Contemporary Methicillin-Resistant *Staphylococcus aureus* Clones in Hong Kong

Margaret Ip,1* R. W. H. Yung,2† T. K. Ng,3 W. K. Luk,4 Cindy Tse,3‡ Philip Hung,1 Mark Enright,3¶ and Donald J. Lyon3§

The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, Hong Kong; Pamela Youde Nethersole Eastern Hospital, Hong Kong; Princess Margaret Hospital, Kowloon, Hong Kong; Tsuen Kwan O Hospital, Hong Kong; and Department of Biology and Biochemistry, University of Bath, Bath, United Kingdom

Received 19 May 2005/Returned for modification 29 June 2005/Accepted 7 July 2005

Two hundred nonduplicate methicillin-resistant *Staphylococcus aureus* (MRSA) isolates causing bacteremia in patients in four major Hong Kong hospitals during the period 2000 to 2001 were characterized by antibiogram, pulsed-field gel electrophoresis (PFGE) using SmaI restriction enzymes, and determination of staphylococcal cassette chromosome *mec* (SCC*mec*) types. Nine PFGE types, A to I, were obtained. PFGE type A constituted 50% (99/200) of all isolates and was present in isolates from all four hospitals. PFGE types A to E, had previously been identified as the major types at one of the hospitals from 1988 to 2000. The majority had a resistance profile to tetracycline (T), erythromycin (E), clindamycin (D), gentamicin (G), tobramycin (To), and ciprofloxacin (Ci), and belonged to SCC*mec* type III; and representatives belonged to clonal complex 239 (CC239) (MRSA with SCC*mec* type III and sequence type 239, designated ST239-MRSA-III). PFGE types F to I were new patterns that had not previously been identified in isolates from Hong Kong. PFGE type F constituted 18% (35/200) of MRSA isolates, had resistance profile TEGToCi, and belonged to CC5 (ST5-MRSA-II). PFGE type G included 13% (26/200) of MRSA isolates, had resistance profile TECi, and belonged to CC45 with SCC*mec* type I or II. PFGE type H had characteristics similar to those of CC239, while PFGE type I included three isolates, two of which expressed resistance to oxacinil and fusidic acid only. Two of these strains had SCC*mec* IVa and carried sequence type 389, with a multilocus sequence typing allelic profile of 3-35-19-2-20-26-39. Contemporary MRSA causing bacteremia in Hong Kong hospitals belong to three clonal complexes (CC5, CC45, and CC239). The most prevalent MRSA clone in Hong Kong belongs to CC239, with PFGE types A to E and SCC*mec* type III, ST239, and a resistance profile of TEDGToCi.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important pathogen and is endemic in most Hong Kong hospitals (7). Previous data indicated that Hong Kong has one of the highest prevalence rates of MRSA among hospitals within the Asia Pacific region (1, 10). Worldwide, there are concerns about the emergence of MRSA in the community setting (8), and bacteremia caused by staphylococci with inducible vancomycin heteroresistance has been reported from a population of 7 million people in Hong Kong. Only blood isolates were obtained from four major Hong Kong hospitals designated hospitals A to D: the Prince of Wales Hospital (PWH; A), the Pamela Youde Nethersole Eastern Hospital (B), Princess Margaret Hospital (C), and Tsuen Kwan O Hospital (D). These hospitals are located in four of the five regional clusters of hospitals operated by the Hospital Authority that serve the whole public sector in providing healthcare to a population of 7 million people in Hong Kong. Only blood isolates were examined; thus, isolates from this collection were deemed representative of the MRSA strains causing invasive disease in Hong Kong. The isolates were stored in...
nutrient agar slants at room temperature, and an additional set was stored in glycerol broth at −70°C. The identities of the *Staphylococcus aureus* isolates were confirmed by colonial morphology, Gram staining, and the coagulase test. Methicillin resistance was screened by oxacillin (1-µg) disk susceptibility testing according to CLSI (formerly NCCLS) (13) and by demonstration of the mecA gene by PCR (22). Representative strains of the United Kingdom epidemic MRSA (eMRSA 1 to 16) (gift of M. Enright, University of Bath, United Kingdom), Brazilian (HS216) (4), Iberian (HPV107) (18), pediatric (HDE288) (17), and the New York-Tokyo (BK2464) (3) clones were included (gifts of H. de Lencastre and A. Tomasz, Laboratory of Microbiology, The Rockefeller University, New York, N.Y.). Representatives of previously well-characterized MRSA types A to E, from PWH, were also included for comparison (11).

**Antimicrobial resistance profiles.** Antibiotics were determined by disk diffusion on Mueller-Hinton agar according to CLSI (13). The antimicrobial agents tested included tetracycline (30 µg), erythromycin (15 µg), clindamycin (2 µg), gentamicin (10 µg), tobramycin (10 µg), ciprofloxacin (5 µg), chloramphenicol (30 µg), cotrimoxazole (25 µg), rifampin (5 µg), netilmicin (30 µg), mupirocin (5 µg), and fusidic acid (10 µg). The interpretation of results was as according to CLSI (13), with the exception of fusidic acid and mupirocin, for which the equivalent breakpoints for resistance were ≥2 mg/liter and ≥8 mg/liter, respectively (zones of inhibition were ≤29 mm and ≤21 mm, respectively). *Staphylococcus aureus* ATCC 25923 was included as a control. Strains with zones of inhibition falling into the category of intermediate susceptibility to a particular antibiotic were considered resistant.

**Pulsed-field gel electrophoresis.** DNA extraction and Smal restriction were performed as previously described (11). DNA fragments were resolved on a 1% agarose gel with a PFGE apparatus, Chef Mapper (Bio-Rad, Richmond, Calif.), at 6 V/cm for 22 h, with switching times ramped from 5 to 35 s at 10°C and an angle of 120°. A lambda DNA-PFGE molecular size standard (Life Technologies) and American Type Culture Collection control strain were included in each gel. The PFGE patterns were examined visually and interpreted according to the criteria of Tenover et al. (20). A pulstotype was designated type A; if the isolates differed by up to a three-band difference, they would be classified as subtypes of the pulstotype. A different pulstotype was indicated if the isolate differed by four or more bands. An epidemic clone was defined as two or more MRSA strains with indistinguishable PFGE fingerprints in a given hospital and the strain types were also present in two or more hospitals in Hong Kong.

**Computer analysis of PFGE profiles.** The similarity of the PFGE fingerprints was also determined by computer comparison and interpreted by using the BioNumerics (version 2.5) software (Applied Maths, Belgium). A dendrogram was generated by the Dice method and by clustering by the unweighted-pair group method using average linkages with 1% band and position tolerance. The major type included isolates which fell into a >80% similarity.

**SCCmec typing.** The SCCmec type was determined by PCR detection using primer pairs for amplification of part of the SCCmec I, II, III, IVa, and IVb genes (23). PCR products were carried out with an initial denaturation at 95°C for 5 min, followed by 30 cycles, each consisting of 95°C for 45 s, 58°C for 45 s, and 72°C for 1 min. The reaction mixture was maintained at 72°C for a further 7 min. For SCCmec IVa and IVb PCRs, an annealing temperature of 53°C was used.

**MLST.** MLST was performed as previously described (6). PCR amplicons of the seven housekeeping genes arc, aroE, gfpF, gmk, pta, tpi, and yqil were obtained from chromosomal DNA. PCR conditions began with initial denaturation at 95°C for 5 min; this was followed by 35 cycles, each consisting of 95°C for 30 s, 55°C for 30 s, and 72°C for 1 min. This was followed by a final extension at 72°C for 10 min. PCR fragments were purified with a PCR purification kit (QIAGEN) and sequenced with an ABI 3700 sequencer.

The sequences of the PCR products were compared with the existing sequences available in the MLST website (http://www.mlst.net) for *Staphylococcus aureus*, and the allelic number was determined for each sequence.

**RESULTS**

**Antibiotic resistance profiles and PFGE.** The PFGE types of MRSA isolates and their respective antibiotic resistance profiles from four Hong Kong hospitals are listed in Table 1. Overall, nine PFGE types, A to I, based on the interpretations of Tenover et al. (20), were obtained. PFGE type A remained the predominant type, constituted 50% (99/200) of all isolates, and was present in all four hospitals. PFGE types C to G were also present in three of the four hospitals. PFGE types A to E had previously been identified to be the major types at PWH during the 13-year period from 1988 to 2000 (11).

PFGE types F to I were new patterns of MRSA isolates, not previously reported in Hong Kong. Isolates with PFGE types F and G were the next most prevalent types and represented 17.5% and 13% of all isolates. PFGE type H differed from type A by a difference of only four bands and was only present in two hospitals, whereas PFGE type I included three isolates from one hospital only.

The PFGE types were generally associated with unique antibiotic resistance profiles and are illustrated in Table 1. PFGE type A and C isolates predominantly were resistant to antibiotics T, E, D, G, To, and Ci and had the major antibiotic resistance profile TEGToCi. PFGE types B, D, and E had additional resistance to antibiotics N, M, or S. PFGE type F had the same resistance profile as type A but was susceptible to clindamycin (TEGToCi). MRSA with PFGE types G and H showed distinctive antibiotic patterns. PFGE type G strains (16/26 strains) were resistant only to antibiotics T, E, and Ci while type H isolates (6/8) were also resistant to D. PFGE type I included only three isolates, two of which were resistant to fusidic acid alone. Overall, the majority of MRSA isolates (>90%) showed susceptibility to netilmicin, mupirocin, fusidic acid, rifampin, cotrimoxazole, and chloramphenicol.

PFGE types A to I of Hong Kong MRSA isolates are illustrated in Fig. 1. PFGE types A to E fell into a cluster of 79.42%
(clusters A to E) when analyzed with the Dice similarity coefficient using optimization with a position band tolerance of 1% and the dendrogram generated by the unweighted-pair group method using average linkages (dendrogram not shown). Several small discrete clusters correlated with PFGE types F to I.

**SCCmec types and MLST analysis.** The distribution of the SCCmec types among MRSA isolates belonging to PFGE types A to I is listed in Table 2. The STs of representatives of the PFGE types as analyzed by MLST are also tabulated. A total of 92% (115/125 isolates) of PFGE types A to E and H isolates carried the SCCmec III gene and were multidrug-resistant, and representatives of these PFGE types had the same sequence type (ST239). Previous studies indicated a difference of up to seven bands in PFGE patterns, and the strains still fell

<table>
<thead>
<tr>
<th>PFGE type (no.)</th>
<th>SCCmec I (%)</th>
<th>SCCmec II (%)</th>
<th>SCCmec III (%)</th>
<th>SCCmec IVa (%)</th>
<th>UID (%)</th>
<th>MLST allele no. (arc-aro-gfp-gmk-pta-psp-yrn)</th>
<th>ST-SCCmec type (isolate no.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (99)</td>
<td>3 (4.0)</td>
<td>90 (90.0)</td>
<td>2 (2.0)</td>
<td>4 (4.0)</td>
<td>2-3-1-1-4-4-3</td>
<td>ST239-III (PWH-A)</td>
<td></td>
</tr>
<tr>
<td>B (1)</td>
<td>1 (100)</td>
<td></td>
<td></td>
<td></td>
<td>2-3-1-1-4-4-3</td>
<td>ST239-III (149)</td>
<td></td>
</tr>
<tr>
<td>C (3)</td>
<td>1 (33.3)</td>
<td>2 (66.7)</td>
<td></td>
<td></td>
<td>2-3-1-1-4-4-3</td>
<td>ST239-III (137)</td>
<td></td>
</tr>
<tr>
<td>D (9)</td>
<td>9 (100)</td>
<td></td>
<td></td>
<td></td>
<td>2-3-1-1-4-4-3</td>
<td>ST239-III (88)</td>
<td></td>
</tr>
<tr>
<td>E (5)</td>
<td>5 (100)</td>
<td></td>
<td></td>
<td></td>
<td>1-4-1-4-12-1-10</td>
<td>ST5-II (36, 61, 71, 227)</td>
<td></td>
</tr>
<tr>
<td>F (35)</td>
<td>3 (8.6)</td>
<td>28 (80)</td>
<td>3 (8.6)</td>
<td>1 (2.9)</td>
<td>10-14-8-6-10-3-2</td>
<td>ST45-II (234, 238)</td>
<td></td>
</tr>
<tr>
<td>G (26)</td>
<td>9 (34.6)</td>
<td>13 (50)</td>
<td>1 (3.8)</td>
<td>2 (7.7)</td>
<td>2-3-1-1-4-4-3</td>
<td>ST239-III (132, 150)</td>
<td></td>
</tr>
<tr>
<td>H (8)</td>
<td></td>
<td>8 (100)</td>
<td></td>
<td></td>
<td>3-35-19-2-20-26-3-9</td>
<td>ST398-IVa (175, 176)</td>
<td></td>
</tr>
<tr>
<td>I (3)</td>
<td>1 (33.3)</td>
<td></td>
<td>2 (66.7)</td>
<td></td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Unique (11)</td>
<td>7 (64)</td>
<td>2 (18)</td>
<td>2 (18)</td>
<td></td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
</tbody>
</table>

*Total number of isolates analyzed, 200. UID, unidentified by SCCmec primers.
within the same cluster by amplified-fragment length polymorphism analysis (11). The antibiotic resistance profile of PFGE type H isolates differed from PFGE types A to E by the loss of aminoglycoside resistance (GTo). This may possibly be due to loss of the resistance determinants often encoded in plasmid or transposon or due to the lack of expression of these genes.

The majority of PFGE type F isolates (31/35) carried the SCCmec II gene; these isolates belonged to ST5. PFGE type G isolates carried SCCmec I or II gene and belonged to ST45. Two of the three PFGE type I isolates had ST398 (allele no. 3-35-19-2-20-26-39) and carried the SCCmec IVa gene. These MRSA strains were resistant to fusidic acid alone and may represent those of community acquisition. However, these strains were few in number and were present only in isolates from one hospital.

DISCUSSION

The contemporary MRSA causing bacteremia in Hong Kong hospitals belong to three clonal complexes (CC5, CC45, and CC239). This study confirmed that the most common and prevalent MRSA clone spreading in Hong Kong belongs to CC239, includes isolates with PFGE types A to E and H belonging to SCCmec type III with ST239, and was multidrug resistant with a profile of TE(DGTo)Ci (the use of parentheses in listing elements of a resistance profile indicates that not all isolates were resistant to a given antibiotic). This clone had established as early as the 1980s in Hong Kong, as indicated by the previous longitudinal study at PWH, which showed by amplified-fragment length polymorphism analysis that strains from 1989 onwards with PFGE patterns A to E were closely related and fell within the cluster of British eMRSA clones 1, 4, 7, and 11 and that these strains belonged to SCCmec type III and ST239 (11). MRSA with CC239 were suggested to be widespread in many countries in southeast Asia (12) and many countries worldwide. These isolates fell into the ST239-MRSA-III group, which represents a distinct branch within clonal complex CC8 in the evolutionary model of emergence of MRSA (16). This lineage includes numerous representatives of eMRSA (clones 1, 4, 7, 9, and 11), Brazilian, Portuguese, and Vienna clones (16).

The second predominant clonal complex belonged to CC5. These isolates had PFGE type F pattern and carried SCCmec type II. Representatives of these isolates belonged to ST5 by MLST and were present in three of the four major hospitals. It was suggested that these isolates fell in the group ST5-MRSA-II, derived from the same ancestor as the New-York-Japan clone within the clonal complex of CC5 (16). MRSA from CC5 has also spread widely to European countries and is the predominant MRSA clone in Korea and Japan (12).

The next prevalent MRSA from this study included isolates from three of the four hospitals and belonged to PFGE type G, with SCCmec types I or II and ST45. Similar isolates belonging to the ST34-MRSA-II group have been documented in the United States and Berlin and belong to the CC45 clonal complex (16). However, strains with SCCmec type I will require further delineation as to their origins. Lastly, the PFGE type I isolates included three isolates isolated from only one hospital; thus, PFGE type I is not considered a Hong Kong-wide clonal type. Two of the three isolates belong to SCCmec type IVa, have a unique MLST allele number of 3-35-19-2-20-26-39 for housekeeping genes arc-aro-glp-gmk-pta-tpi-yqi, and were only recently assigned to a known sequence type (ST398). The strain with ST398 was described as having been isolated from a patient with MRSA in The Netherlands in 2004 (http://www.mlst.net). These may represent isolates that acquired the SCCmec IVa in patients with community-acquired MRSA disease, although they were negative for the Panton-Valentine leukocidin gene by PCR (unpublished data). Further surveillance and SCCmec typing with MLST of MRSA may elucidate further the importance of this ST as a component of a prevalent CA-MRSA in Hong Kong.

Our study documented the major MRSA causing bacteremia in Hong Kong hospitals as belonging to three clonal complexes (CC5, CC45, and CC239). CC239 and CC5 have been identified as being prevalent in Asia (12), although CC5 is predominant in Korea and Japan. The only published case report of CA-MRSA in Hong Kong was in 2004 and described a case of MRSA belonging to ST80 (9). Although we did not identify MRSA with ST80, we have a small number of isolates obtained in 2000 and 2001 with SCCmec IVa, resistant only to oxacillin and fusidic acid and with unique MLST sequence types. Reports of CA-MRSA arising from acquisition of SCCmec type IV in strains of Staphylococcus aureus in both the United States and Australia have been widespread (14, 15). Screening of the MRSA by SCCmec typing and MLST may be necessary to indicate the extent of CA-MRSA in our locality.

ACKNOWLEDGMENTS

The work described in the paper was supported by a grant from the Research Grants Council of the Hong Kong Special Administrative Region (project no. CUHK, direct grant no. 2001.1.069).

We also thank H. de Lencastre and A. Tomasz for the provision of representatives of the international clones. We thank Shirley S. L. Chau and S. L. Lui for excellent technical assistance.

REFERENCES


