we did not examine the amount of toxin produced. Finally, this was a single-center, retrospective study, and the number of cases enrolled was insufficient. No previous reports have examined factors related to infection and severity according to SCCmec type. The infection factors identified herein are simple and easy to apply clinically.

In conclusion, we revealed the clinical characteristics and factors related to infection with SCCmec types II and IV MRSA in a Japanese secondary care facility. SCCmec type II MRSA harboring *psm-mec* –7T>C and SCCmec type IV MRSA harboring *sea* and *cna* have important roles in establishing infection. Classifying MRSA using molecular methods and identifying factors related to infection will help in developing infection control criteria and will allow for better decision-making regarding treatment regimens.

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None declared.

#### 6. Ethical approval

The ethics committees of Kyushu University Hospital (2020-48) and Saiseikai Futsukaichi Hospital (294) approved the study.



## Competing interests

None declared.

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