Panton–Valentine leukocidin-positive meticillin-resistant Staphylococcus aureus in the community in Hong Kong

Meticillin-resistant Staphylococcus aureus (MRSA) strains producing the potent tissue necrotizing toxin Panton–Valentine leukocidin (PVL) encoded by the pvl gene, and harbouring SCCmec type IV or V elements, have been implicated as being associated with MRSA infection acquired in the community setting (Vandenesch et al., 2003; Boyle-Vavra & Daum, 2007). Since the 1990s, community-associated (CA)-MRSA infections have shown a steady increase, and are becoming a significant public-health concern. PVL-positive MRSA isolates have been shown to have distinct genetic backgrounds in different areas, for instance, multilocus sequence typing (MLST) types ST80, ST8 and ST59 were found in the major clones in Europe, USA and Taiwan, respectively (Vandenesch et al., 2003; Tristan et al., 2007; Chen et al., 2005).

In Hong Kong, CA-MRSA infection is a notifiable disease, defined by clinical and epidemiological criteria (Millar et al., 2007), together with molecular characterization of the strain, as belonging to SCCmec type IV or V, and being pvl-positive (CIP, 2008). In an attempt to understand the characteristics and epidemiology of pvl-positive MRSA strains in the community in Hong Kong, we characterized 140 CA-MRSA strains isolated in 2006. Our laboratory is a diagnostic and public health laboratory processing clinical specimens from outpatients and hospital inpatients from both the public and private sector. We also serve as a referral centre for characterization of isolates from other laboratories. For any isolate suspected to be CA-MRSA based on clinical and epidemiological grounds, investigations undertaken included antibiogram determination by the disc diffusion method (CLSI, 2005), SCCmec typing (Okuma et al., 2002; Ito et al., 2004) and pvl PCR (Lina et al., 1999). Isolates found to be pvl-positive were further subjected to molecular typing studies, including spa typing (Shopsin et al., 1999) using the Ridom SpaServer, MLST (Enright et al., 2000) and PFGE (McDougal et al., 2003). One hundred and forty putative CA-MRSA isolates from unique patients were examined during 2006. Forty-two isolates (30 %) were found to carry pvl. Among these, SCCmec IV and V elements were found in 30 (71.4 %) and 12 (28.6 %) isolates, respectively; no other SCCmec element was found in this collection (Table 1). This is in accordance with other reports (Tristan et al., 2007; Boyle-Vavra & Daum, 2007) that pvl carriage is much more frequently associated with SCCmec types IV and V than other types (I, II or III) in S. aureus. In Hong Kong, pvl-positive SCCmec type V isolates have been reported in five MRSA strains (Ho et al., 2007). Our results confirmed the previous findings at an extended scale.

The 42 pvl-positive MRSA strains were mainly recovered from skin and soft tissue specimens (88.1 %), followed by blood culture (7.1 %), joint aspirate (2.4 %) and eye (2.4 %) specimens. As for the 98 pvl-negative MRSA strains, their sites of origin were skin and soft tissue specimens (46.9 %), followed by sputum (25.5 %), urine (8.2 %), blood culture (6.1 %) and other site specimens (13.2 %). PVL-positive MRSA is notoriously associated with skin and soft tissue infection (SSTI). Holmes et al. (2005) reported that the majority (24 %) of pvl-positive S. aureus isolates were linked with SSTI in the UK. A relatively high percentage (44.6 %) of pvl carriage was also seen among the 83 MRSA isolates from soft tissue specimens examined in our study. Our findings showed that SSTI constituted the majority of cases where pvl-positive MRSA was isolated (88.1 %), while the proportion was only 46.9 % among pvl-negative isolates (P<0.0001). These findings from Hong Kong are thus consistent with reports from the literature.

All 42 pvl-positive S. aureus were confirmed to be resistant to β-lactam antibiotics. Resistance to erythromycin and clindamycin was present in 42.9 % (18/42). In addition, 13 isolates were resistant to tetracycline (31 %) and 9 to chloramphenicol (21.4 %). Resistance to ciprofloxacin (3/42), co-trimoxazole (2/42), rifampicin (1/42) and gentamicin (1/42) were less common. Eighteen isolates (42.9 %) were resistant to two or more non-β-lactam agents and seven (16.7 %) were resistant to four or more, whilst one isolate was resistant to five non-β-lactam agents (Table 1). Some studies have suggested that antibiotic resistance in SCCmec IV or V isolates was often limited to resistance to β-lactams (Takano et al., 2007). However, multiple non-β-lactam antimicrobial resistance phenotypes were found in our SCCmec type V isolates, and all of these strains were resistant to at least two, to a maximum of four, non-β-lactam drugs. These data are in line with reports from other localities (Chen et al., 2005; Coombs et al., 2006). In addition, three of our strains were resistant to ciprofloxacin, and susceptibility to fluoroquinolones as a marker for recognizing putative CA-pvl-positive MRSA strains may be unreliable (Millar et al., 2007). These findings further highlight the importance to public health and the clinical importance of pvl-positive MRSA.

Among the 42 strains, 5 MLST types, 5 PFGE types (with 80 % similarity cut-off) and 11 spa types were resolved (Table 1, Fig. 1). The most prevalent MLST types were ST30 and ST59, shared by 22 (52.4 %) and 15 (35.7 %) isolates, respectively. Further expanding on a published report by Ho et al. (2007), our study showed that the predominant CA-MRSA clones in Hong Kong possessed the genetic background ST30-IV. Our pvl-positive MRSA isolates were segregated by PFGE into 5 clusters, with 2 predominant clusters, A and E, comprising 21 and 17 strains, respectively (Fig. 1). The majority of the isolates were thus shown to be genetically related. Within PFGE group A,
<table>
<thead>
<tr>
<th>SCCmec</th>
<th>MLST</th>
<th>Ridom spa typing</th>
<th>Specimen type</th>
<th>Antibiotic resistance profile</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ST type</td>
<td>Allelic profile</td>
<td>spa type (no. of isolates)</td>
<td>spa-repeat</td>
</tr>
<tr>
<td>IV</td>
<td>ST22</td>
<td>7-6-1-5-8-8-6</td>
<td>t852 (1)</td>
<td>r07r23r13r23r31r05r17r25r17r25r16r28</td>
</tr>
<tr>
<td></td>
<td>ST30</td>
<td>2-2-2-2-6-3-2</td>
<td>t019 (18)</td>
<td>r08r16r02r16r02r25r17r24</td>
</tr>
<tr>
<td></td>
<td>ST59</td>
<td>19-23-15-2-19-20-15</td>
<td>t437 (3)</td>
<td>r04r20r17r20r17r25r34</td>
</tr>
<tr>
<td></td>
<td>ST88</td>
<td>22-1-14-23-12-4-31</td>
<td>t690 (1)</td>
<td>r07r12r21r17r33r34r34r34-33</td>
</tr>
<tr>
<td></td>
<td>ST338</td>
<td>19-23-15-48-19-20-15</td>
<td>t437 (2)</td>
<td>r04r20r17r20r17r25r34</td>
</tr>
</tbody>
</table>

CHL, Chloramphenicol; CIP, ciprofloxacin; CLI, clindamycin; ERY, erythromycin; GEM, gentamicin; POF, penicillin/oxacillin/cefotin; RIF, rifampicin; SXT, trimethoprim–sulfamethoxazole; TET, tetracycline.

*Registered as t3382.
†Registered as t3385.
**Fig. 1.** PFGE patterns and phylogenetic tree of 42 *pvl*-positive MRSA isolates from Hong Kong.
all of the strains had an ST30-IV background, while PFGE group E strains were mainly of ST59.

The spa types t019 and t437 were found in 18 (42.9 %) and 14 (33.3 %) isolates, respectively, and 2 novel spa types, t3382 and t3385 were detected in this study. Our results demonstrated that the two dominant spa types t019 and t437 were associated with ST30 and ST59, respectively. These MLST and spa-type associations have been reported in Belgium and the Netherlands as well (Denis et al., 2005; Huijsdens et al., 2006). In addition, five distinct spa types were resolved for the strains belonging to ST30, three distinct types for ST59 and two for ST88 (Table 1).

In conclusion, multilocus sequence typing at lineage level appeared concordant with MLST and PFGE because their results can easily be transferred, exchanged and compared between different laboratories. In general, genetically closely related spa types were grouped together to form a ‘lineage’, and the result of spa typing at lineage level appeared concordant with MLST and PFGE, implying that this typing method is suitable in both outbreak investigations and long-term population studies for MRSA strains.

In conclusion, prel-positive putative CA-MRSA strains in Hong Kong belonged to SCCmec types IV and V, and were mainly associated with SSTIs. Antimicrobial resistance to various groups of agents, including fluoroquinolones, was not uncommon. Most strains belonged to ST30 and ST59, and exhibited spa type t019 and t0437, respectively. Continued monitoring of the epidemiology of such strains is important to provide a basis for control measures and an evaluation of their effectiveness.

Terence Kin Man Cheung, Yiu Wai Chu, Man Yu Chu, Vivien Yee Man Tsang and Janice Yee Chi Lo

Microbiology Division, Public Health Laboratory Services Branch, Centre for Health Protection, Department of Health, Hong Kong SAR

Correspondence: Janice Yee Chi Lo (janicelo@dh.gov.hk)


