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, teicoplanin, 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 SCC*mec* 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 SCC*mec*...
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 t044) 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., 2007). The finding of three ST152 isolates may, however, indicate that this clone is not been detected previously. Given this variation in just three isolates it could strengthen the interpretation that 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 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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