National surveillance reveals findings of Panton–Valentine leukocidin positive meticillin-resistant *Staphylococcus aureus* in Serbia

Panton–Valentine leukocidin (PVL) has been the subject of worldwide attention due to its epidemiological linkage to community-associated meticillin-resistant *Staphylococcus aureus* (CA-MRSA) (Tristan et al., 2007). Surveillance data for meticillin-resistant *S. aureus* (MRSA) has not been reported in Serbia before as the last report was before the splitting of Yugoslavia. We initiated MRSA surveillance in 2008 and report here what is to the best of our knowledge the first finding of PVL-positive MRSA in Serbia. The phenotypic and genotypic characteristics of the isolates and the clinical data related to them are reported in this article and compared to findings in other European and non-European countries.

From January to July 2008, 26 hospitals (mostly acute-care hospitals) in 17 Serbian towns (Fig. 1) agreed to participate in our study of MRSA in Serbia. One isolate per patient was included in the study. MRSA isolates were confirmed phenotypically by the BD Phoenix automated microbiology system (Becton Dickinson Diagnostic Systems), and by detection of the *nuc* and *mecA* genes using PCR (Cirkovic et al., 2008). The PMIC/ID-55 ID/AST panel was used for susceptibility testing of MRSA isolates by the BD Phoenix system (Cirkovic et al., 2008): amoxicillin/clavulanic acid, ampicillin, cefoxitin, ciprofloxacin, clindamycin, erythromycin, fusidic acid, gentamicin, gentamicin synergy, kanamycin, linezolid, mupirocin, high-level mupirocin, nitrofurantoin, oxacillin, penicillin, quinupristin/dalfopristin, rifampicin, telocoplan, tetracycline, tobramycin, trimethoprim, trimethoprim/sulfamethoxazole and vancomycin. The presence of PVL was determined by a PCR protocol described by Lina et al. (1999). *S. aureus* ATCC 49775 was used as the positive control. Determination of SCCmec types I to V was achieved by multiplex PCR (Boye et al., 2007). Multilocus sequence typing (MLST) and *spa* typing were performed for all PVL-positive MRSA isolates detected in this study according to methods described elsewhere (Enright et al., 2000; Harmsen et al., 2003).

In total, 162 MRSA isolates were collected by the 26 participating hospitals, ranging from 1 to 12 isolates per hospital. The presence of PVL genes was demonstrated in four (2.5%) MRSA isolates. These four isolates were isolated in different hospitals from younger outpatients (mean age 28.5 years) with skin infections, i.e. three (75%) with furunculosis and one (25%) with external ear infection. Two isolates were from Belgrade, whereas the other isolates were geographically dispersed (Fig. 1). MLST typing revealed that three of the isolates belonged to ST152, whereas the remaining one was an ST80 isolate. The three ST152 isolates carried a SCCmec...
Table 1. Characterization of PVL-positive MRSA isolates isolated in Serbia in 2008

<table>
<thead>
<tr>
<th>Isolate</th>
<th>MLST</th>
<th>SCCmec type</th>
<th>spa type</th>
<th>Repeat succession</th>
<th>Resistance profile</th>
<th>Town</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ST152</td>
<td>V</td>
<td>f6371</td>
<td>07-56-12-17-17-16-16-33-31-57-31-57-12</td>
<td>Kan, Tob, Gen, Tet</td>
<td>Belgrade</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td>t1183</td>
<td>07-56-13-33-31-57-12</td>
<td>Kan, Tob, Gen</td>
<td>Cacak</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td>t555</td>
<td>07-56-12-17-16-16-33-31-57-12</td>
<td>Kan, Tob, Gen, Ery, Cli</td>
<td>Belgrade</td>
</tr>
<tr>
<td>4</td>
<td>ST80</td>
<td>IV</td>
<td>f044</td>
<td>07-23-12-34-34-33-34</td>
<td>Kan, Tob, Fa</td>
<td>Kragujevac</td>
</tr>
</tbody>
</table>

Cla, Clindamycin; Ery, erythromycin; Fa, fusidic acid; Gen, gentamicin; Kan, kanamycin; Tet, tetracycline; Tob, tobramycin.

t type V element, were resistant to the tested aminoglycosides (kanamycin, tobramycin and gentamicin) and had variable resistance to tetracycline, erythromycin and clindamycin, and had different spa types (Table 1). The variation in spa types indicated that the isolates were not epidemiologically related as they differed by at least two genetic events. However, the two isolates from Belgrade shared PFGE patterns (data not shown). The ST80 (spa f044) isolate carried a SCCmec IV element, and was resistant to kanamycin, tetracycline and fusidic acid.

The proportion of PVL-positive CA-MRSA isolates was low compared to results from other European studies, where ST80-IV is especially common (Larsen et al., 2008). We did find one ST80 but none of the emerging ST8-IV f008 : USA300 isolates (Larsen et al., 2007). Despite the wide dissemination of ST80 in Europe, the Balkan countries have previously been suggested to host a reservoir as ST80 was found in Denmark among refugees from the former Yugoslavian countries (Larsen et al., 2008). The finding of a single isolate in our study confirms its presence but not its predominance in Serbia.

The finding of three ST152 isolates may, however, indicate that this clone is common in Serbia. The first ST152 deposited in the MLST database was recovered from a Danish patient in 2001 who had been hospitalized in Kosovo (Faria et al., 2005). Since then, ST152 has been noted sporadically among CA-MRSA PVL-positive isolates throughout Central Europe, especially Balkan states (former Yugoslavian countries).

In 2005, Müller-Premru et al. (2005) described ST152 (f454) isolated in Slovenia. ST152 was also recovered from 28 patients in the five cantons of Western Switzerland between 1999 and 2004. Most of these patients came from former Yugoslavian countries (Blanc et al., 2007). An association with the former Yugoslavian countries is further supported by reports of Monecke et al. (2007) who noted ST152 (f55) isolated in an immigrant Macedonian child. The ST152 clone thus seems to have spread in most European countries. Although the evidence from the literature suggests that ST152 is still relatively rare, it appears to be associated with PVL-positive CA-MRSA samples, and is distributed from the Balkan through Slovenia (a former Yugoslavian country), Austria and Germany.

Interestingly, Ruimy et al. (2008) noted a high frequency (~24%) of ST152 MRSA among the Malian population, and suggested that PVL-positive ST152 meticillin-susceptible S. aureus originated in Africa and has migrated northwards through the centre of Europe and acquired meticillin resistance.

spa typing data from Ruimy et al. (2008) described a total of 12 spa types among the 21 analysed ST152 isolates (f55, f084, r1149, r1476, 024, r127, r279, 031, r701, r774, r861 and r1215). In our study we found not only the ‘archetype’ f55 but also the rarely described r1183 [Ridom database: Sweden and Germany (www.ridom.de)] and f6371, which had not been detected previously. Given this variation in just three isolates it could strengthen the interpretation that ST152 isolates form a large and diverse reservoir in our part of Europe.

A limitation of our study was that the strains were only collected in the year 2008 and that we have not had the opportunity to continue the surveillance thereafter. However, this report serves as a baseline study for future surveillance programs of MRSA in Serbia.

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