

Methicillin sensitive *Staphylococcus aureus* (MSSA) isolates as a potential source for the emergence of USA 300 methicillin resistant *Staphylococcus aureus* (MRSA) in Malaysia

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Abstract. We investigated the potential of USA300 MRSA emergence in Malaysia by examining 268 MSSA isolates from both community (110) and healthcare (158) settings. Nine isolates from both the environments were similar to the USA300 MRSA background based on MLST, *spa* and PFGE type. These results underscore the importance of continued surveillance to monitor the emergence of USA300 MRSA in Malaysia.

INTRODUCTION

Emerging community-acquired methicillin resistant *Staphylococcus aureus* (CA-MRSA) is an issue of world-wide clinical concern. Originally described in 2001, the CA-MRSA strain USA300 (ST8/ *SCCmecIV*) is one of the most successful lineages, endemic throughout the United States (Tenover *et al.*, 2006). USA 300 CA-MRSA is characterized based on the presence of *PVL* gene (a bicomponent cytotoxin encoded by the *PVL* genes *lukS-PV* and *lukF-PV* that destroys leukocytes), ACME fragment (newly identified arginine catabolic mobile element gene cluster that is involved in bacterial growth and development) (Diep *et al.*, 2006) and the PFGE pulsotype. To date, USA 300 infections have been reported to be associated with minor skin and soft tissue infections as well as rapidly progressing, fatal diseases including necrotizing pneumonia, severe sepsis and necrotizing fasciitis in a variety of different community groups and healthcare settings in the United States (Lowy,

1998). However concerns regarding the incidence of CA-MRSA strains have recently increased as it has disseminated to European countries and the Middle East (Francis *et al.*, 2005; Kourbatova *et al.*, 2005; Diep *et al.*, 2008; Glikman *et al.*, 2010; Tokajian *et al.*, 2010). The majority of MRSA infections in Asian community and healthcare settings have been due to ST239, ST5, and ST30 MRSA (Ko *et al.*, 2005; Hsu *et al.*, 2006) and previous studies on MRSA epidemiology we have conducted in Malaysia found no USA300 MRSA (Nor Shamsudin *et al.*, 2008; Ghaznavi-Rad *et al.*, 2010). However, recent reports of the appearance of USA300 MRSA in Japan and Korea (Park *et al.*, 2008; Nagao *et al.*, 2010) underscore the potential for the increased emergence of USA300 in Southeast Asian countries. The potential for such isolates to appear would not only be expected to occur by strain importation but also by the degree to which suitable resident methicillin susceptible *S. aureus* isolates might acquire *SCCmec* element and *PVL* gene most probably from coagulase negative

staphylococci that are human skin normal flora and frequent nosocomial colonizers, to become MRSA strain such as USA300 (Katayama *et al.*, 2003). In this context, we examined a recently studied population of Malaysian MSSA isolates (Ghasemzadeh-Moghaddam *et al.*, 2011), to determine whether resident MSSA with the USA300 genomic background is present.

MATERIALS AND METHODS

A total of 268 MSSA isolates from a Malaysian MSSA survey collection were examined in our previous study by Multilocus Sequence Typing (MLST) (www.mlst.net), *spa* typing, *agr* typing and panel of virulence genes (Panton-Valentine leukocidin (*PVL*)(*lukS-PV* and *lukF-PV*), *fnb* (fibronectin binding protein), *cna* (collagen adhesin gene), *sea* (Staphylococcal enterotoxin A-G), *seb*, *sec*, *sed*, *see*, *seg*, *tsst* (toxic shock syndrome toxin), *eta*, *etb* (exfoliative toxin a-b) and the arginine catabolic mobile element (*ACME*) gene *arcA* (Ghasemzadeh-Moghaddam *et al.*, 2011).

Out of the 268 MSSA isolates, 9 of the isolates with *spa* type t08 and t24 were investigated for USA 300 background by pulsed field gel electrophoresis (PFGE) according to the method described by Goering *et al.* (2008).

Staphylococcus aureus DNA was digested with *smaI* restriction endonuclease (Fermentas, Axon Scientific, Malaysia) and *XbaI*-digested *Salmonella* Braenderup H2812 DNA was used as a size and gel normalization standard. Electrophoresis was performed in 1% agarose (Seakem Gold, Lonza, Rockland, Me) on a CHEF DRII apparatus (Bio-Rad, Hercules, CA) at 6 V/cm with switching linearly ramped from 5 to 15 seconds for 10 hours and 15 to 60 seconds for next 12 hours. Images of GelRed dye (Biotium Inc, ESSAN HUS sdn, Bhd, Malaysia) stained gels were captured electronically and compared (UPGMA, Dice coefficient) using the Bionumerics v6.6 software (Applied Maths, Sint-Martens-Latem, Belgium). PFGE analysis was repeated in triplicate to confirm reproducibility of the banding patterns. The

PFGE pattern of the Malaysian MSSA isolates were compared with the USA 300 strain FPR3757 isolated from wrist abscess by the Clinical Laboratory of the San Francisco General Hospital (Diep *et al.*, 2006)

RESULTS AND DISCUSSION

As shown in Table 1 nine MSSA isolates with similarity to genomic profile of USA300 strain FPR3757 were identified in the survey collection. Six were healthcare-associated (three from a pediatric ward and one each from Maternity, General Medicine and Orthopedic surgery wards), while three were from the community (rural population). All isolates were sensitive to a group of 24 antibiotics included Ciprofloxacin and Gentamicin. The five healthcare-associated strains were isolated from skin, wound and soft tissue infections, while one was from medical device. Two pediatric strains were isolated from patients with 4 and 13 years old while others patients were more than 13 years old.

All isolates were negative for *ACME-arcA* and *PVL* gene, except for one community isolate which was *PVL* gene positive. Interestingly, all community isolates were *spa* type t008 while healthcare-associated strains were either *spa* type t008 or t024.

As shown in fig. 1, PFGE profiles of all t008 and one of the t024 MSSA isolates were closely related ($\geq 89\%$) to the USA300 MRSA reference pattern, the major difference being the position of the *SmaI* fragment (Fig. 1, double arrow) encoding the *orfX* insertion site for *SCCmec* (Goering *et al.*, 2007). As noted previously (Larsen *et al.*, 2009) one of the t024 isolates was indistinguishable from the t008 MSSA PFGE pattern. In contrast to USA300 MRSA, all of the isolates were *ACME/arcA* and *PVL* gene negative except for one *PVL* positive community isolate (97kp). However, this situation could easily change since *SCCmec* as well as the *ACME* cassette and *PVL* are encoded by mobile elements. These MSSA strains clearly represent a genomic background favourable for the emergence of MRSA. In addition, the pattern of virulence and *agr* genes except for

Table 1. Molecular characterization of USA300 MRSA like MSSA in Malaysia

Sample ID	Source/ ward/ Infection site	Virulence genes													agr				spa type /MLST	Spa-CC (BURP) /MLST CC (e BURST)							
		ACME	PVL	<i>sea</i>	<i>seb</i>	<i>sec</i>	<i>seg</i>	<i>seh</i>	<i>sei</i>	<i>tesf</i>	<i>efa</i>	<i>efb</i>	<i>ecaA</i>	<i>ecaD</i>	<i>ena</i>	<i>fibA</i>	I	II			III	IV					
21c	H/Paediatric/ Soft tissue																								t024/ST8	spa-CC08 /CC8	
31c	H/ Maternity/ Skin wound																									t024/ST8	spa-CC08 /CC8
17d	H/ Paediatric/ Skin wound																									t008/ST8	spa-CC08 /CC8
30d	H/ Paediatric/ Medical device																									t008/ST8	spa-CC08 /CC8
15E	H/ Orthopaedic surgery/ Skin wound																									t008/ST8	spa-CC08 /CC8
23E	H/ General medicine/ Skin wound																									t024/ST8	spa-CC08 /CC8
97kp	C/ village people		+																							t008/ST8	spa-CC08 /CC8
195kp	C/ village people																									t008/ST8	spa-CC08 /CC8
167kp	C/ village people																									t008/ST8	spa-CC08 /CC8

H: Hospital, C: Community, +: presence of gene, blank: absence of gene

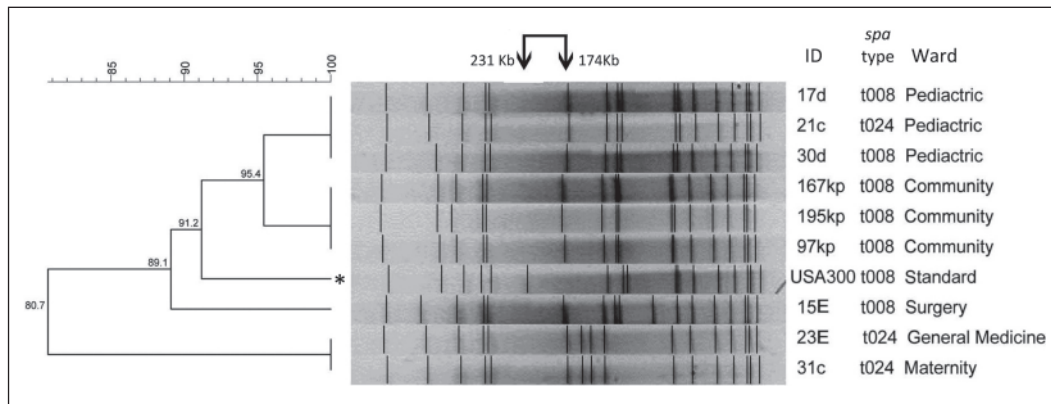


Figure. 1. *Sma*I-digested chromosomal DNA from Malaysian MSSA isolates in comparison to a reference USA300 MRSA strain (FPR3757) analyzed by BioNumerics, v6.6. Relative band sizes (in kilobases) and the position of the *Sma*I fragment containing *mecA* are indicated by the double arrow

PVL gene found in these isolates was consistent with USA300 MRSA as reported by others (Diep *et al.*, 2006; Limbago *et al.*, 2009). Interestingly, 5/6 healthcare-associated isolates were collected from skin and soft tissue infection as USA300 has trend to cause same kind of infection (Lowy, 1998). Apart from USA 300 background, the MSSA collection also contained isolates with MRSA ST1, ST3, ST5, ST8, ST239, and ST121 background based on MLST and *spa* types (Ghasemzadeh-Moghaddam *et al.*, 2011)

An obvious limitation of this study is the small sample size which is far from a nationwide survey. Nevertheless, these results confirm the presence of the USA300 genetic background in the Malaysian MSSA population found in both healthcare and community settings. While the majority of these isolates were *spa* type t008, both t008 and t024 ST8 MRSA isolates are clinically

significant (Larsen *et al.*, 2009). Thus, the potential for USA 300 MRSA emergence clearly exists in Malaysia. This finding underscores the importance of continued surveillance of Malaysian staphylococcal populations with the hope of not only monitoring but also preventing the widespread emergence of this problematic (successful lineage carrying *pvl* gene and *acme* fragment) MRSA strain like USA 300.

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