JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 2010, p. 867–872 Vol. 48, No. 3
0095-1137/10/$12.00 doi:10.1128/JCM.01112-09
Copyright © 2010, American Society for Microbiology. All Rights Reserved.

Predominance and Emergence of Clones of Hospital-Acquired Methicillin-Resistant *Staphylococcus aureus* in Malaysia▽

Ehsanollah Ghaznavi-Rad,[1] Mariana Nor Shamsudin,[1] Zamberi Sekawi,[1] Liew Yun Khoon,[1] Mohammad Nazri Aziz,[4] Rukman Awang Hamat,[1] Norlijah Othman,[3] Pei Pei Chong,[2] Alex van Belkum,[5] Hamed Ghasemzadeh-Moghaddam,[1] and Vasanthakumari Neela[1]*

Department of Medical Microbiology and Parasitology,[1] Department of Biomedical Sciences,[2] and Department of Paediatrics,[3] Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Serdang 43400, Selangor, Malaysia; Pathology Laboratories, Hospital Kuala Lumpur, Jalan Pahang, 50586, Wilayah Persekutuan, Kuala Lumpur, Malaysia[4]; and Erasmus MC, Department of Medical Microbiology and Infectious Diseases, Gravendijkwal 230, 3015 CE Rotterdam, the Netherlands[5]

Received 8 June 2009/Returned for modification 1 September 2009/Accepted 18 December 2009

We define the epidemiology of predominant and sporadic methicillin-resistant *Staphylococcus aureus* (MRSA) strains in a central teaching and referral hospital in Kuala Lumpur, Malaysia. This is done on the basis of *spa* sequencing, multilocus sequence typing (MLST), staphylococcal cassette chromosome *mec* (SCC*mec*) typing, and virulence gene profiling. During the period of study, the MRSA prevalence was 44.1%, and 389 MRSA strains were included. The prevalence of MRSA was found to be significantly higher in the patients of Indian ethnicity ($P < 0.001$). The majority (92.5%) of the isolates belonged to ST-239, *spa* type t037, and possessed the type III or IIIA SCC*mec*. The arginine catabolic mobile element (ACME) *arcA* gene was detected in three (1.05%) ST-239 isolates. We report the first identification of ACME *arcA* gene-positive ST-239. Apart from this predominant clone, six (1.5%) isolates of ST-22, with two related *spa* types (t032 and t4184) and a singleton (t3213), carrying type IVh SCC*mec*, were detected for the first time in Asia. A limited number of community-acquired (CA) MRSA strains were also detected. These included ST-188/t189 (2.1%), ST-1/t127 (2.3%), and ST-7/t091 (1%). Panton-Valentin leukocidin (PVL) was detected in all ST-1 and ST-188 strains and in 0.7% of the ST-239 isolates. The majority of the isolates carried *agr* I, except that ST-1 strains were *agr* III positive. Virulence genes *seg* and *sei* were seen only among ST-22 isolates. In conclusion, current results revealed the predominance of ST-239-SCC*mec* III/IIIA and the penetration of ST-22 with different virulence gene profiles. The emergence in Malaysia of novel clones of known epidemic and pathogenic potential should be taken seriously.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an established human pathogen that causes both health care-associated (HA) and community-acquired (CA) infections. Modern MRSA has evolved from several successful clonal lineages of methicillin-susceptible *S. aureus* strains via acquisition of a mobile genetic element called staphylococcal cassette chromosome *mec* (SCC*mec*). This element contains the *mecA* gene, which encodes penicillin-binding protein 2′ (PBP2′) with significantly reduced affinity for β-lactams (36).

Increased emergence of multidrug resistance among MRSA strains has become a major concern in the hospital environment, as it invokes a tremendous financial burden and enhanced morbidity and mortality due to hard-to-treat systemic infections (7). Genotyping data from large international studies have shown that a limited number of major clones of MRSA display an enhanced propensity to spread and cause opportunistic human infections in various parts of the world (5, 13, 33). MRSA was introduced into Malaysia in the early 1970s (26), and a review of the records of the microbiology laboratories of all state hospitals in Malaysia showed that the proportion of MRSA isolates from *S. aureus*-infected individuals had been approximately 21% throughout the last few years.

Phenotypic MRSA typing methods are not suited to detailed epidemiological surveillance (20, 40). Several superior molecular typing methods can be used for supporting infection control and for tracing the nosocomial sources and transmission routes of bacterial pathogens. Among these methods, multilocus sequence typing (MLST) has recently been proven to be the best for long-term global epidemiological and bacterial population genetics studies. It is a discriminatory genotypic method where isolates are typed by sequencing variable regions of housekeeping genes. A combination of alleles from the seven loci forms a sequence type (ST). Similar STs are grouped into clonal complexes (13). *spa* typing has been shown to be as discriminatory as the current gold standard method, pulsed-field gel electrophoresis (PFGE) (10). *spa* types can be determined via amplification and sequencing of the X region of the protein A gene (*spa*), which contains polymorphic direct repeats. StaphType (version 1.4; Ridom GmbH, Würzburg, Germany) provides a software tool enabling straightforward sequence analysis and designation of *spa* types by synchronization via a central server (42). *spa* typing is useful in analyzing hospital outbreaks and in identifying genetic changes that occur over a relatively short time span (14, 43). Molecular typing data on HA MRSA isolates in Malaysia are sparse in compar-

* Corresponding author. Mailing address: Department of Medical Microbiology and Parasitology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400, Serdang, Selangor, Malaysia. Phone: 0060-3-89472507. Fax: 0060-3-89413802. E-mail: neela2000@hotmail.com.

▽ Published ahead of print on 20 January 2010.

ison with those on strains deriving from Europe, the United States, or Japan. This is notwithstanding the steadily increasing but already high rates of MRSA infections in Malaysia (38). Pilot data hinted at the predominance of a single MRSA genotype in Malaysia. This is similar to the situation in many other Asian countries (1, 23, 31).

For the present study, clinical MRSA isolates associated with various infections were collected from the largest public tertiary referral hospital in Kuala Lumpur, the capital of Malaysia. The epidemiology of MRSA in this hospital (HKL) will most likely reflect the nation's epidemiology as it is the major government referral hospital for patients from all states in Malaysia. Each year at least 1 million people (~3.8% of the total Malaysian population) are treated here. HKL records an annual MRSA prevalence over 40%. Many of these MRSA strains are multidrug resistant. Additional epidemiological studies are clearly warranted in order to increase the insight into the dynamics of MRSA epidemiology in Malaysia.

The aims of the present study were to characterize the current Malaysian MRSA isolates and determine their molecular epidemiology by MLST, *spa* typing, SCC*mec* typing, and virulence gene profiling.

MATERIALS AND METHODS

Clinical setting and bacterial strains. Hospital Kuala Lumpur (HKL) is the largest government tertiary referral hospital, with 81 wards and 2,502 beds. It is located in Kuala Lumpur. An average of 40% MRSA prevalence is recorded annually in this hospital. For conducting an adequate statistical analyses, taking into account the prevalence of MRSA in this hospital, the sample size (SS) was determined using the equation $SS = Z^2 \times p \times (1 - p)/c^2$, where Z is the statistic for the level of confidence (for a level of confidence of 95% or a 5% type I error, which is conventional, Z is 1.96), p is the estimated proportion of an attribute that is present in the population (in the present study, $p = 0.468$), and c is the desired level of precision, which is 0.05 (8, 25).

MRSA samples used in the current study were collected from October 2007 to September 2008. Out of a total number of 1,887 MRSA strains, a random selection of 389 strains was investigated in molecular detail. Thirty-two nonrepetitive MRSA samples per month (approximately eight per week) were collected from clinical samples obtained from hospitalized patients. The first 8 isolates (irrespective of the ward) per week were collected. Demographic and culture data such as age, gender, ethnicity, ward, site of isolation, and type of sample were all recorded. All isolates previously identified to the species level by the hospital laboratory were reconfirmed in the microbiology laboratory at Universiti Putra Malaysia by standard methods such as Gram staining, catalase testing, tube coagulation, mannitol testing, and MRSA screen latex agglutination testing (Denka Seiken Co., Ltd., Tokyo, Japan). Isolates were confirmed as MRSA by oxacillin and cefoxitin susceptibility testing according to the CLSI guidelines (6). All isolates were also reevaluated for the presence of the *mecA* gene by PCR (32). Confirmed MRSA isolates were then stored at −80°C in Luria-Bertani broth supplemented with 20% glycerol awaiting further investigation.

SCC*mec* typing, *spa* typing, and MLST. Chromosomal DNA was extracted using the DNeasy kit (Qiagen Inc.) according to the manufacturer's instructions. SCC*mec* typing was carried out according to the protocol described by Zhang et al. (50). Confirmation of SCC*mec* type V and subtyping of type IV were done based on the variation in the *ccrC* and *ccrA2B2* genes, as recently described by Kondo et al. (24) and Milheiriço et al. (29).

All MRSA isolates, irrespective of their SCC*mec* types, were subjected to *spa* typing according to the method of Shopsin et al. (41) using the primers SpaF (AGACGATCCTTCGGTGA) and SpaR (CAGCAGTAGTGCCGTTTG). The amplified *spa* gene fragments were purified and sequenced. *spa* types were assigned by using StaphType software (version 1.4; Ridom GmbH, Würzburg, Germany), as described by Harmsen et al. (16). By application of the BURP (based upon repeat pattern) algorithm, *spa* types with more than five repeats were clustered into distinct groups.

MLST was conducted for representatives of each *spa* lineage, including the new and uncommon ones, as described previously (13). Twenty-six selected strains were processed for MLST. Sequence types (STs) and clonal clusters (CCs) were assigned using the *S. aureus* MLST database (www.mlst.net) hosted by Imperial College in London, United Kingdom.

PCR-based assays for virulence factors of *S. aureus*. Four different categories of virulence factors were screened. These included cytolytic toxin genes such as *pvl* (28); superantigen genes including *sea*, *seb*, *sec*, *sed*, *see*, *seg*, *seh*, *sei*, and *tsst* (21); exotoxin genes such as *eta* and *etb* (26); genes encoding adhesions such as *cna* (2), *fnbA* (51), *ica*, and *icaD* (37); and the newly identified arginine catabolic mobile element (ACME) gene cluster, which is involved in bacterial growth and development (4). Multiplex PCR-based protocols for allotyping *agr* classes I to IV were performed as described elsewhere (27).

Statistical analysis. Statistical analyses were made with SPSS version 14 software (SSPS, Inc., Chicago, IL). Categorical variables between two groups were compared by means of chi-square or Fisher exact tests. The latter was done if 20% of the expected values were smaller than 5. A P value of <0.05 indicated statistical significance. A goodness-of-fit test was used to test the prevalence of MRSA strains among different ethnic groups in Malaysia. The model used for the test is the Malaysian population statistics (http://www.statistics.gov.my), which shows that 65%, 26%, 7.7%, and 1.3% of the Malaysian population are Malay (bumiputras), Chinese, Indians, and others, respectively.

TABLE 1. Distribution of MRSA isolates based on the ward of isolation

Ward	No. (%) of MRSA isolates characterized in the present study	Total no. (%) of MRSA isolates during the study period
General medicine	97 (24.9)	378 (20.1)
Pediatrics	30 (7.7)	174 (9.2)
General surgery	54 (13.9)	212 (11.2)
Urology/nephrology	60 (15.4)	297 (15.7)
Neurosurgery	51 (13.1)	286 (15.2)
Orthopedic surgery	68 (17.5)	243 (12.8)
Maternity	2 (0.5)	15 (0.8)
ICU[a]	27 (6.9)	94 (5.1)
Unknown		188 (9.9)
Total	389 (100)	1,887 (100)

[a] ICU, intensive care unit.

RESULTS

Demographical characterization of MRSA strains. From October 2007 to September 2008, 44.1% (n = 1,887) of all *S. aureus* strains (n = 4,280) causing infections in the hospital were identified as MRSA and 389 of them were collected for molecular investigation. The frequencies of MRSA isolates obtained from various wards and different type of specimens are summarized in Table 1 and Fig. 1.

All patients were divided into four groups according to age. The infant group ages ranged from 1 to 11 months (9 subjects, 2.3%), the child group ages ranged from 1 to 15 years (31 subjects, 8%), the adult group ages ranged from 16 to 65 years (271 subjects, 69.7%), and the elderly were more than 65 years old (78 subjects, 20.1%). The median age of patients was 47.8 years, and the age range was 4 days to 88 years (standard deviation [SD], ±21.1 years). The male-to-female patient ratio was 61.2:38.8, which showed no significance ($\chi^2 = 0.282$) for gender relatedness of MRSA infection. The highest prevalence of MRSA was seen in the adult category. When data were stratified according to the three major ethnic groups in Malaysia, it was found that MRSA was isolated from 207 (53.2%) Malays, 88 (22.6%) Chinese, and 94 (24.2%) Indians. The prevalence of MRSA infection among Indians was found to be significantly elevated (goodness $\chi^2 = 88.09$, $P < 0.001$).

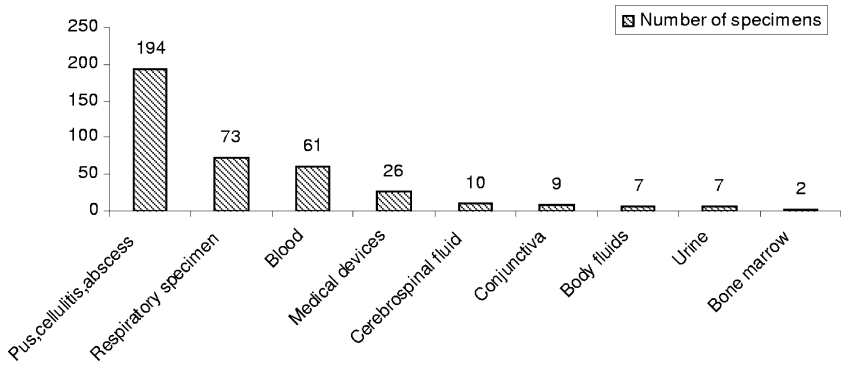

FIG. 1. Number of MRSA strains isolated from different types of specimens: pus, cellulites, and abscess, referred as wound and soft-tissue infections; respiratory specimens (pulmonary secretion, sputum, and tracheal aspirate); medical devices (central nervous system tip and catheter); cerebrospinal fluid (CSF); conjunctiva; body fluids (peritonea and synovial); and bone marrow.

SCC*mec* typing, *spa* typing, and MLST. SCC*mec* typing identified that the vast majority (92.8%) of isolates carried either SCC*mec* type III (n = 78 or 20%) or its variant IIIA (n = 283 or 72.8%), while 22 (5.7%) and 6 (1.5%) isolates harbored SCC*mec* types V and IVh, respectively.

spa typing of the 389 isolates revealed 13 different *spa* types. Repeats among the *spa* types varied from 4 (t4150) to 17 (t4184). The majority (73.5%) of the isolates belonged to t037. The BURP algorithm was used to group all isolates into definite clonal lineages on the basis of sequence relatedness. Since clustering parameters excluded *spa* types shorter than five repeats, one *spa* type (t4150) was excluded from BURP grouping. The phylogenetic tree produced two different groups, and 4 other *spa* types were singletons. Group 1 contained 6 *spa* types, with t037 as the founder, while group 2 contained 2 *spa* types (t032 and t4184), with t032 as the founder. Among the 13 *spa* types 3 new *spa* types were identified and deposited in the *spa* database. Two of these new types clustered with t037, while one resembled t032.

MLST (60 isolates) was performed on all isolates belonging to the different *spa* types except for the two predominant ones (t037 and t421), for which 10 and 6 isolates, respectively, were MLST typed. This identified six STs, ST-239 (CC8) (n = 360; 92.5%), ST-1 (CC1) (n = 9; 2.3%), ST-188 (CC1) (n = 8; 2.1%), ST-22 (CC22) (n = 6; 1.5%), ST-7 (CC7) (n = 4; 1%), and ST-1283 (CC8), confirming the existence of six distinct clones among the 389 MRSA isolates. Application of the eBURST algorithm to the six STs clustered both ST-239 and ST-1283 in CC8. ST-1283 was described for the first time in this study and deposited at the MLST database. This ST was recovered from a cerebrospinal fluid sample and is a single-locus variant (SLV) of ST-239, differing from this genotype by a single synonymous point mutation in the gene encoding guanylate kinase (*gmk*). The other four STs and CCs were ST-1 (CC1), ST-22 (CC22), ST-188 (CC1), and ST-7 (CC7). ST-188 is closely related to ST-1, with differences only in the two loci *arcC* (carbamate kinase gene) and *gmk* (http://saureus.mlst.net/). Figure 2 illustrates the molecular characterization of strains representing the different SCC*mec* types, *spa* types, and STs.

Toxin gene profile. All MRSA strains irrespective of STs and *spa* types were examined for the presence of several virulence factors (Table 2). All strains were commonly positive for adhesin genes such as the fibronectin binding protein gene (*fnbA*) and the biofilm-related genes *icaA* and *icaD*, while the collagen adhesin gene (*cna*) was amplified in 384 (98.7%) strains. Among the superantigen genes, *sea* was the most prevalent one and was detected in 337 (86.6%) strains (ST-239, -1, -188, and -7), followed by *seh*, detected in 9 strains (ST-1), while *sec* was found in 4 strains (ST-22). *seg* and *sei* genes were demonstrated only in ST-22 isolates. None of the other superantigenic toxin genes such as *seb*, *sed*, and *tsst* were observed in the strains investigated. The *pvl* gene was amplified in 19 (4.9%) strains (0.7% of ST-239 strains and 100% of ST-1 and ST-188 strains). Three ST-239 strains (1.1%) harbored the ACME-associated *arcA* gene, two from wounds and one from a respiratory infection. Analyzing the relationship between the toxin genes and the STs by χ^2 test revealed that there is an association between MLST and certain virulence genes: *sea* and ST-239 are correlated ($\chi^2 = 54.84$, $P < 0.001$) and ST-22 and *sec*, *seg*, and *sei* are also correlated ($\chi^2 = 389$, $P < 0.001$), whereas the association between ST-188, ST-1, and *pvl* ($\chi^2 = 389$, $P < 0.001$) is clearly significant. *agr* typing grouped the majority of the isolates in the *agr* I category, except that ST-1 strains were of the *agr* III class.

DISCUSSION

In agreement with earlier reports, the prevalence of MRSA in the study hospital did not vary by gender (22). However, the MRSA infection rates are significantly different between ethnic groups and the rate is clearly elevated in the Indian population. A similar observation was reported for CA MRSA in African-American children (39).

The highest rate of MRSA, as reported from our general medicine ward, is in the dermatology ward, with a high rate of soft-tissue infection. The short hospital stay and adequate antibiotic therapy in otherwise healthy patients in the maternity ward explain the low prevalence in that ward (31, 46).

Several international epidemiological investigations demonstrated that most hospital-acquired MRSA infections are caused by a relatively small number of epidemic MRSA clones spread worldwide. The most frequently encountered clones are the Iberian (ST-247-IA), Brazilian/Hungarian (ST-239-III),

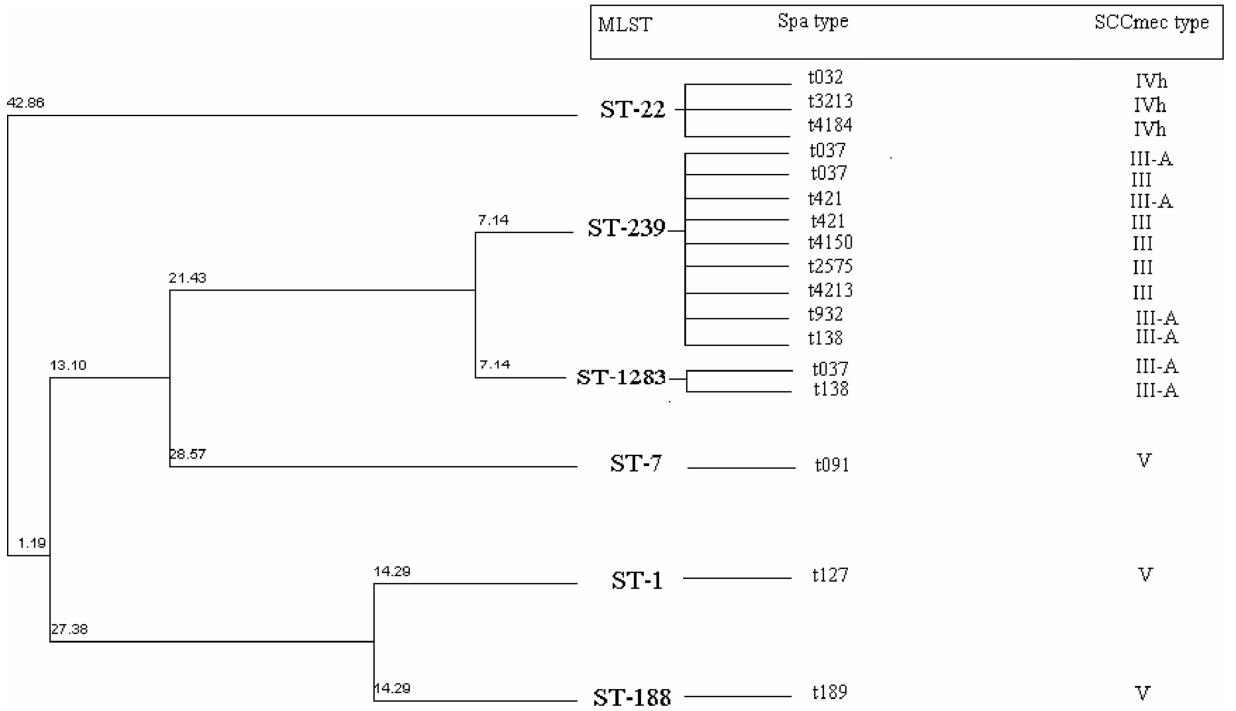

FIG. 2. Phylogenic tree of MRSA strains included in the present study showing the combination of data from *spa* typing, MLST, and SCC*mec* typing. The tree is a dendrogram based on the unweighted pair group method using average linkages (UPGMA) and constructed based on the pairwise differences in the MLST allelic profiles. The numbers above the row lines demonstrate the genetic distances between the strains: two isolates that differ at one of seven loci differ at a distance of 14.3% (1/7). The diagram shows distances along each branch as 7.1%, half that of the single-locus difference. In this way the distance between ST-239 and ST-1283, defined by a single locus, is 7.1% whereas for ST-1 and ST-188, with two different loci, the distance is again 14.3%.

Berlin (ST-45-IV), New York/Japan (ST-5-II), pediatric (ST-5-IV), EMRSA-15 (ST-22-IV), and EMRSA-16 (ST-36-II) clones (33). We here show the high endemicity of a limited number of clones including ST-239 (CC8), which is the predominant Malaysian clone. This clone usually is SCC*mec* type III/IIIA and *spa* type t037, which is dominant in most other Asian countries except South Korea and Japan (23). Six *spa* types were obtained for ST-239, which shows that there are at least 6 subtypes circulating in a single hospital. Surprisingly, ST-22 (CC22) of SCC*mec* type IVh, *spa* t032, seems to have recently penetrated Malaysia.

We present the first cases of infection by ST-22, SCC*mec*

TABLE 2. Distribution of different virulence factors among MRSA isolates

No. of isolates	MLST	*spa* type[a]	No. (%) of isolates carrying:									*agr* type
			ACME *arcA*	*sea*	*seh*	*sec*, *seg*, and *sei*	*pvl*	*icaA*	*icaD*	*fnbA*	*cna*	
284	ST-239	t037 (10)	3 (1.05)	243 (85.5)			2 (0.7)	284 (100)	284 (100)	284 (100)	284 (100)	I
1	ST-1283	t037 (1)		1 (100)				1 (100)	1 (100)	1 (100)	1 (100)	I
61	ST-239	t421 (6)		59 (96.7)				61 (100)	61 (100)	61 (100)	61 (100)	I
5	ST-239	t4213 (5)		5 (100)				5 (100)	5 (100)	5 (100)	5 (100)	I
4	ST-239	t4150 (4)		4 (100)				4 (100)	4 (100)	4 (100)	4 (100)	I
1	ST-239	t138 (1)		1 (100)				1 (100)	1 (100)	1 (100)	1 (100)	I
1	ST-1283	t138 (1)		1 (100)				1 (100)	1 (100)	1 (100)	1 (100)	I
1	ST-239	t932 (1)		1 (100)				1 (100)	1 (100)	1 (100)	1 (100)	I
1	ST-239	t2575 (1)		1 (100)				1 (100)	1 (100)	1 (100)	1 (100)	I
4	ST-22	t032 (4)				4 (100)		4 (100)	4 (100)	4 (100)	4 (100)	I
1	ST-22	t4184 (1)				1 (100)		1 (100)	1 (100)	1 (100)	1 (100)	I
1	ST-22	t3213 (1)		1 (100)		1 (100)		1 (100)	1 (100)	1 (100)	1 (100)	
9	ST-1	t127 (9)		9 (100)	9 (100)		9 (10)	9 (100)	9 (100)	9 (100)	9 (100)	III
8	ST-188	t189 (8)		8 (100)			8 (100)	8 (100)	8 (100)	8 (100)	8 (100)	I
4	ST-7	t091 (4)		4 (100)				4 (100)	4 (100)	4 (100)		I
3	ST-239	NT[b] (3)		3 (100)				3 (100)	3 (100)	3 (100)	3 (100)	I

[a] The numbers in parentheses indicate the numbers of isolates that were typed by MLST for that particular *spa* type.
[b] NT, nontypeable.

type IVh, with three different *spa* types (t932, t3213, and t4184) in Malaysia. These strains were mainly isolated from blood (66.6%) or skin and soft-tissue infection (33.3%). SCC*mec* IVh, present in a recently described specific subtype of EMRSA-15 (ST-22), was reported for the first time in Portugal (29) but shortly after from places as distant as Sweden (3) and lately from Spain (48). The SCC*mec* IV element is small in comparison to elements such as SCC*mec* type III; the small size enables easier spread among *S. aureus* strains. As a possible consequence of its enhanced mobility, it is also more variable than other SCC*mec* types. The detection of ST-22 (which is one of the predominant MRSA clones in Europe, Australia, and New Zealand [40]) in Singapore (18) and later in Malaysia strengthens the case for the epidemic nature of this clone and also suggests that it may easily spread to other Southeast Asian countries.

S. aureus produces a wide variety of exoproteins that contribute to its ability to colonize and cause disease in mammalian hosts. The majority of the virulence factors produced in *S. aureus* are under the control of the *agr* system. Although *agr* II and III are found in some well known clones (pediatric, New York/Japan, and EMRSA-16) (15, 21), the most predominant *agr* group among worldwide MRSA clones is *agr* group I (47). A similar situation was observed in our isolates, where 96.7% of the isolates were *agr* I while only 3.3% carried *agr* III. Our results illustrate that the *pvl* locus is more common (80.9%) among CA MRSA strains that belonged to ST-1- SCC*mec* V-*agr* III and ST-188-SCC*mec* V-*agr* I, while a small percentage (0.7%) were seen among the ST-239-SCC*mec* III and III-A-*agr* I isolates. *pvl*-positive ST-1-SCC*mec* IV-*agr* III isolates were first reported in the United States, but strains recently detected from Europe and Asia are of *agr* type I or II (44). Recently, ST-1 and ST-188 MRSA strains with type V SCC*mec* were reported from Singapore (17, 19). Furthermore, *pvl*-positive HA MRSA isolates harboring SCC*mec* type III were found in Algeria (34) and China (49). In general, *pvl* is carried in less than 5% of the MRSA strains that harbor SCC*mec* types I to III (9).

The current study for the first time reports the presence of the ACME *arcA* gene in ST-239 SCC*mec* type III MRSA isolates. ACME *arcA*, which contributes to growth and survival by encoding resistance and virulence determinants that enhance fitness and pathogenicity, was first detected in USA300 (ST-8) (4, 11).

Exotoxins with superantigenic properties are responsible for strong inflammatory responses that contribute to the severity of staphylococcal infections. Regardless of the STs, most (86.6%) of the isolates studied carried the *sea* gene. Our results are in accordance with those obtained for Russian isolates, where 88% of the MRSA strains from clinical samples were *sea* positive (12). Although in previous studies *sea* was not detected in the Brazilian clone in general (45), it was found in our isolates which matched the Brazilian and Hungarian clones in MLST and SCC*mec* type. Enterotoxin H was found to be present only among the Malaysian ST1 isolates. Enterotoxin H has a particularly high affinity for binding to the major histocompatibility complex type II molecules and is usually reported in CA MRSA strains (35). Among all isolates, only the ST-22 clone shared the enterotoxin gene cluster locus (*egc*), comprising five superantigenic enterotoxin genes (*seg*, *sei*, *sem*, *sen*, and *seo*). The combination of classical (enterotoxin C-positive) (21) and variant (enterotoxin C-negative) strains (30) among our isolates reconfirms that diverse ST-22 strains have penetrated our hospital. Despite of high percentages of wound and soft-tissue infections, exfoliative toxin genes (*eta* and *etb*) were not detected in any of our strains, indicating the role of other virulence factors involved in establishing the infections.

In conclusion, this study identified three main types of MRSA in HKL. The majority of strains are ST-239, which is predominant in several other Asian countries as well. ST-22, first described in the United Kingdom and later in other European countries, Australia, New Zealand, and Singapore, has now penetrated Malaysia, and this emphasizes the epidemic nature of strains with this ST. In addition, minor groups of CA MRSA strains detected in the current study indicate the ability of such strains to settle in the hospital environment. The combination of ST-1-SCC*mec* V-*agr* III and ST-188-SCC*mec* V-*agr* I was commonly detected among CA MRSA strains. This is the first study to report the ACME *arcA* gene in ST-239-SCC*mec* type III MRSA. The presence of a series of virulence determinants in the local MRSA strains suggests the ability of these strains to cause a broad spectrum of infectious diseases under favorable conditions.

ACKNOWLEDGMENTS

We are grateful to Teruyo Ito (Juntendo University) for providing reference SCC*mec* type strains and confirming the SCC*mec* type IVh MRSA isolates. We express our special thanks to Dag Harmsen (University Hospital Munster) and Alexander Friedrich for helping us to implement BURP analysis for *spa* types.

We take this opportunity to thank Hospital Kuala Lumpur for kindly providing all of the MRSA isolates. This work was supported by the Ministry of Science, Technology and Innovation Malaysia (MOSTI) Science Fund with grant numbers 06-01-04-SF0885 and 02-01-04-SF0853.

REFERENCES

1. **Aires de Sousa, M., M. I. Crisóstomo, I. S. Sanches, J. S. Wu, J. Fuzhong, A. Tomasz, and H. de Lencastre.** 2003. Frequent recovery of a single clonal type of multidrug-resistant *Staphylococcus aureus* from patients in two hospitals in Taiwan and China. J. Clin. Microbiol. **41:**159–163.
2. **Arciola, C. R., D. Campoccia, S. Gamberini, L. Baldassarri, and L. Montanaro.** 2005. Prevalence of cna, fnbA and fnbB adhesin genes among Staphylococcus aureus isolates from orthopedic infections associated to different types of implant. FEMS Microbiol. Lett. **246:**81–86.
3. **Berglund, C., T. Ito, X. X. Ma, M. Ikeda, S. Watanabe, B. Söderquist, and K. Hiramatsu.** 2009. Genetic diversity of methicillin-resistant Staphylococcus aureus carrying type IV SCCmec in Orebro County and the western region of Sweden. J. Antimicrob. Chemother. **63:**32–41.
4. **Campbell, S., H. Deshmukh, C. Nelson, I. Bae, M. Stryjewski, J. Federspiel, G. Tonthat, T. Rude, S. Barriere, R. Corey, and V. J. Fowler.** 2008. Genotypic characteristics of *Staphylococcus aureus* isolates from a multinational trial of complicated skin and skin structure infections. J. Clin. Microbiol. **46:**678–684.
5. **Chung, M., G. Dickinson, H. De Lencastre, and A. Tomasz.** 2004. International clones of methicillin-resistant *Staphylococcus aureus* in two hospitals in Miami, Florida. J. Clin. Microbiol. **42:**542–547.
6. **Clinical and Laboratory Standards Institute.** 2007. Performance standards for antimicrobial susceptibility testing; 17th informational supplement. CLSI M100-S17. Clinical and Laboratory Standards Institute, Wayne, PA.
7. **Cosgrove, S. E., G. Sakoulas, E. N. Perencevich, M. J. Schwaber, A. W. Karchmer, and Y. Carmeli.** 2003. Comparison of mortality associated with methicillin-resistant and methicillin-susceptible Staphylococcus aureus bacteremia: a meta-analysis. Clin. Infect. Dis. **36:**53–59.
8. **Daniel, W.** 1999. Biostatistics: a foundation for analysis in the health sciences. John Wiley & Sons, New York, NY.
9. **Deresinski, S.** 2005. Methicillin-resistant *Staphylococcus aureus*: an evolutionary, epidemiologic, and therapeutic odyssey. Clin. Infect. Dis. **40:**562–573.
10. **Deurenberg, R. H., and E. E. Stobberingh.** 2008. The evolution of Staphylococcus aureus. Infect. Genet. Evol. **8:**747–763.

11. **Diep, B. A., S. R. Gill, R. F. Chang, T. H. Phan, J. H. Chen, M. G. Davidson, F. Lin, J. Lin, H. A. Carleton, E. F. Mongodin, G. F. Sensabaugh, and F. Perdreau-Remington.** 2006. Complete genome sequence of USA300, an epidemic clone of community-acquired meticillin-resistant *Staphylococcus aureus*. Lancet **367:**731–739.
12. **Dmitrenko, O. A., V. Prokhorov, F. S. Fluer, T. N. Suborova, I. I. Volkov, V. I. Karabak, and A. L. Gintsburg.** 2006. Detection of the genes of pyrogenic toxins of superantigens in clinical isolates of methicillin resistant Staphylococcus aureus. Zh. Mikrobiol. Epidemiol. Immunobiol. **2:**36–42.
13. **Enright, M. C., N. P. Day, C. E. Davies, S. J. Peacock, and B. G. Spratt.** 2000. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. J. Clin. Microbiol. **38:**1008–1015.
14. **Faria, N. A., J. A. Carrico, D. C. Oliveira, M. Ramirez, and H. de Lencastre.** 2008. Analysis of typing methods for epidemiological surveillance of both methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* strains. J. Clin. Microbiol. **46:**136–144.
15. **Gomes, A. R., S. Vinga, M. Zavolan, and H. de Lencastre.** 2005. Analysis of the genetic variability of virulence-related loci in epidemic clones of methicillin-resistant *Staphylococcus aureus*. Antimicrob. Agents Chemother. **49:** 366–379.
16. **Harmsen, D., H. Claus, W. Witte, J. Rothgänger, H. Claus, D. Turnwald, and U. Vogel.** 2003. Typing of methicillin-resistant *Staphylococcus aureus* in a university hospital setting by using novel software for *spa* repeat determination and database management. J. Clin. Microbiol. **41:**5442–5448.
17. **Hsu, L. Y., Y. L. Koh, N. L. Chlebicka, T. Y. Tan, P. Krishnan, R. T. Lin, N. Tee, T. Barkham, and T. H. Koh.** 2006. Establishment of ST30 as the predominant clonal type among community-associated methicillin-resistant *Staphylococcus aureus* isolates in Singapore. J. Clin. Microbiol. **44:**1090–1093.
18. **Hsu, L. Y., T. H. Koh, K. Singh, M. L. Kang, A. Kurup, and B. H. Tan.** 2005. Dissemination of multisusceptible methicillin-resistant *Staphylococcus aureus* in Singapore. J. Clin. Microbiol. **43:**2923–2925.
19. **Hsu, L. Y., T. H. Koh, T. Y. Tan, T. Ito, X. X. Ma, R. T. Lin, and B. H. Tan.** 2006. Emergence of community-associated methicillin-resistant *Staphylococcus aureus* in Singapore: a further six cases. Singapore Med. J. **47:**20–26.
20. **Ip, M., D. J. Lyon, F. Chio, M. C. Enright, and A. F. Cheng.** 2003. Characterization of isolates of methicillin-resistant *Staphylococcus aureus* from Hong Kong by phage typing, pulsed-field gel electrophoresis, and fluorescent amplified-fragment length polymorphism analysis. J. Clin. Microbiol. **41:** 4980–4985.
21. **Jarraud, S., G. Cozon, F. Vandenesch, M. Bes, J. Etienne, and G. Lina.** 1999. Involvement of enterotoxins G and I in staphylococcal toxic shock syndrome and staphylococcal scarlet fever. J. Clin. Microbiol. **37:**2446–2449.
22. **Kairam, N., M. E. Silverman, D. F. Salo, E. Baorto, B. Lee, and C. S. Amato.** 8 July 2009, posting date. Cutaneous methicillin-resistant Staphylococcus aureus in a suburban community hospital pediatric emergency department. J. Emerg. Med. [Epub ahead of print.] doi:10.1016/j.jemermed.2009.05.015.
23. **Ko, K. S., J. Y. Lee, J. Y. Suh, W. S. Oh, K. R. Peck, N. Y. Lee, and J. H. Song.** 2005. Distribution of major genotypes among methicillin-resistant *Staphylococcus aureus* clones in Asian countries. J. Clin. Microbiol. **43:**421–426.
24. **Kondo, Y., T. Ito, X. X. Ma, S. Watanabe, B. N. Kreiswirth, J. Etienne, and K. Hiramatsu.** 2007. Combination of multiplex PCRs for staphylococcal cassette chromosome *mec* type assignment: rapid identification system for *mec*, *ccr*, and major differences in junkyard regions. Antimicrob. Agents Chemother. **51:**264–274.
25. **Lawanga, S. K., and S. Lemeshow.** 1991. Sample size determination in health studies: a practical manual. WHO, Geneva, Switzerland.
26. **Lim, V. K., and H. I. Zulkifli.** 1987. Methicillin resistant Staphylococcus aureus in a Malaysian neonatal unit. Singapore Med. J. **28:**176–179.
27. **Lina, G., F. Boutite, A. Tristan, M. Bes, J. Etienne, and F. Vandenesch.** 2003. Bacterial competition for human nasal cavity colonization: role of staphylococcal *agr* alleles. Appl. Environ. Microbiol. **69:**18–23.
28. **Lina, G., Y. Piémont, F. Godail-Gamot, M. Bes, M. O. Peter, V. Gauduchon, F. Vandenesch, and J. Etienne.** 1999. Involvement of Panton-Valentine leukocidin-producing Staphylococcus aureus in primary skin infections and pneumonia. Clin. Infect. Dis. **29:**1128–1132.
29. **Milheiriço, C., D. C. Oliveira, and H. de Lencastre.** 2007. Multiplex PCR strategy for subtyping the staphylococcal cassette chromosome mec type IV in methicillin-resistant *Staphylococcus aureus*: 'SCC*mec* IV multiplex.' J. Antimicrob. Chemother. **60:**42–48.
30. **Moore, P. C. L., and J. A. Lindsay.** 2002. Molecular characterization of the dominant UK methicillin-sensitive *Staphylococcus aureus* strains, EMRSA-15 and EMRSA-16. J. Med. Microbiol. **151:**516–521.
31. **Nor Shamsudin, M., Z. Sekawi, A. van Belkum, and V. Neela.** 2008. First community-acquired meticillin-resistant *Staphylococcus aureus* in Malaysia. J. Med. Microbiol. **57:**1180–1181.
32. **Oliveira, D. C., and H. de Lencastre.** 2002. Multiplex PCR strategy for rapid identification of structural types and variants of the *mec* element in methicillin-resistant *Staphylococcus aureus*. Antimicrob. Agents Chemother. **46:** 2155–2161.
33. **Oliveira, D. C., A. Tomasz, and H. de Lencastre.** 2002. Secrets of success of a human pathogen: molecular evolution of pandemic clones of meticillin-resistant Staphylococcus aureus. Lancet Infect. Dis. **2:**180–189.
34. **Ramdani-Bouguessa, N., M. Bes, H. Meugnier, F. Forey, M. E. Reverdy, G. Lina, F. Vandenesch, M. Tazir, and J. Etienne.** 2006. Detection of methicillin-resistant *Staphylococcus aureus* strains resistant to multiple antibiotics and carrying the Panton-Valentine leukocidin genes in an Algiers hospital. Antimicrob. Agents Chemother. **50:**1083–1085.
35. **Ren, K., J. D. Bannan, and V. Pancholi.** 1994. Characterization and biological properties of a new staphylococcal exotoxin. J. Exp. Med. **180:**1675–1683.
36. **Robinson, D. A., and M. C. Enright.** 2003. Evolutionary models of the emergence of methicillin-resistant *Staphylococcus aureus*. Antimicrob. Agents Chemother. **47:**3926–3934.
37. **Rohde, H., J. K. Knobloch, M. A. Horstkotte, and D. Mack.** 2001. Correlation of *Staphylococcus aureus icaADBC* genotype and biofilm expression phenotype. J. Clin. Microbiol. **39:**4595–4596.
38. **Sam, I. C., M. Kahar-Bador, Y. F. Chan, S. K. Loong, and G. Mohd Nor.** 2008. Multisensitive community-acquired methicillin-resistant *Staphylococcus aureus* infections in Malaysia. Diagn. Microbiol. Infect. Dis. **62:**437–439.
39. **Sattler, C. A., E. J. Mason, and S. L. Kaplan.** 2002. Prospective comparison of risk factors and demographic and clinical characteristics of community-acquired, methicillin-resistant versus methicillin-susceptible *Staphylococcus aureus* infection in children. Pediatr. Infect. Dis. J. **21:**910–916.
40. **Schlichting, C., C. Branger, J. M. Fournier, W. Witte, A. Boutonnier, C. Wolz, P. Goullet, and G. Döring.** 1993. Typing of *Staphylococcus aureus* by pulsed-field gel electrophoresis, zymotyping, capsular typing, and phage typing: resolution of clonal relationships. J. Clin. Microbiol. **31:**227–232.
41. **Shopsin, B., M. Gomez, S. O. Montgomery, D. H. Smith, M. Waddington, D. E. Dodge, D. A. Bost, M. Riehman, S. Naidich, and B. N. Kreiswirth.** 1999. Evaluation of protein A gene polymorphic region DNA sequencing for typing of *Staphylococcus aureus* strains. J. Clin. Microbiol. **37:**3556–3563.
42. **Strommenger, B., C. Kettlitz, T. Weniger, D. Harmsen, A. W. Friedrich, and W. Witte.** 2006. Assignment of *Staphylococcus* isolates to groups by *spa* typing, SmaI macrorestriction analysis, and multilocus sequence typing. J. Clin. Microbiol. **44:**2533–2540.
43. **Tenover, F. C., R. D. Arbeit, R. V. Goering, P. A. Mickelsen, B. E. Murray, D. H. Persing, and B. Swaminathan.** 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J. Clin. Microbiol. **33:**2233–2239.
44. **Tristan, A., M. Bes, H. Meugnier, G. Lina, B. Bozdogan, P. Courvalin, M. E. Reverdy, M. C. Enright, F. Vandenesch, and J. Etienne.** 2007. Global distribution of Panton-Valentine leukocidin-positive methicillin-resistant Staphylococcus aureus, 2006. Emerg. Infect. Dis. **13:**594–600.
45. **Tristan, A., T. Ferry, G. Durand, O. Dauwalder, M. Bes, G. Lina, F. Vandenesch, and J. Etienne.** 2007. Virulence determinants in community and hospital meticillin-resistant *Staphylococcus aureus*. J. Hosp. Infect. **65:**105–109.
46. **Udo, E. E., N. Al-Sweih, S. Mohanakrishnan, and P. W. West.** 2006. Antibacterial resistance and molecular typing of methicillin-resistant *Staphylococcus aureus* in a Kuwaiti general hospital. Med. Princ. Pract. **15:**39–45.
47. **van Leeuwen, W., W. van Nieuwenhuizen, C. Gijzen, H. Verbrugh, and A. Van Belkum.** 2000. Population studies of methicillin-resistant and -sensitive *Staphylococcus aureus* strains reveal a lack of variability in the *agrD* gene, encoding a staphylococcal autoinducer peptide. J. Bacteriol. **182:**5721–5729.
48. **Vindel, A., O. Cuevas, E. Cercenado, C. Marcos, V. Bautista, C. Castellares, P. Trincado, T. Boquete, M. Pérez-Vázquez, M. Marín, and E. Bouza.** 2009. Methicillin-resistant *Staphylococcus aureus* in Spain: molecular epidemiology and utility of different typing methods. J. Clin. Microbiol. **47:**1620–1627.
49. **Yu, F., Z. Chen, C. Liu, X. Zhang, X. Lin, S. Chi, T. Zhou, Z. Chen, and X. Chen.** 2008. Prevalence of *Staphylococcus aureus* carrying Panton-Valentine leukocidin genes among isolates from hospitalized patients in China. Clin. Microbiol. Infect. **14:**381–384.
50. **Zhang, K., J. A. McClure, S. Elsayed, T. Louie, and J. M. Conly.** 2005. Novel multiplex PCR assay for characterization and concomitant subtyping of staphylococcal cassette chromosome *mec* types I to V in methicillin-resistant *Staphylococcus aureus*. J. Clin. Microbiol. **43:**5026–5033.
51. **Zhou, H., Z. Y. Xiong, H. P. Li, Y. L. Zheng, and Y. Q. Jiang.** 2006. An immunogenicity study of a newly fusion protein Cna-FnBP vaccinated against *Staphylococcus aureus* infections in a mice model. Vaccine **24:**4830–4837.