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Original Article

Comparison of Methicillin-Resistant and Methicillin-Sensitive *Staphylococcus aureus* Strains Isolated from a Tertiary Hospital in Terengganu, Malaysia

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SUMMARY: *Staphylococcus aureus* is a persistent human pathogen responsible for a variety of infections ranging from soft-tissue infections to bacteremia. The objective of this study was to determine genetic relatedness between methicillin-resistant *S. aureus* (MRSA) and methicillin-susceptible *S. aureus* (MSSA) strains. We isolated 35 MRSA and 21 MSSA strains from sporadic cases at the main tertiary hospital in Terengganu, Malaysia, screening them for the presence of virulence genes. Their genetic relatedness was determined by accessory gene regulator (*agr*) types, PCR-restriction fragment length polymorphism (RFLP) of the *coa* gene, pulsed-field gel electrophoresis (PFGE), *S. aureus* protein A (*spa*), and multilocus-sequence typing (MLST). We found that 57% of MRSA and 43% of MSSA strains harbored enterotoxin genes. The majority (87.5%) of the strains were *agr* type I. PCR-RFLP and PFGE genotyping of the *coa* gene revealed that MRSA strains were genetically related, whereas MSSA strains had higher heterogeneity. The combined genotype, MLST-*spa* type ST239-t037, was shared among MRSA and MSSA strains, indicating that MRSA strains could have evolved from MSSA strains. Two combined MLST-*spa* types were present in MRSA strains, whereas 7 different MLST-*spa* types were detected in MSSA strains, including 2 combined types (ST779-t878 and ST1179-t267) that have not been reported in Malaysia. In conclusion, enterotoxin genes were more prevalent in MRSA than in MSSA strains in the Terengganu hospital. The MSSA strains were genetically more diverse than the MRSA strains.

INTRODUCTION

Staphylococcus aureus is one of the most important bacterial pathogens isolated from the community and healthcare settings in Malaysia and other parts of the world. *S. aureus* is known to produce a variety of virulence factors that are responsible for specific acute staphylococcal toxemia syndromes, septic shock, infective endocarditis, arthritis, and necrotizing pneumonia (1–3).

Staphylococcal enterotoxins, which belong to a large family of staphylococcal and streptococcal pyrogenic exotoxins, are known to stimulate non-specific T-cell proliferation (4). More than 20 different types of enterotoxins (SEA to SEE, SEG to SEI, SEIJ, SEIK-SEIQ, SER to SET, SEW, SEIU) have been reported so far (5).

Methicillin-resistant *S. aureus* (MRSA) is known to have evolved from methicillin-susceptible *S. aureus* (MSSA) after acquiring the staphylococcal cassette chromosome *mec* (SCC*mec*) element. SCC*mec* generally consists of 2 essential components, the *ccr* gene complex (*ccr*) and the *mec* gene complex (*mec*), which harbors the *mecA* gene (6). The *mecA* gene encodes a 78-kDa penicillin-binding protein (PBP) 2a that confers methicillin resistance as well as resistance toward other β-lactam antibiotics (7). Currently, 11 different SCC*mec* types have been reported worldwide (http://www.sccmec.org/Pages/SCC_TypesEN.html).

The accessory gene regulator (*agr*) controls the expression of virulence factors in *S. aureus* (8). Four different *agr* types (*agr* types I–IV) have been reported (8).

Various methods are available to subtype *S. aureus*, and these include PCR-resriction fragment length polymorphism (RFLP) of the *coa* gene (9), pulsed-field gel electrophoresis (PFGE) (10), multilocus-sequence typing (MLST) (11), *S. aureus* protein A (*spa*) typing (12), and *mec*-associated direct repeat unit (*dru*) typing (13).

PCR-RFLP of *coa* is based on the *Alu*I restriction heterogeneity of the coagulase region that contains 81-bp tandem repeats at the 3′ coding region (14). On the other hand, *spa* and *dru* typing are based on sequence analysis of the polymorphic region X of the *spa* gene and the *mec* region of MRSA, respectively, and are commonly used for subtyping this organism (7). The data generated by *spa* and *dru* typing are also highly

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comparable between laboratories and are analyzed via Ridom SpaServer (7) and dru-typing.org (13), respectively. Similarly, MLST, which is based on sequence analysis of 7 housekeeping genes, is also highly comparable between laboratories because designation of sequence types (STs) is performed via the curator at www.mlst.net.

Several studies have documented the molecular characterization of hospital-acquired MRSA and hospital-acquired MSSA in countries such as Russia (15), Japan (16), North America, Europe, and others (17). Hu et al. (16) demonstrated that all the Japanese MRSA strains investigated harbored superantigenic toxin genes as compared with only 76% of their MSSA strains. Baranovich et al. (15) indicated that some of their Russian MSSA strains had an identical genetic background with pandemic MRSA clones, whereas Goering et al. (17) demonstrated that some of their MSSA genotypes were associated with MRSA outbreak strains. However, no report has compared hospital-acquired MRSA and hospital-acquired MSSA from the east coast region of Peninsular Malaysia. The objective of the study was to characterize and determine the genetic relatedness among 35 MRSA and 21 MSSA strains isolated from the main tertiary hospital in Terengganu, a state located on the east coast of Peninsular Malaysia.

MATERIALS AND METHODS

Bacterial strains: All of the strains isolated from 2008 to 2010 were retrieved from glycerol stocks; however, we could revive only 56 viable *S. aureus* strains, including 35 MRSA and 21 MSSA strains from sporadic cases. The strains had been isolated from inpatients (patients who had stayed at the hospital for at least one night) at Hospital Sultanah Nur Zahirah, an 821-bed referral hospital that has 9 specialist clinics, 16 operation theaters, and 28 patient wards, and which is the main tertiary hospital in Kuala Terengganu, the state capital of Terengganu.

The organisms had been isolated from swab samples (n = 28, 50%), blood (n = 14, 25%), pus (n = 7, 12.5%), tissue (n = 3, 5.4%), urine (n = 2, 3.6%), sputum (n = 1, 1.8%), and unknown sites (n = 1, 1.8%).

The strains had been identified by standard biochemical methods, including coagulase test by the hospital's clinical laboratory staff. We carried out purity and confirmation testing of the strains using a cefoxitin disk diffusion test and mannitol-salt agar as described by Lim et al. (18). All strains were cultured in tryptone soy broth and stored in CryoVials containing 25% v/v glycerol (Invitrogen, Carlsbad, Calif., USA) at −85°C.

PCR detection of virulence genes and *agr* types: Genomic DNA from MRSA and MSSA strains was extracted using the Wizard Genomic DNA Purification Kit (Promega, Madison, Wis., USA). A 5-μL aliquot was used as the DNA template. Detection of adhesion genes (*efb, fnbA, fnbB, cna, hlg, ica*, and *sdrE*) (19–21), toxin genes (*sea, seb, sec, sed, see, seg, seh, sei, sej, eta, etb, etd, tst*, and *pvl*) (22–24), and *agr* types (*agr* type I–IV) (25) was performed as previously described (19,21–25). All PCR experiments were repeated once to confirm their reproducibility.

Genotyping by PCR-RFLP of *coa* gene, PFGE, *spa*, and MLST: PCR amplification of the *coa* gene was performed using genomic DNA, primers, and conditions as described by Hookey et al. (9), with minor modifications. Briefly, PCR amplification was performed in a final volume of 25 μL containing 0.4 μM of each primer pair (Operon Biotechnologies GmbH, Ebersberg, Germany), 35 μM of each deoxynucleoside triphosphate, 1.5 mM $MgCl_2$, and 0.5 U *Taq* DNA polymerase (Promega).

The amplicons were digested with *Alu*I (Promega) as described by Hookey et al. (9). Digested products were separated in a 1.5% agarose gel at 90 V for 3 h. Gels were photographed under UV light after staining with ethidium bromide (0.5 μg/mL) and destaining with distilled water.

PFGE was performed as described by Lim et al. (26). The banding patterns generated were analyzed using BioNumerics version 6.0 (Applied Maths, Kortrijk, Belgium). Cluster analysis was performed using the unweighted pair-group method with arithmetic averages (UPGMA) with a position tolerance of 0.15. All DNA profiles were assigned arbitrary designation and analyzed by defining a similarity (Dice) coefficient, F (26).

We performed *spa* and heteroduplex PCR for identification of MLST ST239 as described by Harmsen et al. (12) and Feil et al. (27). The *spa* amplicons were purified using a Qiagen DNA purification kit (Qiagen GmbH, Hilden, Germany) and sequenced to validate their identities. Nucleotide sequences of *spa* type were analyzed using BioNumerics version 6.0.

MLST was conducted on the representatives of each *spa* type using conditions as described by Enright et al. (11). The amplicons were purified using a commercial kit (Qiagen), and sequenced. The allelic number and STs were assigned using the *S. aureus* MLST database (http://saureus.mlst.net), whereas the clustering of related STs (defined as clonal complexes [CCs]) was analyzed with the BURST algorithm (http://eburst.mlst.net).

SCC*mec* and *mec*-associated *dru* typing: Characterization of SCC*mec* types was performed on all 35 MRSA strains using conditions as described by Milheirico et al. (28). Five strains—NCTC10442, N315, 85/2082, JCSC4744, and WI5—were used as positive controls for SCC*mec* types I, II, III, IV, and V, respectively (26).

All MRSA strains were further characterized by *dru* typing under the conditions described by Goering et al. (17). The *dru* amplicons were purified using a commercial DNA purification kit (Intron Biotechnology, Kyunggki-do, Korea) and sequenced to validate their identities. Nucleotide sequences of *dru* type were analyzed using BioNumerics version 6.0. The *dru* types were determined using the TRST-Tandem Repeat Sequence Analysis plugin (available in BioNumerics version 6.0) that could identify dr and dt sequences from ab1.files (www.dru-typing.org). The *dru* types could also be identified by using stand-alone *dru* typing tools, which can be downloaded from the *dru* server (http://www.mystrains.com/druid).

Statistical analysis: STATISTICA (version 8.0) was used for data analysis. The associations between different virulence factors were determined by Spearman's

rank order correlation coefficient test. *r*-value was taken as the type of association between the variables. The breakpoints for the association of virulence factors were defined as follows: perfect association with $r = 1$, no association with $r = 0$ and inverted correlation with $r = -1$ (http://www.graphpad.com/articles/interpret/corl_n_linear_reg/correlation.htm).

RESULTS

Prevalence of virulence genes among MRSA and MSSA strains: The majority of the strains were positive for adhesion genes such as fibrinogen-binding protein (*efb*), fibrinogen-binding protein A (*fnbA*) (62.5% each), and intracellular adhesion (*ica*) (44.6%). Only 4 strains (7.1%) were positive for the *sdrE* gene, whereas hemolysin (*hlg*), collagen adhesin (*cna*), or fibrinogen-binding protein B (*fnbB*) genes were not detected.

Based on Spearman's rank correlation coefficient test, a correlation between *ica* and the enterotoxin gene was observed ($r = 0.08$, $P < 0.05$). Other enterotoxin (*sed, see, seg, seh, sei*, and *sej*) and exfoliative toxin (*eta, etb*, and *etd*) genes were not detected in any of the strains. Two MSSA strains harbored 2 enterotoxin genes within their respective genomes (*seb* and *sec* for MSSA13, *sea* and *sec* for MSSA21; Table 1). In total, 18 MRSA and 6 MSSA strains tested positive for the *sea* enterotoxin gene, which was the predominant enterotoxin gene detected, whereas the *seb* enterotoxin gene was only detected from 2 MSSA strains. The *sec* enterotoxin gene was present in 3 MSSA and 2 MRSA strains.

We detected 3 *agr* genotypes, with *agr* type I predominanting (87.5%; 34 MRSA and 15 MSSA), followed by *agr* type III (7.1%; 4 MSSA) and *agr* type II (5.4%; 1 MRSA and 2 MSSA). We did not detect *agr* type IV. In total, 22 *agr* type I strains harbored the *sea* gene, whereas one *agr* type II (1/3) strain harbored the *sec* enterotoxin gene. All 4 *agr* type III strains harbored enterotoxin genes (*sea, seb*, or *sec*).

Molecular characterization of *S. aureus* strains: *Alu*I digestion of *coa*-positive PCR products yielded 29 different restriction profiles ($F = 0.18$–1.0). Fifteen strains could not be typed by *coa*-RFLP typing despite repeated attempts because no *coa* gene was amplified. A similar observation was reported by Sanjiv et al. (29), where 1 of 21 *S. aureus* strains studied did not produce any *coa* genes. Identical profiles were obtained in separate experiments using the same set of strains, indicating that this subtyping method is reproducible. Nine strains: 8 MRSA (MRSA16, MRSA17, MRSA23, MRSA27, MRSA12, MRSA33, MRSA14, and MRSA35) and one MSSA (MSSA17), shared identical PCR-RFLP *coa* profiles. These 9 strains were cultured from different years (2008–2010) and different sources, including swabs ($n = 4$), blood ($n = 1$), pus ($n = 2$), urine ($n = 1$), and unknown ($n = 1$) (Table 1). Similarly, 3 strains (2 MRSA and 1 MSSA) also shared an identical PCR-RFLP *coa* profile.

On the other hand, PFGE of *Sma*I-digested genomic DNA from 56 strains resulted in 38 distinct pulsed-field profiles (PFPs) ($F = 0.62$–1.0) comprising 10–16 restriction fragments. Based on 80% similarity in the PFGE profile analysis (10), we observed 4 clusters: Cluster 1–4 (Fig. 1). Cluster 1–3 consisted of both MRSA and MSSA strains. Eleven strains were not grouped in any of the clusters.

Two MRSA strains (MRSA16 and MRSA17) that had identical PCR-RFLP *coa* profiles were similarly indistinguishable by their PFPs, with both sharing 14 restriction fragments. Both strains were cultured from different patient wards (orthopedic and surgical) and different specimens (swab and pus). Four other MRSA strains (MRSA22, MRSA23, MRSA24, and MRSA25) indistinguishable by PFGE were distinguishable by *coa* PCR-RFLP because their PCR-RFLP *coa* profiles shared only 18% similarity (data not shown). Of these 4 MRSA strains (MRSA22 to MRSA25), 3 were cultured from swab samples and one from a pus sample, and all were isolated from 3 different wards.

The *spa* typing of the 56 *S. aureus* (35 MRSA and 21 MSSA) strains revealed 9 different *spa* types. The most prevalent *spa* type was t037 (82.1%). MLST was performed on representative strains for each *spa* types ($n = 9$). These 9 *spa* types were ST1 (CC1), ST7 (CC7), ST30 (CC30), ST239 (CC8), ST508 (CC45), ST772 (CC15), ST779 (CC97), ST1659 (CC15), and ST1179 (CC97).

The discriminatory power for *coa* PCR-RFLP, PFGE, *spa* typing, and MLST was 0.91, 0.98, 0.33, and 0.33, respectively.

Clonal characterization of MRSA strains: All 35 MRSA strains were grouped into 3 SCC*mec* types: type III (91.4%), type IV (2.9%), and type V (5.7%). PCR-RFLP of the *coa* gene yielded 19 subtypes; PFGE-*Sma*I produced 22 PFPs. Seven strains were not typeable by PCR-RFLP of the *coa* gene.

A combination of *spa* and MLST typing identified 2 different MLST-*spa* types: ST239-t037 ($n = 33$) and ST772-t657 ($n = 2$). The higher discriminatory power of *mec*-associated *dru* further differentiated strains within the ST2399-t037 type into 8 distinct *dru* types (dt13g, dt13d, dt14d, dt11a, dt10h, dt10a, dt11c, and dt14i), including one novel *dru* type (dt14i). ST772-t657 was associated only with dt10ao. The discriminatory power for *dru* typing was 0.79. Although 2 strains (MRSA31 and MRSA32) were from the same *spa* type (ST772-t657), SCC*mec* type (type V), and *dru* type (dt10ao), they could be distinguished based on their *coa* PCR-RFLP and PFGE profiles. Furthermore, MRSA31 belonged to *agr* type II, whereas MRSA32 was categorized as *agr* type I.

Clonal characterization of MSSA strains: The characterization of 21 MSSA strains by PFGE and PCR-RFLP of the *coa* gene resulted in 18 PFPs and 13 PCR-RFLP profiles, respectively. The combined analysis of both MLST and *spa* typing further differentiated these 21 MSSA strains into 8 combined types: ST239-t037 ($n = 13$), ST779-t878 ($n = 1$), ST1179-t267 ($n = 1$), ST1-t127 ($n = 2$), ST508-t550 ($n = 1$), ST7-t796 ($n = 1$), ST1659-t084 ($n = 1$), and ST30-t122 ($n = 1$). All 8 MLST types were from 8 different lineages (CC1, CC7, CC8, CC15, CC30, CC45, CC97, and CC779).

DISCUSSION

This report describes the virulotypes and genetic association of MRSA and MSSA isolated from the main tertiary hospital in the east coast state of Terengganu,

Table 1. Characterization of the 56 MRSA and MSSA strains from Terengganu, Malaysia, based on virulotypes, PCR-RFLP of *coa*, PFGE profiles, *agr*, SCC*mec*, *dru*, MLST, and *spa* types

Strain	Source	*spa* type	MLST type	*coa*-RFLP profile	*Sma*I-PFGE profile	*agr* type	SCC*mec* type	*dru* type	Virulence gene
MRSA									
MRSA1	blood	t037	ST239	C18	S3	I	III	dt13d	*sea, efb, fnbA*
MRSA2	swab	t037	ST239	C17	S3	I	III	dt13g	
MRSA3	sputum	t037	ST239	C21	S4	I	III	dt13g	*efb, fnbA*
MRSA4	swab	t037	ST239	C17	S12	I	III	dt13g	*sea, fnbA*
MRSA5	swab	t037	ST239	Untypeable	S13	I	III	dt13g	*sea, efb, fnbA*
MRSA6	swab	t037	ST239	C28	S14	I	III	dt13g	*sea, ica, efb*
MRSA7	tissue	t037	ST239	C15	S14	I	III	dt13g	*sea, efb*
MRSA8	tissue	t037	ST239	C24	S16	I	III	dt13g	*sea, efb, fnbA*
MRSA9	swab	t037	ST239	C6	S17	I	III	dt13g	*efb, fnbA, sea*
MRSA10	swab	t037	ST239	C1	S18	I	III	dt13g	*efb, fnbA*
MRSA11	blood	t037	ST239	Untypeable	S19	I	III	dt14d	
MRSA12	swab	t037	ST239	C8	S20	I	III	dt13g	*sea, efb, fnbA, sdrE*
MRSA13	blood	t037	ST239	C27	S22	I	III	dt13g	*sea, efb, fnbA, ica*
MRSA14	unknown	t037	ST239	C8	S22	I	III	dt13d	*sea, efb, ica*
MRSA15	swab	t037	ST239	Untypeable	S23	I	III	dt13d	*sea, efb*
MRSA16	swab	t037	ST239	C8	S24	I	III	dt13d	*sea, efb, fnbA, ica, sdrE*
MRSA17	pus	t037	ST239	C8	S24	I	III	dt13d	*sea, efb, ica*
MRSA18	swab	t037	ST239	C12	S24	I	III	dt13d	*sea, efb, ica*
MRSA19	blood	t037	ST239	C10	S24	I	III	dt13d	*sea, efb, fnbA, sdrE*
MRSA20	pus	t037	ST239	C21	S24	I	III	dt13d	*sea, efb, fnbA, ica*
MRSA21	blood	t037	ST239	C13	S25	I	III	dt13d	*sea, fnbA, ica*
MRSA22	pus	t037	ST239	C26	S26	I	III	dt14i	*efb, fnbA*
MRSA23	swab	t037	ST239	C8	S26	I	III	dt13g	*efb*
MRSA24	swab	t037	ST239	C23	S26	I	III	dt14i	*ica, sdrE*
MRSA25	swab	t037	ST239	Untypeable	S26	I	III	dt14i	*efb*
MRSA26	blood	t037	ST239	Untypeable	S27	I	III	dt10h	
MRSA27	swab	t037	ST239	C8	S28	I	III	dt14i	*efb, fnbA, ica*
MRSA28	pus	t037	ST239	Untypeable	S28	I	III	dt14i	*efb, ica*
MRSA29	tissue	t037	ST239	Untypeable	S28	I	III	dt14i	*efb, fnbA*
MRSA30	blood	t037	ST239	C16	S28	I	III	dt11a	
MRSA31	blood	t657	ST772	C9	S29	II	V	dt10ao	*sec*
MRSA32	blood	t657	ST772	C4	S30	I	V	dt10ao	*sec, fnbA*
MRSA33	pus	t037	ST239	C8	S31	I	III	dt13g	*sea, efb, fnbA*
MRSA34	blood	t037	ST239	C25	S33	I	IV	dt10a	*fnbA*
MRSA35	blood	t037	ST239	C8	S34	I	III	dt11c	
MSSA									
MSSA1	swab	t127	ST1	C11	S1	II	—	—	*efb, fnbA, ica*
MSSA2	swab	t037	ST239	C2	S2	III	—	—	*sea, efb, ica*
MSSA3	swab	t037	ST239	Untypeable	S4	III	—	—	*efb, fnbA, ica, sec*
MSSA4	swab	t550	ST508	C4	S5	I	—	—	*sea, fnbA, ica*
MSSA5	swab	t037	ST239	Untypeable	S5	I	—	—	*seb*
MSSA6	swab	t037	ST239	C14	S6	I	—	—	*sea, fnbA, ica*
MSSA7	swab	t796	ST7	C17	S7	I	—	—	*fnbA*
MSSA8	blood	t037	ST239	untypeable	S8	I	—	—	*sea, efb, fnbA, ica*
MSSA9	swab	t037	ST239	C3	S9	I	—	—	*efb*
MSSA10	pus	t037	ST239	C7	S9	I	—	—	*sea, efb, fnbA*
MSSA11	swab	t267	ST1179	C19	S10	I	—	—	*efb, ica*
MSSA12	swab	t037	ST239	C5	S11	I	—	—	*efb, fnbA, ica*
MSSA13	swab	t127	ST1	Untypeable	S15	III	—	—	*seb, sec, efb, fnbA, ica*
MSSA14	blood	t878	ST779	C20	S21	I	—	—	*fnbA*
MSSA15	swab	t122	ST30	Untypeable	S24	I	—	—	*fnbA*
MSSA16	pus	t037	ST239	Untypeable	S32	I	—	—	*pvl, ica*
MSSA17	urine	t037	ST239	C8	S35	I	—	—	*efb, fnbA, ica*
MSSA18	swab	t084	ST1659	Untypeable	S36	II	—	—	*efb, fnbA, ica*
MSSA19	swab	t037	ST239	Untypeable	S37	I	—	—	*fnbA, ica*
MSSA20	blood	t037	ST239	C22	S37	I	—	—	*fnbA, ica*
MSSA21	urine	t037	ST239	C29	S38	III	—	—	*sea, sec, fnbA*

—, negative result.

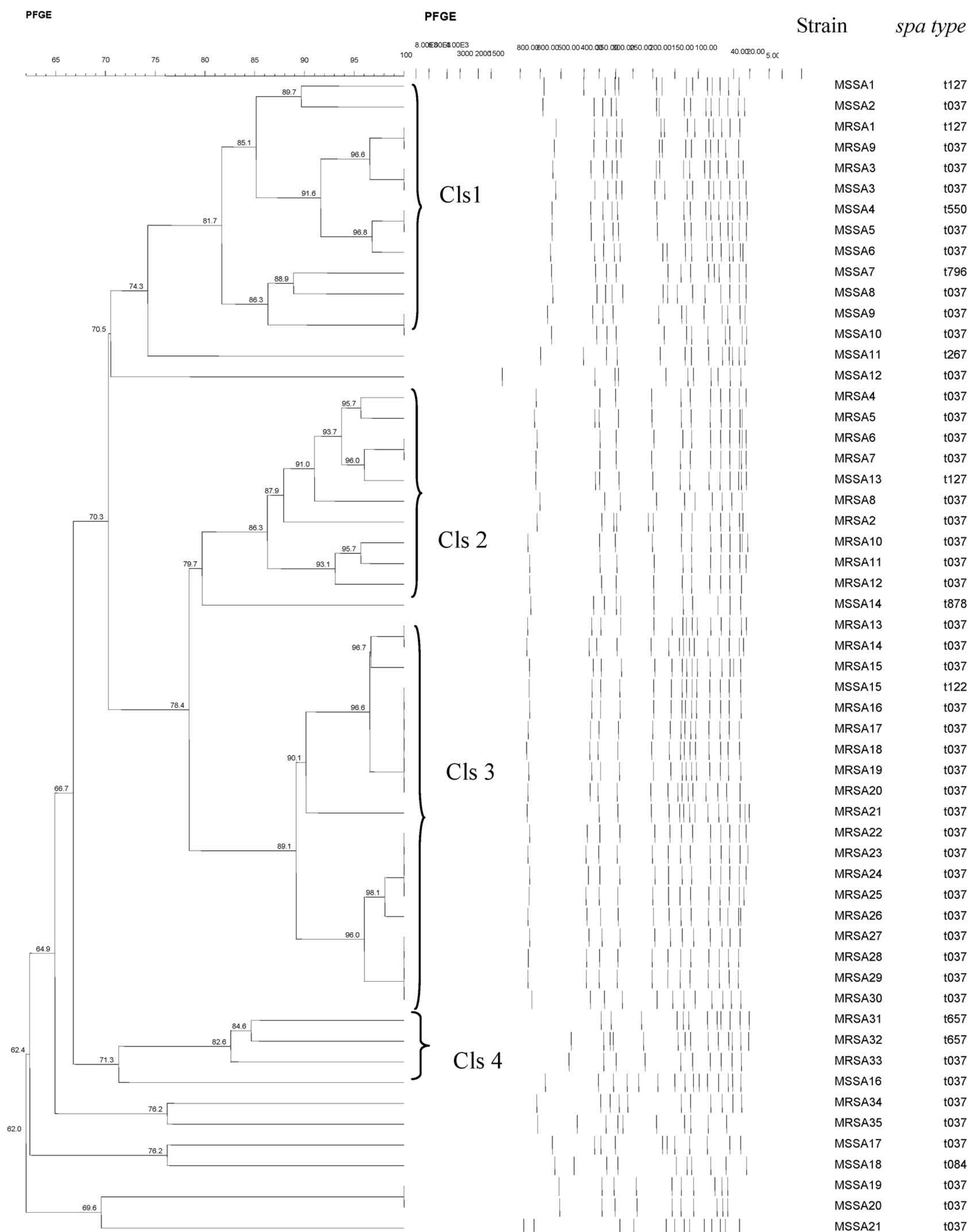

Fig. 1. Dendrogram of PFGE-*Sma*I profiles of the 56 MRSA and MSSA strains from Terengganu, Malaysia. Cls, Cluster.

Malaysia, from 2008 to 2010.

Overall, 57% of the MRSA strains and 43% of the MSSA strains harbored at least one type of enterotoxin (*sea, seb*, or *sec*) gene. This indicates that enterotoxin genes were more prevalent in MRSA than in MSSA strains in the Terengganu hospital. The presence of enterotoxin genes is often associated with food poisoning and staphylococcal purpura fulminans (4,30).

In total, 14 MSSA and 11 MRSA strains were positive for the *ica* gene, which is required for the formation of biofilms on host surfaces (31). Furthermore, Spearman's rank correlation tests indicated that MRSA and MSSA strains with the *ica* gene exhibited higher virulence potential because these strains also harbored the *sea*, *seb*, or *sec* enterotoxin genes. The possible association of *ica* with the enterotoxin (*sea*, *seb*, or *sec*) gene is a cause for concern because biofilm-associated bacteria are normally resistant to the host immune system, which can be further weakened by the presence of enterotoxin genes (32).

The *sea* gene was the most common enterotoxin gene present among the Terengganu strains (51% of MRSA strains and 29% of MSSA strains). This concurred with the recent report by Ghaznavi-Rad et al. (33), who found a much higher prevalence of the *sea* gene (86.6%) among 337 *S. aureus* isolates from a tertiary hospital in

Kuala Lumpur, the capital city of Malaysia. They also reported the presence of the sec, *seg*, *she*, and *sei* enterotoxin genes among the Kuala Lumpur *S. aureus* isolates, whereas we detected the *seb* and *sec* genes, but not the *seg*, *seh*, and *sei* genes in the Terengganu isolates.

The predominant *agr* type among the strains was *agr* type I, and this is consistent with the report of Ghaznavi-Rad et al. (33). This indicates that *agr* type I is predominant among MRSA strains isolated from the east and west coasts of Peninsular Malaysia. Although Collery et al. (34) reported that strains possessing the *tst* gene are often associated with *agr* type III, none of our *agr* type III strains harbored the *tst* gene. This is important because the *tst* gene encodes for toxic shock syndrome toxins, causing neonatal toxic shock syndrome-like exanthematous disease and staphylococcal purpura fulminans (30,35).

Some MRSA and MSSA strains shared similar *spa* (t037), MLST (ST239), and PCR-RFLP *coa* profiles (C8) although they were cultured from different years and sources. This indicates the persistence of particular MLST, *spa*, and PCR-RFLP *coa* genotypes in the hospital.

Cluster 1–3 of the PFGE analysis consisted of MRSA and MSSA strains that were cultured from different years and sources. MRSA1, which was isolated from a blood sample in 2010, could have evolved from MSSA1 (cultured from a swab sample in 2009) because both strains were clonally related as determined by PFGE, and shared similar *spa* and MLST types. On the other hand, MRSA3, which was cultured from sputum in 2010, could also have originated from MSSA3 (cultured from a swab sample in 2009) because both strains shared the same *spa*, MLST, and PFGE profiles. This further supports the notion of the circulation of particular clones in the hospital.

The MSSA strain (MSSA15) that shared similar PFGE profiles (S24) with 4 MRSA strains was cultured from different years (i.e., 2008, 2009, 2010) and sources (swab, pus, blood), and was associated with different *spa* types (t037 and t122). Both *spa* types (t037 and t122) were closely related because they shared 5 *spa*-type repeats succession (16-02-25-17-24), indicating that both MRSA and MSSA strains shared similar genetic properties. The 2 ST772-t657 MRSA strains were cultured just 3 days apart from blood samples from patients in the pediatric wards. Both strains were clonally related because they shared more than 80% similarity, suggesting the spread of a particular clone in the same patient wards.

The predominant SCC*mec* type was type III (91%). This is consistent with the results reported from 2 tertiary hospitals (i.e., Hospital Kuala Lumpur and University Malaya Medical Centre) in Kuala Lumpur (26,36). Similarly, SCC*mec* type III is also common in clinical *S. aureus* isolates in neighboring Southeast Asian countries (Singapore, Thailand, and Indonesia) and Taiwan (6,37).

The *dru* types dt10a and dt10ao were associated with SCC*mec* type IV and type V, respectively. The remaining 7 *dru* types (dt13d, dt13g, dt14d, dt14i, dt10h, dt11a, and dt11c) were found in SCC*mec* type III strains. Among the 7 *dru* types, 3 (dt13g, dt13d, and dt14d) were also detected in MRSA strains isolated from 2 tertiary hospitals in Kuala Lumpur (18,38). Three *dru* types (dt14i, dt10h, and dt11c), including one novel *dru* type (dt11c), have never been reported in Malaysia.

Genotyping of the *coa* gene by PCR-RFLP using *Alu*I and PFGE using *Sma*I showed that most of the MRSA strains were genetically related. Both *spa* and MLST typing also yielded less heterogeneity because only 2 different combined MLST and *spa* types were observed among the MRSA isolates (i.e., ST239-t037 and ST772-t657).

On the other hand, both PCR-RFLP of the *coa* gene and PFGE subtypes showed that most of the MSSA strains were genetically diverse and heterogeneous. Furthermore, the 21 MSSA strains were also grouped into 8 different MLST-*spa* types. This indicated that the MSSA strains were more genetically diverse than the MRSA strains, which is in agreement with other reports that MSSA strains were more diversely distributed and highly heterogeneous as compared with MRSA strains (17,39,40).

Some strains within PFGE Cluster 1 harbored different types of enterotoxin genes even though they were considered clonally related (their PFPs shared more than 80% similarity). This shows that *S. aureus* strains, regardless of whether they are resistant or sensitive to methicillin, are able to acquire or lose enterotoxin genes because these genes are likely located on mobile genetic elements such as pathogenicity islands, plasmids, and prophages (16).

MLST type ST239, which is a single-locus variant of ST8, remained the predominant clone in the Terengganu hospital and accounted for 82% of the strains, including 13 MSSA and 33 MRSA strains. This is similar to what has been reported in other tertiary hospitals in Malaysia (26,33), China (41), Germany (42), and Russia (15). This Brazilian/Hungarian MRSA clone (MLST ST239) has advantageous genetic properties that enhance the ability of biofilm formation, leading to the adherence and invasion of human airway cells (43). Thirty-two (96%) of the ST239 MRSA strains were SCC*mec* type III, inferring the possibility that ST239 MRSA strains in this hospital might have evolved from ST239 MSSA strains via acquisition of the SCC*mec* mobile element.

Although Ghaznavi-Rad et al. (33) reported that MLST type ST7 was only present among MRSA strains isolated from Kuala Lumpur, we detected MLST type ST7 among the Terengganu MSSA strains. This MLST type has also been reported in MSSA from Nigeria (44). MLST type ST1 (CC1) and ST508 (CC45) among clinical MSSA strains were also present in a tertiary hospital in Kuala Lumpur (34), whereas MLST types ST1179 and ST779 are new in Malaysia.

Strains of MLST type ST22 and SCC*mec* type IV have been isolated from 5 major referral hospitals in Malaysia (Hospital Kuala Lumpur and University Malaya Medical Centre, Kuala Lumpur; Selayang Hospital, Selangor; Queen Elizabeth Hospital, Sabah; and Kota Bharu Hospital, Kelantan) (26,45), but were absent in the Terengganu hospital.

In conclusion, enterotoxin genes were more prevalent in MRSA strains than MSSA strains isolated from the Terengganu tertiary hospital; *sea* was the predominant enterotoxin gene. The Brazilian/Hungarian ST239

clone, which is predominant in other tertiary hospitals throughout Malaysia, was also predominant in the Terengganu hospital. The Terengganu MSSA strains were genetically more diverse than the MRSA strains. PFGE is more discriminative than PCR-RFLP of the *coa* gene, *spa*, and MLST in the subtyping of both MRSA and MSSA strains.

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Conflict of interest None to declare.

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