Characteristics of community- and hospital-acquired meticillin-resistant *Staphylococcus aureus* strains carrying SCCmec type IV isolated in Malaysia

Norazah Ahmad,¹ Izayu Nurfarha Ruzan,¹ Mohamed Kamel Abd Ghani,² Azura Hussin,³ Salbiah Nawati,⁴ Mohamad Nazri Aziz,⁵ Nurahan Maning⁶ and Victor Lim Kok Eow⁷

Correspondence

Norazah Ahmad
norazah@imr.gov.my

¹Institute for Medical Research, Kuala Lumpur, Malaysia

²Department of Biomedical Sciences, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia

³Department of Microbiology, Queen Elizabeth Hospital, Sabah, Malaysia

⁴Department of Microbiology, Selayang Hospital, Selangor, Malaysia

⁵Department of Microbiology, Kuala Lumpur Hospital, Kuala Lumpur, Malaysia

⁶Department of Microbiology, Kota Bharu Hospital, Kelantan, Malaysia

⁷International Medical University, Kuala Lumpur, Malaysia

Community-acquired meticillin-resistant *Staphylococcus aureus* (CA-MRSA) occurring among hospital isolates in Malaysia has not been reported previously. As CA-MRSA reported worldwide has been shown to carry SCCmec types IV and V, the aim of this study was to determine the SCCmec types of MRSA strains collected in Malaysia from November 2006 to June 2008. From a total of 628 MRSA isolates, 20 were SCCmec type IV, whilst the rest were type III. Further characterization of SCCmec type IV strains revealed 11 sequence types (STs), including ST22, with the majority being ST30/Panton–Valentine leukocidin positive. Eight out of nine CA-MRSA were ST30, one was ST80, and all were sensitive to co-trimoxazole and gentamicin. Five new STs designated ST1284, ST1285, ST1286, ST1287 and ST1288 were discovered, suggesting the emergence of novel clones of MRSA circulating in Malaysian hospitals. The discovery of the ST22 strain is a cause for concern because of its ability to replace existing predominant clones in certain geographical regions.

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INTRODUCTION

Meticillin-resistant *Staphylococcus aureus* (MRSA) infections affecting the community have been increasingly reported worldwide. Published reports of community-acquired MRSA (CA-MRSA) infections have mainly involved children, active young adults and the indigenous population who have no previous established risk factors (CDC, 1999; Lindenmayer *et al.*, 1998; Maguire *et al.*, 1996). Whilst the majority of patients infected with CA-MRSA present with minor skin and soft-tissue infections, some cases are severe, including necrotizing infections that warrant hospital admission, and can be fatal (Dickson *et al.*, 2008).

The incidence of CA-MRSA infection in Asia has been widely reported. In Malaysia, the occurrence of CA-MRSA among hospital isolates has not been reported previously. CA-MRSA is defined as MRSA isolated from patients in an outpatient or community setting or within 48 h of hospital admission where the patient was not recently associated with any healthcare facilities nor received antibiotic treatment and does not have other risk factors for MRSA (Millar *et al.*, 2007; Nathwani *et al.*, 2008).

Surveillance for MRSA in Malaysian hospitals is included as part of the National Surveillance of Antibiotic Resistance programme, which was started in 1999. In 2007, a total of 3014 MRSA isolates were collected from 13 state hospitals and the rate of meticillin resistance in *S. aureus* in these hospitals ranged from 6.8 to 44.1% (Ministry of Health, 2007). However, these surveillance data did not differentiate between hospital-acquired (HA) infections and CA infections; thus, the true incidence of infections caused by CA-MRSA is not known.

Abbreviations: CA, community-acquired; CDC, Centers for Disease Control and Prevention; HA, hospital-acquired; MLST, multilocus sequence typing; MRSA, meticillin-resistant *Staphylococcus aureus*; PVL, Panton–Valentine leukocidin; ST, sequence type.

Meticillin resistance in *S. aureus* is encoded by the *mecA* gene and is located in the staphylococcal cassette chromosome *mec* (SCC*mec*), which is incorporated in the *S. aureus* chromosome. Most CA-MRSA reported worldwide have been shown to carry SCC*mec* types IV and V. Therefore, this study was conducted to determine the presence of SCC*mec* types IV and V among a collection of MRSA strains at the Bacteriology Unit, Institute for Medical Research, Kuala Lumpur, received between November 2006 and June 2008. As CA-MRSA is distinguished from HA-MRSA by its possession of different genetic characteristics, we also determined the sequence types (STs), the presence of Panton–Valentine leukocidin (PVL)-encoding genes and the antibiotic susceptibility profiles of these isolates.

METHODS

Bacterial isolates. A total of 628 MRSA strains at the Bacteriology Unit, Institute for Medical Research, collected between November 2006 and June 2008, was analysed. These strains were received from 9 hospitals and the number of strains from each hospital varied from 1 to 347 isolates. Most of the strains were from four major referral hospitals, namely Kuala Lumpur (347 isolates), Selayang (154 isolates), Hospital Queen Elizabeth (80) and Kota Bharu (42 isolates). The strains were isolated in the hospital microbiology laboratories from clinical samples obtained from the various clinical departments. At the Institute for Medical Research, MRSA was confirmed by a positive tube coagulase test using rabbit plasma with EDTA (BBL; Becton Dickinson), a positive DNase test and the detection of the *mecA* gene by PCR.

SCC*mec* typing, PVL-encoding gene detection and multilocus sequence typing (MLST). Chromosomal DNA was extracted from the isolates using a DNeasy kit (Qiagen) following the instructions of the manufacturer. SCC*mec* typing was carried out using primers specific for SCC*mec* types I–V as described by Ito *et al.* (2004) and Lim *et al.* (2003). The strains were first screened for the *ccr* gene complex, followed by *mec* complex determination. Strains with SCC*mec* IV were further characterized for the presence of PVL-encoding genes as described by Lina *et al.* (1999). MLST was conducted as described by Enright *et al.* (2000). ST was determined by comparing the allelic profiles with the database at the MLST website (<http://saureus.mlst.net>).

Antibiotic susceptibility testing. Antibiotic susceptibility testing was carried out at the Institute for Medical Research following Clinical and Laboratory Standards Institute guidelines. Disc susceptibility testing was carried out using erythromycin, gentamicin, clindamycin, ciprofloxacin, rifampicin, co-trimoxazole, fusidic acid and linezolid. For fusidic acid, a zone-size breakpoint of ≥ 21 mm was used to define susceptibility (Toma & Barriault, 1995). All susceptibility tests were performed using Mueller–Hinton II agar (BBL; Becton Dickinson). For vancomycin, the MIC was determined using vancomycin Etest strips (AB Biodisk).

Patient data. For SCC*mec* type IV isolates, the patient's record was reviewed to determine whether the patient fulfilled the epidemiological criteria for CA-MRSA, namely the isolation of MRSA within 48 h of hospitalization, no history of antibiotic intake and no history of hospitalization or being in chronic-care health facilities within the last year.

RESULTS AND DISCUSSION

From the total of 628 MRSA strains screened for SCC*mec* types, 20 were found to be SCC*mec* type IV, whilst 608 strains were type III. None of the strains was SCC*mec* type I, II or V. Among the 20 SCC*mec* type IV MRSA strains, 9 were CA-MRSA, based on the definition above, and 11 were HA-MRSA (Table 1). The occurrence of SCC*mec* types among MRSA varies in hospitals worldwide. Most HA-MRSA infections are SCC*mec* types I, II and III, whilst SCC*mec* types IV and V have been associated with CA infections (Boyle-Vavra *et al.*, 2005; Karahan *et al.*, 2008; Vandenesch *et al.*, 2003; Wang *et al.*, 2004). Types VI and VII are SCC*mec* types that have been discovered more recently (Berglund *et al.*, 2008; Oliveira *et al.*, 2006). In our study, the majority of the hospital isolates were SCC*mec* type III and only a minority was SCC*mec* type IV. SCC*mec* type III is common in the hospitals of neighbouring countries, namely Singapore, Indonesia and Thailand (Chongtrakool *et al.*, 2006). SCC*mec* type II MRSA strains, which are predominant in Korea and Japan, were not observed among our strains (Chongtrakool *et al.*, 2006; Ko *et al.*, 2005a, b). MRSA with SCC*mec* type IV was first described in CA-MRSA (Ma *et al.*, 2002) and has since commonly been reported in CA-MRSA. However, this SCC*mec* type has been shown to be prevalent among hospital isolates (Berglund *et al.*, 2005; Denis *et al.*, 2005; Huang *et al.*, 2007).

The CA-MRSA strains were all isolated from skin and soft-tissue infections, whilst the HA-MRSA strains were isolated from a variety of sources including blood. Eight of the nine CA-MRSA isolates and three of the eleven HA-MRSA isolates of SCC*mec* type IV were positive for the PVL-encoding gene. None of the bacteraemia cases was PVL positive. Although the presence of PVL-encoding genes cannot be used to define CA-MRSA, it has been identified in many CA-MRSA strains isolated worldwide, such as in Japan, Korea, Singapore, Taiwan, Australia and Belgium (Boyle-Vavra *et al.*, 2005; Coombs *et al.*, 2004; Denis *et al.*, 2005; Hsu *et al.*, 2006).

MLST of the 20 SCC*mec* type IV strains revealed 11 STs. Most of the STs were ST30, whilst the other STs were each represented by a single strain (ST22, ST45, ST80, ST101, ST188, ST1284, ST1285, ST1286, ST1287 and ST1288). Eight of the nine CA-MRSA strains were ST30 and one was ST80. The ST30 strains were all PVL positive, whilst the ST80 strain was PVL negative. The HA-MRSA strains that were ST30 were also PVL positive, and were isolated from skin and wound infections. PVL is a cytotoxin that causes leukocyte destruction and tissue necrosis. It has been suggested that the integration of the PVL-encoding gene locus and SCC*mec* type IV into ST30 MRSA strains could be responsible for making these strains a particularly successful community-based pathogen (Vandenesch *et al.*, 2003). The finding of ST80 among our local CA-MRSA is of interest. This ST has been associated with CA-MRSA isolates from France and Switzerland, and with hospital

Table 1. Clinical and demographical data of patients with SCCmec type IV and strain characteristics

Isolate	Ward	Year of isolation	Age (years)/sex	Infection type	MRSA type	Antibiogram* (sensitivity to)	ST	PVL-encoding gene
MY1	Outpatient	2007	48/M	Skin infection	CA	E, C, R, S, F, G	30	+
MY2	SCN	2006	Neonate/F	Bacteraemia	HA	C, R, F	101	–
MY3	ICN	2007	Neonate /M	Wound infection	HA	E, C, R, S, F, Cp	1285	–
MY4	ICN	2007	Neonate /M	Bacteraemia	HA	E, C, R, S, F, Cp	1286	–
MY5	Medical	2008	42/F	Bacteraemia	HA	R, S, G	1287	–
MY6	Surgical	2007	20/F	Breast abscess	CA	E, C, R, S, G	30	+
MY7	Outpatient	2008	12/M	Toe abscess	CA	C, R, S, G	30	+
MY8	Medical	2007	20/F	MRSA	HA	E, C, S, F, G	1288	–
MY9	Outpatient	2008	33/M	Sacral abscess	CA	C, R, S, F, G	30	+
MY10	Medical	2008	23/M	Intravenous line infection	HA	C, R, S, G	30	+
MY11	Outpatient	2008	29/F	LSCS wound infection	HA	C, R, S, F, G	30	+
MY12	Orthopaedic	2008	69/F	Wound infection	HA	E, C, R, S, G	45	–
MY13	Psychiatric	2008	45/M	Bed sore	HA	E, C, R, S, F, Cp, G	188	–
MY14	Outpatient	2008	28/F	Abdominal wall abscess	CA	E, C, R, S, G	30	+
MY15	Outpatient	2008	75/F	Breast abscess	CA	E, C, R, S, G	30	+
MY16	Outpatient	2008	29/M	Leg cellulitis	CA	E, C, R, S, F, G	30	+
MY17	Orthopaedic	2008	24/M	Wound infection	HA	E, C, R, S, F, G	1284	–
MY18	Outpatient	2008	76/M	Skin infection	CA	E, R, S, F, G	30	+
MY19	Surgical	2008	52/M	Wound infection	HA	R, S, G	22	+
MY20	Orthopaedic	2008	20/M	Left arm abscess	CA	E, C, R, S, G	80	–

F, Female; ICN, intensive care nursery; LSCS, lower segment caesarean section; M, male; SCN, special care nursery.

*All strains were sensitive to linezolid and vancomycin. C, Clindamycin; Cp, ciprofloxacin; E, erythromycin; F, fusidic acid; G, gentamicin; R, rifampicin; S, co-trimoxazole.

isolates in Greece and Norway (Aires de Sousa & de Lencastre, 2003; Hanssen *et al.*, 2005). Its discovery among our local CA-MRSA isolates may suggest the successful establishment of this European clone in a new locality or country.

The discovery of ST30 in two of the HA-MRSA strains is a cause for concern. CA-MRSA may spread in hospitals and establish itself as a nosocomial infection along with HA-MRSA. Huang *et al.* (2007) reported that SCCmec type IV (ST59), which is usually CA, has become an important nosocomial pathogen in hospitals in Taiwan. Conversely, a retrospective study of MRSA strains disseminated in Japanese hospitals isolated between 1979 and 1985 showed a predominance of strains that belonged to ST30 with SCCmec type IV. These strains were replaced in the 1990s by a different clone of ST5 and SCCmec type II. This transition of a predominant clone has been suggested to be due to selective pressure and the successful ability of ST5 type IV SCCmec MRSA strains, which carry several

antibiotic-resistance genes, to adapt to environmental change (Ma *et al.*, 2006).

The isolation of ST22 among our local hospital MRSA strains is of particular interest because it has the same ST as EMRSA-15, a dominant clone that is commonly found in the UK (Witte *et al.*, 2001). This clone has spread into European countries and established itself as the dominant clone, replacing the Iberian and Brazilian clones that were for some time the dominant type of MRSA in Europe (Aires de Sousa *et al.*, 1998; Amorim *et al.*, 2007; Heym *et al.*, 2002). This ability to replace a well-established clone is indeed a concern, as this is the first time that this strain has been reported among Malaysian MRSA isolates. This ST22 isolate was sensitive to gentamicin and tetracycline (results not shown), and resistant to erythromycin and ciprofloxacin, which is also a feature of EMRSA-15. Rapid travel and freedom of movement from one country to another has been suggested as helping dissemination of this clone to countries outside the UK (Melter *et al.*, 2006). ST22 is

easily spread because of the small size of SCCmec type IV, and may eventually replace the predominant SCCmec type III strains in our hospitals.

Five newly discovered STs were designated ST1284 (151-123-93-2-145-150-2), ST1285 (2-3-1-1-4-4-11), ST1286 (2-31-1-2-13-4-11), ST1287 (2-123-93-2-13-4-11) and ST1288 (151-1-14-5-145-150-11), and were deposited in the MLST database. Three of the new STs (ST1285, ST1286 and ST1287) were discovered in one hospital in West Malaysia, whilst another two (ST1284 and ST1288) were discovered in a hospital in East Malaysia, which is separated from West Malaysia by the South China Sea (Fig. 1). Using eBURST, ST1284, ST1286, ST1287 and ST1288 were shown to be individual unlinked STs that were not single-locus variants of any other STs in the MLST database. However, ST1285 is a single-locus variant of ST239 and is therefore associated with clonal complex 239. ST239 is a common ST found among HA-MRSA in the Asia Pacific region. The discovery of these new STs suggests that there are novel clones of MRSA circulating in Malaysian hospitals. These novel clones were sensitive to most of the antibiotics tested, except for ST1287, which showed multiple antibiotic resistance.

SCCmec type IV was originally described as being smaller in size than SCCmec I, II or III, and devoid of any antibiotic-resistance genes except *mecA*, making it susceptible to various non- β -lactam antibiotics (Ma *et al.*, 2002). Most of the SCCmec type IV strains in this study were sensitive to four or more non- β -lactam antibiotics, with the exception of three strains that showed multiple antibiotic resistance. All of the CA-MRSA strains in this study were sensitive to four or more non- β -lactam antibiotics, whilst the strains that showed multiple antibiotic resistance were HA-MRSA strains. Interestingly,

the MRSA strain isolated from the bed-sore swab of an in-patient who had been in the ward for more than 120 days was sensitive to seven antibiotics. MRSA isolated from two bacteraemic cases showed sensitivity to only three commonly used antibiotics. It has been suggested that SCCmec type IV may have subsequently acquired additional genes following exposure to antibiotic selection pressures in hospitals (Berglund *et al.*, 2005). The Malaysian National Surveillance of Antibiotic Resistance programme for the year 2007 reported that MRSA strains from 12 major hospitals were resistant to erythromycin, gentamicin and co-trimoxazole at rates of 95, 93.5 and 89.3 %, respectively (Ministry of Health, 2007). The CA-MRSA strains in this study were mainly sensitive to these antibiotics and the occurrence of strains with non-multiple antibiotic resistance should alert physicians to the possibility of encountering CA-MRSA as the cause of infection. Ciprofloxacin susceptibility has been used as a phenotypic marker of CA-MRSA (Otter & French, 2008); however, this could not be applied to our local CA-MRSA strains as they were all resistant to ciprofloxacin. Instead, susceptibility to gentamicin was used as the phenotypic marker of CA-MRSA in our local strains.

In conclusion, SCCmec type IV was found in our hospital isolates and also among our CA-MRSA strains. Our CA-MRSA strains were mainly ST30 and were all PVL-encoding gene positive and did not show multiple antibiotic resistance. ST30 was also discovered among hospitalized patients. New STs were discovered, which suggests that there are novel clones of MRSA circulating in Malaysian hospitals. The discovery of MRSA strains with ST22 among our hospital isolates raises concern, as this clone has been reported to have the ability to replace the existing predominant clones of certain geographical regions.

Fig. 1. Map of Malaysia showing the locations of the hospitals where the SCCmec type IV strains were isolated. Symbols represent the ST present at each location.

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