Impact of *psm-mec* in the mobile genetic element on the clinical characteristics and outcome of SCC*mec*-II methicillin-resistant *Staphylococcus aureus* bacteraemia in Japan

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Abstract

Over-expression of alpha-phenol-soluble modulins (PSMs) results in high virulence of community-associated methicillin-resistant *Staphylococcus aureus* (MRSA). The *psm-mec* gene, located in the mobile genetic element SCC*mec*-II, suppresses PSMαs production. Fifty-two patients with MRSA bacteraemia were enrolled. MRSA isolates were evaluated with regard to the *psm-mec* gene sequence, bacterial virulence, and the minimum inhibitory concentration (MIC) of vancomycin and teicoplanin. Fifty-one MRSA isolates were classified as SCC*mec*-II, and 10 had one point mutation in the *psm-mec* promoter. We compared clinical characteristics and outcomes between mutant MRSA and wild-type MRSA. Production of PSMα3 in mutant MRSA was significantly increased, but biofilm formation was suppressed. Wild-type MRSA caused more catheter-related bloodstream infections (30/41 vs. 3/10, p 0.0028), whereas mutant MRSA formed more deep abscesses (4/10 vs. 3/41, p 0.035). Bacteraemia caused by mutant MRSA was associated with reduced 30-day mortality (1/10 vs. 13/41, p 0.25), although this difference was not significant. The MIC_{90} of teicoplanin was higher for wild-type MRSA (1.5 mg/L vs. 1 mg/L), but the MIC of vancomycin was not different between the two groups. The 30-day mortality of MRSA with a high MIC of teicoplanin (≥1.5 mg/L) was higher than that of strains with a lower MIC (≤0.75 mg/L) (6/10 vs. 6/33, p 0.017). Mutation of the *psm-mec* promoter contributes to virulence of SCC*mec*-II MRSA, and the product of *psm-mec* may determine the clinical characteristics of bacteraemia caused by SCC*mec*-II MRSA, but it does not affect mortality.

Keywords: clinical characteristics, MRSA, phenol-soluble modulins, *psm-mec* mutation, teicoplanin, vancomycin
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Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) has rapidly spread in healthcare settings worldwide [1]. Community-associated (CA) MRSA, the USA300 (SCC*mec*-IV) and USA400 (SCC*mec*-V) strains, has been observed in especially North America over the last decade [2]. The virulence of CA-MRSA is higher than that of hospital-associated (HA)-MRSA, possibly because of the production of Panton-Valentine leukocidine (PVL) [3]. However, there is controversy as to whether the role of PVL affects disease severity and clinical outcome in MRSA infection [4–6].

Recently, Wang *et al.* [7] found novel cytolytic peptides to be the alpha-type phenol-soluble modulins (PSMs), which are encoded in an operon present in all sequenced *S. aureus* strains. PSMαs consist of 20–25 amino acids and contribute to evasion of the innate immune system by MRSA [7]. Production

of PSMαs is elevated in the most prevalent CA-MRSA strains, and PSMαs contribute to CA-MRSA virulence *in vivo* [7]. Recent reports suggest that the transcription and translation products of *psm-mec*, which is located in the mobile genetic elements SCC*mec*-II and -III in HA-MRSA, suppress the production of PSMαs [8,9].

Limited surveillance data on the genetic characteristics of MRSA are available in Japan; the NY/Japan clone (SCC*mec*-II) was predominantly clinically isolated. SCC*mec*-IV MRSA was detected in only 4–20% of samples, and these strains did not contain the *lukS/F-PV* gene [10,11]. Thus, the characteristics of CA-MRSA strains in Japan are different from those of strains reported in other countries.

Mutations in the *psm-mec* promoter found in HA-MRSA (SCC*mec*-II) attenuated the transcription of *psm-mec*. These strains showed increased expression of PSMαs to levels observed in the USA300 and USA400 strains [9]. We hypothesized that PSMαs may be important in determining the manifestation and severity of invasive MRSA infections. Our aim in this study was to examine whether SCC*mec*-II MRSA strains with mutations in the *psm-mec* promoter, which produce high amounts of PSMαs, are associated with clinical characteristics and outcome in bacteraemic patients.

Patients and Methods

Patients and study design

In total, 72 patients with MRSA bacteraemia were recruited retrospectively from March 2009 to December 2011 at Tohoku University Hospital, a 1300-bed tertiary-care teaching hospital in Japan. Bacteraemia was defined based on positive blood culture with systemic manifestation of infection. We excluded patients who (i) presented other pathogens in the blood culture during one MRSA bacteraemia episode; (ii) were dead within 24 h after the blood culture was obtained; and (iii) were under 18 years of age. Thus, 52 patients were ultimately enrolled.

The following data were collected from each patient's electric medical record: age, sex, hospitalization data, housing, co-morbidities, infection site, risk factors for MRSA infection, including recent surgery, wounds and insertion of catheters, and site of MRSA infection. Complications of MRSA bacteraemia were evaluated using radiological records such as computed tomography in addition to surgical pathology and additional culture results. The outcomes studied included 30-day mortality and length of hospital stay (LOHS). Data collection was approved by the Ethics Committees of Tohoku University.

Microbiological molecular analysis

Methicillin-resistant *Staphylococcus aureus* was confirmed by *mecA* gene carriage, and the presence of the *lukS/F-PV* gene was investigated by polymerase chain reaction (PCR) [12]. Type of SCC*mec* was determined by using SCC*mec*-II specific primers [12] or the class of the *mec* and the *ccr* gene complex [13]. DNA fragments including the *psm-mec* were sequenced by using primers as previously reported [8]. Type of polymorphic region of the protein A gene (*spa*) was determined by sequenced *spa* PCR [14]. We present the primers used in this study in Table S1.

Measurement of PSMs

The amount of PSMαs was measured as previously described [9]. Briefly, MRSA culture supernatants were evaporated and the remaining solid was dissolved in 40% acetonitrile. The supernatants remaining after subsequent centrifugation were evaporated, and the evaporated products were dissolved in water and subjected to reversed-phase high-performance liquid chromatography (HPLC). δ-hemolysin (Hld) and PSMα1 were not separated in this system.

Biofilm formation assay

Methicillin-resistant *Staphylococcus aureus* was cultured in tryptic soy broth containing 0.25% glucose in 96-well polystyrene plates for 3 days at 37°C. Cells attached to the plate were stained with safranin; staining was measured according to the absorbance at 490 nm.

Expression of AgrA

Protein of AgrA was obtained through sodium dodecyl sulphate (SDS)-polyacrylamide gel. Expression of AgrA was performed by western blotting assay as previously described [9]. The band intensity was measured by densitometry scanning (Image J 1.45 s, NIH).

Antimicrobial susceptibility

The minimal inhibitory concentration (MIC) of vancomycin and teicoplanin was determined by the *E*-test (range 0.016–256 mg/L; bioMérieux, Lyon, France). The MIC breakpoint for vancomycin and teicoplanin resistance was >2 mg/L according to EUCAST [15].

Statistical analysis

Descriptive statistics, such as means, standard deviations, frequencies and percentages, were collected. The chi-squared test, Fisher's exact test and unpaired *t*-test were conducted using Graphpad prism 5 (GraphPad Software, La Jolla, CA, USA). $p < 0.05$ was considered statistically significant.