Comparison of methicillin-resistant \textit{Staphylococcus aureus} strains isolated in 2003 and 2008 with an emergence of multidrug resistant ST22: SCCmec IV clone in a tertiary hospital, Malaysia

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\textbf{KEYWORDS}

MDR; MLST; MRSA; PFGE; PVL; SCCmec

\textbf{Background/Purpose:} Infections caused by methicillin-resistant \textit{Staphylococcus aureus} (MRSA) continue to be a problem for clinicians worldwide. The objective of this study was to determine the changes in antibiograms of MRSA and their genotypic characteristics.

\textbf{Methods:} The antibiograms of 162 MRSA isolates (52 from 2003 and 110 from 2008) from a tertiary hospital were analyzed by antimicrobial susceptibility tests, the Panton-Valentine leukocidin (PVL) and staphylococcal cassette chromosome \textit{mec} (SCC\textit{mec}) types were determined by polymerase chain reaction, and genetic relatedness by pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST).

\textbf{Results:} All the isolates were sensitive to vancomycin. Resistance to ciprofloxacin, clindamycin, erythromycin, and gentamicin remained high throughout the study period, although a small decrease was observed in 2008 for ciprofloxacin (96\% to 90\%) and gentamicin (90\% to 83\%). Similarly, a slight decrease in resistance toward fusidic acid (10\% to 9\%), linezolid (2\% to 1\%), rifampicin (8\% to 4\%), and teicoplanin (4\% to 0\%) was observed between 2003 and 2008. In contrast, there was a significant increase ($p < 0.05$) in resistance rates toward trimethoprim-sulfamethoxazole, netilmicin, and tetracycline between 2003 and 2008.
Methicillin-resistant Staphylococcus aureus (MRSA) is an important bacterial pathogen associated with community (CA) and health care (HA) infections in Malaysia and worldwide. In a local study, the MRSA infection rate in a tertiary hospital was reported at 10.0 among 1000 hospital admissions. The incidence of MRSA hospital infections in Japan was between 0.7 and 0.8 per 100 admissions from 1999 to 2003. The rate of MRSA infections among hospitalized patients in the Asia-Pacific region was 45.9%.

The prevalence of MRSA in Malaysian hospitals has increased from 17% in 1986 to 44.1% in 2007. An increase of 62% in MRSA was reported in the United States. Because most MRSA is also resistant to many commonly used antibiotics, this has raised concern over the limited choice of antimicrobial agents for the treatment of life-threatening cases. This could lead to a prolonged hospital stay and increase the cost of care. A death rate of 34% within 30 days was observed among patients with MRSA infections as compared to 27% in patients with methicillin-sensitive Staphylococcus aureus (MSSA). MRSA has evolved from methicillin-susceptible S. aureus via acquisition of mobile genetic elements called staphylococcal cassette chromosome mec (SCCmec) which has two essential components, the ccr gene complex (ccr) and the mec gene complex (mec).

Previous studies in Malaysia have shown that HA-MRSA strains are associated with SCCmec type III whereas CA-MRSA is associated with SCCmec type IV and SCCmec type V. There are no reports on the presence of other SCCmec types in Malaysia.

Panton-Valentine leukocidin (PVL) positive S. aureus is normally associated with CA-MRSA infections such as skin and mucous membrane infections, necrotizing pneumonia, urinary tract infections, and endocarditis.

Rapid and discriminative subtyping methods are essential for determining the epidemiology of pathogenic strains and are useful in the design of rational pathogen control methods. Several methods are available and these include pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST), direct repeat unit (DRU) typing, spa typing, and other polymerase chain reaction (PCR)-associated typing methods. MLST has been shown to be useful in global epidemiologic studies of S. aureus. Ghaemmaghami et al. reported that more than 90% of MRSA infections in a tertiary hospital (HKL) in Malaysia belonged to SCCmec type ST239, whereas Ahmad et al. reported that most of the CA-MRSA reported in Malaysia belonged to SCCmec type ST30.

The changing epidemiology of MRSA is an important public health concern as infections caused by CA-MRSA are increasing. The objective of this study was to determine the changes in the antimicrobial susceptibility profiles of MRSA in a Malaysian tertiary teaching hospital between the years 2003 and 2008 and their genotypic characteristics as determined by PFGE, MLST, SCCmec, and PVL typing.

Materials and methods

Bacterial isolates

One hundred sixty-two nonduplicate MRSA isolates obtained from 158 patients and four healthcare workers (52 from 2003 and 110 from 2008) from the University Malaya Medical Centre (UMMC), Kuala Lumpur, Malaysia, were analyzed. UMMC is a 980-bed referral teaching hospital in Malaysia, which has orthopedic, pediatric, medical, surgical, obstetrics and gynecology, and psychiatry wards, and general intensive care units and neurology intensive care units. All MRSA isolates that could be revived from 2003 and 2008 stock cultures were included for analysis. The isolates were identified as MRSA by standard methods in UMMC and were checked for purity before analysis. All the isolates were cultured in Luria-Bertani broth and stored in cryovials with 50% glycerol (Invitrogen, Grand Island, NY, USA) at −20°C and −85°C.

Ninety-nine percent (161 of 162) of MRSA isolates were HA-MRSA. HA-MRSA refers to cases with positive culture obtained 48 hours after admission to the hospital, whereas
CA-MRSA refers to cases with no association with healthcare setting. The organisms were isolated from nasal swabs (n = 37; 23%), tissue (n = 13; 8%), wound swabs (n = 28; 17%), urine (n = 6; 4%), pus (n = 12; 7%), body fluids (n = 24; 15%), sputum (n = 20; 12%), nasopharyngeal secretion (n = 7; 4%), catheter tip (n = 3; 2%), bone (n = 4; 3%), blood (n = 7; 4%), and chest tube "drainage" (n = 1; 1%).

Antimicrobial susceptibility testing

The antimicrobial susceptibility of S. aureus isolates to 13 antimicrobial agents [vancomycin (30 µg), oxacillin (1 µg), ciprofloxacin (5 µg), tetracycline (30 µg), erythromycin (15 µg), fusidic acid (75 µg), netilmicin (30 µg), teicoplanin (30 µg), gentamicin (10 µg), linezolid (30 µg), rifampicin (5 µg), trimethoprim-sulfamethoxazole (75 µg), and clindamycin (2 µg) (Oxoid Ltd, Basingstoke, Hampshire, UK)] was determined by the disk diffusion method according to Clinical and Laboratory Standard Institutes guidelines. The minimum inhibitory concentration (MIC) for vancomycin was confirmed using Etest (Ab Biodisk, Solna, Sweden). S. aureus isolate ATCC25923 was used as the quality control strain for the antimicrobial susceptibility test as recommended by the Clinical and Laboratory Standards Institute.

The D-zone test method, which is used for the detection of inducible clindamycin resistance (known as inducible macrolides, lincosamides, and streptograminB, i-MLSb) and constitutive clindamycin resistance (known as constitutive macrolides, lincosamides, and streptograminB [c-MLSb]) was performed on all erythromycin-resistant isolates according to established protocols and the results were interpreted as described previously.20,21

PCR detection of pvl gene and SCCmec types

Genomic deoxyribonucleic acid (DNA) from MRSA was extracted by using the Wizard Genomic DNA purification kit (Promega, Madison, WI, USA). An aliquot (approximately 5 ng) was used as a DNA template for PCR analysis. Primers for the detection of pvl and SCCmec types were previously described by Lina et al. and Milheiroco et al. (Operon Biotechnologies GmbH, Cologne, Germany) were used. Specific primers 4a1, 4a2, 4b1, 4b2, 4c1, 4c2, 4d1, and 4d2 as described by Hisata et al. and Okuma et al. were used for further subgrouping the SCCmec IV isolates. Eight positive-control MRSA isolates, NCTC10442, N315, 85/2082, JCSC4744, JCSC2172, JCSC4469, JCSC4788 and WIS were used for SCCmec types I, II, III, IVa, IVb, IVc, IVd, and V, respectively (courtesy of Dr. Teruyo Ito, Japan). All PCR experiments were repeated once to confirm their reproducibility.

PFGE and MLST

PFGE was performed according to published work. The chromosomal DNA was digested with 10 U Smal followed by separation on CHEF DRIII (Bio-Rad, Hercules, CA, USA) in 0.5 × Tris-borate-ethylenediaminetetraacetic acid (EDTA) at 14°C for 22 hours with pulse times of 5–60 seconds. Gels were photographed under ultraviolet light after staining with ethidium bromide (0.5 µg/mL).

Cluster analyses of PFGE profiles were analyzed with BioNumerics Version 6.0 (Applied Maths, Kortrijk, Belgium) based on the unweighted pair group method with arithmetic averages (UPGMA) with a position tolerance of 0.15. All PFGE profiles were assigned an arbitrary designation, and differences were defined by the Dice coefficient of similarity, F.

MLST was conducted on all SCCmec type IV, PVL positive, and representative isolates of each predominant PFGE type as described earlier by Enright et al. The sequence types (STs) were assigned via the S. aureus MLST database (www.mlst.net).

Statistical analysis

A statistical software package STATISTICA (Version 8.0, StatSoft, Inc, Tulsa, OK, USA; http://www.statsoft.com/products/) was used for data analysis. Comparison of certain variables was determined by the Fisher exact test. A p value <0.05 (two-tailed) was taken as the level of significance for the Fisher exact test.

Results

Antimicrobial susceptibility profiles

All the isolates were sensitive to vancomycin. Linezolid, teicoplanin, and rifampicin resistance remained low (1–5%). In contrast, resistance to ciprofloxacin, erythromycin, clindamycin, and gentamicin remained high throughout the study period. A summary of the antimicrobial resistance rates for MRSA isolates is shown in Table 1. There was a significant increase in the rates of resistance to trimethoprim-sulfamethoxazole (p < 0.01), netilmicin (p = 0.01), and tetracycline (p < 0.01) for 2008 isolates compared with the 2003 isolates. There was no significant difference in the resistance rates in 2003 and 2008 for the following antimicrobial agents: ciprofloxacin (96% in 2003 and 90% in 2008), gentamicin (90% in 2003 and 83% in 2008), rifampicin (8% in 2003 and 4% in 2008), fusidic acid (10% in 2003 and 9% in 2008), teicoplanin (4% in 2003 and 0% in 2008), clindamycin (94% in 2003 and 96% in 2008), and linezolid (2% in 2003 and 1% in 2008) (p > 0.05). The resistance rates for erythromycin remained the same.

Most (n = 109, 99%) of the 2008 isolates were sensitive to teicoplanin except for one nasopharyngeal secretion isolate, which showed intermediate susceptibility. Ninety-nine percent (n = 148) of the ciprofloxacin-resistant isolates were also resistant to erythromycin. Similarly, 99% (n = 137) of gentamicin-resistant isolates were also resistant to erythromycin except for a nasal swab isolate MRSA0805-10 from a patient in the orthopedic ward.

A total of 106 isolates (96%) from 2008 and 50 (96%) isolates from 2003 were multidrug resistant (MDR) (resistant to more than three classes of antimicrobial agents). Three isolates (MRSA0308-23, MRSA0805-15, and MRSA0812-33) were resistant to more than seven classes of antimicrobial agents, including penicillins, macrolides, aminoglycosides, lincosamides, fluoroquinolones, tetracyclines, folate pathway inhibitors, and fusidic acid.

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Based on the D-zone test, 96% (150 of 156) and 3% (4 of 156) of the erythromycin-resistant isolates showed inducible clindamycin resistance and constitutive clindamycin resistance, respectively. Two erythromycin-resistant isolates did not have any flattening of the clindamycin zone adjacent to the erythromycin disk.

**pvl Gene and SCCmec types**

The pvl gene was detected in three isolates from 2008 (Table 1). No pvl gene was detected in 2003 isolates. Three SCCmec types were observed: SCCmec type III (90%, n = 146), SCCmec type IV (9%, n = 15), and SCCmec type V (1%, n = 1) (Table 1). Thirteen SCCmec type IV isolates were further subtyped as SCCmec type IVa. A significant difference in the numbers of SCCmec type III and SCCmec type IV between 2003 and 2008 was observed.

A hundred and forty-one SCCmec type III isolates (50 from 2003 and 91 from 2008) were MDR. There was no significant difference in the percentage of isolates with MDR status among SCCmec type III in 2003 and 2008 (96% vs. 97%, p = 1.0). Most (93%, n = 14) SCCmec type IV isolates were MDR and also resistant to ciprofloxacin.

### Genotypes of MRSA based on PFGE and MLST

Smal-digested genomic DNA of the 162 MRSA isolates resulted in 84 distinct pulsed-field profiles (PFPs) comprising 10–18 restriction fragments (F = 0.57–1.00). Based on the interpretation proposed by Tenover et al., these 84 PFPs were further grouped into 63 PFGE types where closely related isolates with a similarity of more than 97% (1–2 band difference) was considered as a unique PFGE type (Fig. 1). Most of the isolates were genetically related (F > 0.8). Thirty-five isolates shared the same PFGE type even though they were cultured from different sources and occasions (Fig. 1). Some isolates from 6 years apart shared similar PFGE profiles, indicating the persistence of a particular genotype.

MLST was performed on all SCCmec type IV (n = 15), SCCmec type V (n = 1), and representative isolates for each predominant PFGE type (n = 18). This identified five STs (ST239, ST772, ST22, ST6, and ST1178). MLST type ST239 was observed from both 2003 (n = 9) and 2008 (n = 9) isolates, whereas new MLST types ST772 (n = 1), ST22 (n = 12), ST6 (n = 1), and ST1178 (n = 2) were identified among the 2008 isolates. All 12 ST22 isolates carried SCCmec type IV (Table 2).

### Combined analysis

A dendrogram based on combined PFPs and resistotypes is shown in Fig. 2. All 162 MRSA isolates were differentiated into 110 combined subtypes. Some isolates (i.e., MRSA0807-19, MRSA0808-19, MRSA0305-10, and MRSA0312-17) shared the same combined subtypes even though they were cultured independently from different time periods and sources. Based on 70% similarity, five clusters were observed. Cluster 1 consists of 127 isolates; Clusters 2 and 3 consist of three isolates; Cluster 4 consists of 12 isolates; and Cluster 5 consists of seven isolates. Nine isolates were not grouped into any of the clusters.

Fifty-nine isolates within Cluster 1 were clonally related (shared more than 80% similarity) although they were from different sources and years. These isolates were also resistant to gentamicin, erythromycin, and clindamycin and 98% of them were of SCCmec type III. Some isolates that were indistinguishable by PFGE were further differentiated in the combined analysis as they had different resistotypes. For example, MRSA0801-26, MRSA0805-22, MRSA0812-37, MRSA0309-10, MRSA0809-25, MRSA0806-33, MRSA0810-9, MRSA0807-19, MRSA0808-19, MRSA0305-10, and MRSA0312-17 were further differentiated by their resistotypes although they shared similar PFGE profiles.

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### Table 1 Resistance rates, PVL, and SCCmec types of Malaysian MRSA isolates in 2003 and 2008

<table>
<thead>
<tr>
<th>Antimicrobial agents</th>
<th>Year 2003, n = 52 (%)</th>
<th>Year 2008, n = 110 (%)</th>
<th>Total (%)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciprofloxacin</td>
<td>50 (96)</td>
<td>99 (90)</td>
<td>149 (92)</td>
<td>0.23</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>49 (94)</td>
<td>106 (96)</td>
<td>155 (96)</td>
<td>0.68</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>50 (96)</td>
<td>106 (96)</td>
<td>156 (96)</td>
<td>1.00</td>
</tr>
<tr>
<td>Fusidic acid</td>
<td>5 (10)</td>
<td>10 (9)</td>
<td>15 (9)</td>
<td>1.00</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>47 (90)</td>
<td>91 (83)</td>
<td>138 (85)</td>
<td>0.24</td>
</tr>
<tr>
<td>Linezolid</td>
<td>1 (2)</td>
<td>1 (1)</td>
<td>2 (1)</td>
<td>0.54</td>
</tr>
<tr>
<td>Netilmicin</td>
<td>14 (27)</td>
<td>55 (50)</td>
<td>69 (43)</td>
<td>0.01</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>4 (8)</td>
<td>4 (4)</td>
<td>8 (5)</td>
<td>0.27</td>
</tr>
<tr>
<td>Teicoplanin</td>
<td>2 (4)</td>
<td>0 (0)</td>
<td>2 (1)</td>
<td>0.11</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>10 (19)</td>
<td>71 (65)</td>
<td>81 (50)</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Trimethoprim-sulfamethoxazole</td>
<td>19 (37)</td>
<td>80 (73)</td>
<td>99 (61)</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1.00</td>
</tr>
<tr>
<td><strong>SCCmec type</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCCmec type III</td>
<td>52 (100)</td>
<td>94 (85)</td>
<td>146 (90)</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>SCCmec type IV</td>
<td>0 (0)</td>
<td>15 (14)</td>
<td>15 (9)</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>SCCmec type V</td>
<td>0 (0)</td>
<td>1 (1)</td>
<td>1 (1)</td>
<td>1.00</td>
</tr>
<tr>
<td><strong>pvl</strong></td>
<td>0 (0)</td>
<td>3 (3)</td>
<td>3 (2)</td>
<td>0.55</td>
</tr>
</tbody>
</table>
Based on PFGE analysis, the only SCCmec type V isolate was clonally related (shared 81.8% similarity) with one SCCmec type III (MRSA0807-13). However, they only shared 79.8% in the combined analysis as these two isolates had unique resistotypes.

Among the 15 SCCmec type IV isolates, one was grouped in Cluster 1, 12 in Cluster 4, and two isolates were not grouped in any cluster. The 12 isolates within Cluster 4 belonged to MLST type ST22, with seven of them sharing the same resistotype (Fig. 2, Table 2).

Two teicoplanin-resistant isolates (MRSA0311-23 and MRSA0312-35) shared 78.2% similarity and were grouped in Cluster 1 (Fig. 2). Both were SCCmec type III but had different resistotypes. MRSA0311-23 was resistant to erythromycin, gentamicin, ciprofloxacin, oxacillin, and trimethoprim-sulfamethoxazole, whereas MRSA0312-35 was resistant to mupirocin, tetracycline, oxacillin, and fusidic acid.

Discussion

Infections caused by MRSA continue to be a problem in Malaysian hospitals. Although several studies have documented the antimicrobial resistance trends of MRSA in other countries, reports comparing resistance trends and
their genetic characteristics between two periods of time in Malaysia are scarce. This report shows detailed antimicrobial resistance trends and the genetic characteristic of MRSA isolated in a tertiary hospital in 2003 and 2008.

In this study, tetracycline-resistant MRSA had significantly increased over a 6-year period ($p < 0.01$), possibly because of an increased usage of tetracycline or doxycycline in the hospital. Thong et al.\textsuperscript{13} reported tetracycline resistance rates of 81.2\% in Singapore, Thailand, Taiwan, and Indonesia.\textsuperscript{11,29} All 15 SCCmec type IV MRSA isolates (9\%) were HA-MRSA (unpublished hospital records), although SCCmec type IV MRSA isolates were mostly associated with CA-MRSA.\textsuperscript{5} SCCmec type IV HA-MRSA has been previously reported in Denmark.\textsuperscript{30} In addition, all SCCmec type IV isolates were susceptible to rifampicin, mupirocin, teicoplanin, and vancomycin. This is in agreement with a previous report by Ahmad et al.\textsuperscript{6} that SCCmec type IV isolates are susceptible to four or more non-$\beta$-lactam antibiotics. Similarly, D’Souza et al.\textsuperscript{31} also reported that 83\% of their SCCmec type IV isolates from India were susceptible to many classes of antimicrobials.

In this study, 15 MRSA isolates were resistant to gentamicin. This is concordant with previously reported erythromycin-resistant isolates from India were susceptible to many classes of antimicrobials. Two of 12 ST22 SCCmec type IV MRSA isolates reported here were resistant to gentamicin. This differs from the report of Ahmad et al.\textsuperscript{5} (Malaysia) and Conceição et al.\textsuperscript{32} (Atlantic Azores islands), where the ST22 isolates were sensitive to gentamicin and tetracycline.

Table 2  Resistotypes, PFGE, and MLST types of SCCmec type IV and V isolates

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Resistotypes</th>
<th>MLST</th>
<th>PFGE type</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRSA0801-21</td>
<td>CIP, ERY, LZD, CN, SXT, DA, NET</td>
<td>ST22</td>
<td>51</td>
</tr>
<tr>
<td>MRSA0803-28</td>
<td>CIP, ERY, SXT, DA</td>
<td>ST22</td>
<td>28</td>
</tr>
<tr>
<td>MRSA0804-1</td>
<td>CIP, ERY, SXT, DA</td>
<td>ST22</td>
<td>54</td>
</tr>
<tr>
<td>MRSA0805-9</td>
<td>CIP, ERY, SXT, DA</td>
<td>ST22</td>
<td>55</td>
</tr>
<tr>
<td>MRSA0806-21</td>
<td>CIP, ERY, SXT, DA</td>
<td>ST22</td>
<td>56</td>
</tr>
<tr>
<td>MRSA0810-10</td>
<td>CIP, ERY, FD</td>
<td>ST22</td>
<td>50</td>
</tr>
<tr>
<td>MRSA0810-17</td>
<td>CIP, ERY, FD, DA</td>
<td>ST22</td>
<td>49</td>
</tr>
<tr>
<td>MRSA0810-22</td>
<td>CIP, ERY, SXT, DA</td>
<td>ST22</td>
<td>51</td>
</tr>
<tr>
<td>MRSA0811-22</td>
<td>CIP, ERY, SXT, DA</td>
<td>ST22</td>
<td>53</td>
</tr>
<tr>
<td>MRSA0811-30</td>
<td>CIP, TE, ERY, SXT, DA</td>
<td>ST22</td>
<td>52</td>
</tr>
<tr>
<td>MRSA0812-1</td>
<td>CIP, ERY, SXT, DA</td>
<td>ST22</td>
<td>54</td>
</tr>
<tr>
<td>MRSA0812-23</td>
<td>CIP, ERY, TE, CN, SXT, DA, NET</td>
<td>ST22</td>
<td>54</td>
</tr>
<tr>
<td>MRSA0805-10</td>
<td>CIP, CN, SXT</td>
<td>ST1178</td>
<td>39</td>
</tr>
<tr>
<td>MRSA0805-1</td>
<td>ERY, FD, SXT</td>
<td>ST1178</td>
<td>3</td>
</tr>
<tr>
<td>MRSA0806-11</td>
<td>All\textsuperscript{a}</td>
<td>ST6</td>
<td>1</td>
</tr>
<tr>
<td>MRSA0808-17</td>
<td>CIP, ERY, CN, SXT</td>
<td>ST772</td>
<td>46</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Sensitive to all the antimicrobial agents tested.

CIP = ciprofloxacin; CN = gentamicin; DA = clindamycin; ERY = erythromycin; FD = fusidic acid; LZD = linezolid; MLST = multilocus sequence typing; NET = netilmicin; PFGE = pulsed-field gel electrophoresis; SXT = trimethoprim-sulfamethoxazole; TE = tetracycline.
Figure 2. Computed dendrogram derived from combined data analysis of PFGE and resistotypes for 2003 and 2008 MRSA isolates. The dotted vertical line indicates 70% similarity level. (A) MRSA0807-19, MRSA0808-19, MRSA0305-10, MRSA0312-17. (B) MRSA0804-1. (C) MRSA0805-9. CIP = ciprofloxacin; CN = gentamicin; DA = clindamycin; E = erythromycin; FD = fusidic acid; LZD = linezolid; NET = netilmicin; RD = rifampicin; SXT = trimethoprim-sulfamethoxazole; TE = tetracycline; TEC = teicoplanin; VA = vancomycin.
The SCCmec IV: ST22 from the Atlantic Azores islands belonged to SCCmec type IVh (Conceição et al.\textsuperscript{[32]}), whereas all the SCCmec type IV: ST22 in the current study were SCCmec type IVa. Even though ST30 (SCCmec type IV) and ST1 are known as pandemic MRSA clones,\textsuperscript{37} they are absent in this study.

The high ciprofloxacin resistant rates among SCCmec type IV isolates in this hospital might be because of the high usage of antibiotics in the hospital (unpublished data). High erythromycin- and trimethoprim-sulfamethoxazole resistance rates were also observed among SCCmec type IV isolates, and similar observations had been previously reported in a hospital in Southern Iran by Japoni et al.\textsuperscript{36} Coombs et al.\textsuperscript{34} reported the presence of SCCmec type IV, MLST type ST22 isolates with erythromycin and ciprofloxacin resistance among MRSA isolated from western Australia.

The only SCCmec type V isolate (MRS A0812-36) found in this study was CA-MRSA, PVL negative, ST772 and MDR.
SCCmec type V was first reported in CA-MRSA from nasal swabs of healthy local university students. Ahmad et al and Otter and French proposed gentamicin and ciprofloxacin, respectively, as phenotypic markers for CA-MRSA. However, the only CA-MRSA isolates in this study were resistant to both the antimicrobial agents. Therefore, both antimicrobial agents might not be suitable to be used as the phenotypic marker of CA-MRSA in this tertiary hospital. Similarly, more than 90% of MRSA isolates from another tertiary hospital in Malaysia belonged to this pandemic clone.

Combined analysis based on PFPS and resistotypes showed that most of the MRSA isolates were clonally related even though they were cultured at different times. This suggests that several clones of MRSA isolates were circulating in this tertiary hospital during the study period. Some other 2003 and 2008 isolates also shared similar combined subtypes and this further implies persistence of certain clones within the hospital environment. The presence of three indistinguishable clinical isolates, which were cultured during the same month (July) but from different wards, further supports the notion of the circulation of a particular clone in the hospital.

Most of the isolates resistant to tetracycline (90% in both 2003 and 2008) and trimethoprim-sulfamethoxazole (68% in 2003 and 79% in 2008) in 2003 and 2008 belong to Cluster 1. The tetracycline and trimethoprim-sulfamethoxazole isolates might be a part of the persistent clone in the hospital environment.

In conclusion, correlation between PFGE profiles and resistotypes was observed. Isolates with indistinguishable PFGE profiles often have similar antibiotic susceptibility patterns even though there are variations in certain antibiograms in distinct clones of MRSA. The antibiotic resistance rates had increased over the years and the persistence of MDR isolates remains a problem. Although MRSA with SCCmec type III with MLST type ST239 is predominant in Malaysia, SCCmec type IV with MLST type ST22 is gaining prominence. The MDR MRSA clinical isolates from UMMC were mostly genetically related, suggesting that few predominant clones of the species are involved in infection.

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