Prevalence of Panton-Valentine leukocidin genes in community-associated methicillin-resistant Staphylococcus aureus in the District of Pomoravlje

Ljiljana Petrović Jeremić, Nada Kuljić Kapulica, Dragana Jošić, Zorica Lešanović

*Center for Clinical Microbiology, Public Health Institution, Ćuprija, Serbia; †Institute for Microbiology, Military Medical Academy, Belgrade, Serbia; ‡Faculty of Medicine of the Military Medical Academy, University of Defence, Belgrade, Serbia

Abstract

Background/Aim. Community-associated methicillin-resistant Staphylococcus aureus (CA-MRSA) strains appear to have rapidly disseminated among population in the community without established risk factors for MRSA worldwide. Panton–Valentine leukocidin (PVL) is a cytolytic toxin, encoded by the lukF-PV/ and lukF-PV' genes. PVL may be the key toxin responsible for enhanced virulence of CA-MRSA. The aim of this study was to detect the genes encoding PVL in CA-MRSA isolates from healthy people from the District of Pomoravlje. Methods. We took throat and nose swabs from healthy, employed persons with mandatory sanitary examinations and analyzed the presence of MRSA, between January 2011 and December 2012 in the District of Pomoravlje. Susceptibility of isolated strains to cefoxitin was investigated by using disc diffusion according to the recommendations of CLSI (Clinical Laboratory Standard Institute), and by E test. The presence of penicillin–binding protein 2a (PBP2a) in Staphylococcus was detected using latex agglutination Slide®MRSA Detection test. The gold standard, polymerase chain reaction (PCR) assay, was used for detection of mecA gene and PVL gene, and typing of SCCmec region. Results. Our investigation showed that staphylococcal carrier state was present in 2.58% of 52,910 throat and nasal swabs, and in 50 of them (3.67%) MRSA was isolated. Among these MRSA, 2 (4%) isolates were PVL-positive. Conclusion. The prevalence of CA-MRSA and the presence of PVL gene among healthy, employed population in the District of Pomoravlje were low. The values obtained in this study show that, our region is not significantly different from the other parts of our country, nor from the other European countries.

Key words: methicillin resistance; staphylococcus aureus; community acquired infections; polymerase chain reaction; panton-valentine leukocidin; serbia.
Introduction

Until recently, methicillin-resistant *Staphylococcus aureus* (MRSA) was considered as the prototype of a nosocomial pathogen 1. Since the mid-1990s 2, this pathogen has emerged as a cause of infection in young, previously healthy people in general community, and the term community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) was established. From their health care-associated MRSA (HA-MRSA) counterparts, these isolates differ clinically, in the virulence factors, epidemiology and frequency of occurrence 3,4.

Methicillin resistance is conferred by the mecA gene, which is part of a mobile genetic element called the "staphylococcal cassette chromosome (SCC) mec". CA-MRSA and HA-MRSA can be distinguished by molecular methods, based on the differences of SCCmec region. HA-MRSA isolates carry a relatively large SCCmec belonging to type I, II, or III. Besides methicillin, they are often resistant to many classes of non-β-lactam antimicrobials. In contrast, CA-MRSA isolates harbor smaller SCCmec elements, type IV or V 5, having a size up to 30 kb, and are presumable more mobile. They are resistant to fewer non-β-lactam classes of antimicrobials.

MRSA, like other *S. aureus* strains, has numerous mechanisms to produce disease and to evade host defense. In establishing an infection, numerous surface proteins mediate adherence to host tissues or prosthetic materials. After adherence, it is able to grow and persist in various ways: it can form biofilms, invade and survive inside epithelial cells, including endothelial cells; form small-colony variants which may contribute to persistent and recurrent infection, produce antiphagocytic microcapsule that help it evade the host immune system or produce leukocidins that cause leukocyte destruction 6.

Panton-Valentine leukocidin (PVL) is a two-component *S. aureus* protein encoded by the lukF-PV and lukS-PV genes. Its ability to lyse leukocytes was first described in 1894 by Van de Vald 7. Panton and Valentine in 1932 linked the presence of leukotoxin with skin and soft tissue infections (SSTI) 8. Some authors indicate that infections with PVL-positive strains are more severe: pneumonia caused by PVL-positive MRSA or methicillin-sensitive *Staphylococcus aureus* (MSSA) strains is accompanied by high fever, sepsis, hemoptysis, pleural effusion, and even death 9. PVL is commonly observed in CA-MRSA strains, and the frequency of PVL in the United States is increasing along with the spread of CA-MRSA clones 10, 11. Subsequently, there have been reports of PVL-positive clones emerging in the hospital 12. While some authors proposed PVL as a genetic marker of CA-MRSA 13, a group of authors from Australia did not find a significant association between CA-MRSA-SCCmec type IV and PVL gene 14.

The objective of this study was to establish the prevalence of PVL in MRSA isolates associated with community.

Methods

Bacterial isolates

During 2011 and 2012 we analysed 52,910 throat and nose swab samples taken from adult, healthy population from 16 to 60 years of age, from the District of Pomoravje.

The swabs were cultured on blood agar (Bio-Merieux, France) and then incubated for 24 h aerobically at 37°C. All isolates were stored frozen in dextrose broth at -20°C, and re-cultivated on blood agar prior to each experiment. The isolates of *S. aureus* were identified by tube coagulase test with rabbit plasma (Tolrlak, Belgrade) after incubation for 4 h and 24 h. Test negative after 4 h had to be reexamined after 24 h.

Antibiotic susceptibility test

The sensitivity of *S. aureus* to methicillin and other groups of antibiotics was tested by the disk diffusion (DD) method according to the recommendation of Clinical Laboratory Standard Institute (CLSI) 15. Mueller-Hinton agar (MHA) (Bio-Merieux, France) was inoculated with suspension of 24-hour culture of staphylococci, density of 0.5 McFarland. After 15 min antibiogram disks were placed: cefoxitin (30 µg), gentamicin (30 µg), amikacin (30 µg), tetracycline (30 µg), ofloxacin (5 µg), erythromycin (15 µg), clindamycin (2 µg), trimethoprim-sulfamethoxazole – SXT (1.25 + 23.75 µg), fusidic acid (30 µg), vancomycin (30 µg) (BD, England), and incubated for 18–24 h at 35–37°C.

Methicillin resistance was also determined by agglutination test "Slidex MRSA Detection" to prove PBP2a (Bio-Merieux, France). The “Slidex MRSA Detection” test is a rapid slide agglutination assay designed to detect the presence of PBP2a in *S. aureus*. Test was performed as recommended by the manufacturer.

MIC of cefoxitin was determined by E test (Bio-Merieux, France). The test conditions recommended by the manufacturer are based and providing results comparable with CLSI methods and include incubation of inoculum whose density is equivalent to 0.5 McFarland standards, on MH agar with 2% NaCl, for 24 h at 35–37°C.

The isolates were considered CA-MRSA according to criteria established by Centers for Disease Control and Prevention (CDC) 16: they were derived from healthy people that had not been hospitalized within the preceding year.

PCR detection of the mecA gene and PVL genes and typing of SCCmec region

For PCR amplification, bacterial DNA was prepared by the use of kit for DNA isolation (B-DNA Sorb, Sacace, Italy). The resistance to methicillin was confirmed by amplifying a 162 bp fragment of mecA gene by primers and conditions described previously Oliveira et al. 17. The primers used to amplify a 433 bp region of lukF-PV genes and PCR conditions were previously described by Lina et al. 18. Typing of SCCmec region was performed using the primers and protocols described by Milheiro ando et al. 19.

Results

A total of 52,910 throat and nose swabs were analysed, and in 1,363 (2.58%) *S. aureus* was isolated. By the use of antibiogram disks with cefoxitin, E test for cefoxitin, and agglutination test for MRSA detection, and according to the
criteria established by CDC, among these *S. aureus* isolates 50 (3.67%) of them belonged to CA-MRSA. In PCR amplification with primers specific for *mecA* gene, all 50 isolates were positive and proven MRSA.

Beside cefoxitin, CA-MRSA isolates were tested for sensitivity to other groups of antibiotics: fusidic acid, SXT, quinolones, aminoglycosides, macrolides and tetracyclines. The least number of isolates was resistant to fusidic acid, only 4 (8%), to SXT 8 (16%) isolates, and to amikacin 9 (18%) isolates. Resistance to ciprofloxacin was detected in 15 (30%) of isolates, to gentamicin and clindamycin in 26 (52%) of isolates each, to erythromycin in 27 (54%), and to tetracyclin in 28 (56%) of isolates (Table 1). Our CA-MRSA isolates showed multiple drug resistance (MDR) patterns: 24 (48%) of the isolates were resistant to three or more antibiotics, 9 (18%) were resistant to two, 7 (14%) showed resistance to one antibiotic, but 10 isolates (20%) were susceptible to non-β-lactam antibiotics such as fusidic acid, SXT, quinolones, aminoglycosides, macrolides and tetracyclines.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Resistant n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetracyclin</td>
<td>28 (56)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>27 (54)</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>26 (52)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>26 (52)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>15 (30)</td>
</tr>
<tr>
<td>Amikacin</td>
<td>9 (18)</td>
</tr>
<tr>
<td>SXT</td>
<td>8 (16)</td>
</tr>
<tr>
<td>Fusidic acid</td>
<td>4 (8)</td>
</tr>
</tbody>
</table>

SXT – sulfamethoxazole/ trimethoprim.

PCR amplification with primers specific for genes encoding PVL detected these genes in only two CA-MRSA isolates (Figure 1), so the prevalence of the PVL-positive isolates was 4%. Molecular typing of two PVL-positive isolates reveal that one of them contained type IV SCCmec region, specific for CA-MRSA. Another PVL-positive isolate contained type V SCCmec region, that is also specific for CA-MRSA.

One of the PVL positive isolates was resistant to erythromycin, clindamycin gentamicin and tetracyclin, and other, except resistance to erythromycin and gentamicin, showed inducible clindamycin resistance.

**Discussion**

The anterior nares are the most frequent site of colonization for *S. aureus*. It is estimated that in some individuals (about 20%) this site is persistently colonized with *S. aureus*, while in others (about 30%) colonization is only periodical. Colonized individuals represent the main reservoir of *S. aureus*, and they contribute to the spreading of this bacteria in hospitals and community. Beside that, colonized strains are increasing the rate of infection especially in the case of host defence weakening, when they can easily be introduced.

The results of prevalence examination of *S. aureus* nasal carriage vary, depending on the studied population and study design. In this study *S. aureus* was isolated from 2.58% throat and nose swabs from healthy and employed population from the the District of Pomoravlje. In another study on healthy population in Belgrade, similar results were obtained.

The prevalence of commensal *S. aureus* nasal colonization differed significantly in European countries, and the differences could not be explained by differences in age, gender or general practitioner (GP) practice, according to a recent research published in The Lancet Infectious Diseases. A total of 32,206 nasal swabs from patients in nine countries were analysed in the study, and *S. aureus* was isolated from 6,956 (21.6%). The most extreme prevalence was in Hungary (12.1%) and in Sweden (29.4%).

In the study of von Eiff et al., conducted at a single institution, 1,640 *S. aureus* strains were isolated from nasal swabs, and 5.8% of them were methicillin resistant. Among 1,363 *S. aureus* isolates in our study, 50 (3.67%) were methicillin resistant. The nasal carriage rate of MRSA in the population of medical students in Belgrade was low: 0.37%. The discordant rates of colonization, probably, were driven by changes in the ecology and epidemiology of MRSA.

The strains of CA-MRSA carry SCCmec IV or SCCmec V, which are the smallest of the SCCs. In contrast to the multidrug-resistant nosocomial MRSA strains that carry larger SCCmec types, CA-MRSA strains are generally susceptible to several non-β-lactam antibiotics. But for some CA-MRSA strains, like epidemic clone USA300, it was noted that are becoming resistant to several non-β-lactam antibiotics.
The same situation is in the District of Pomoravlje. Compared to a similar research in 2009 25, percentage of macrolide-resistant and aminoglycoside-resistant CA-MRSA isolates is higher in this study: 54% CA-MRSA isolates resistant to erythromycin versus 42.4% in 2009, and 52% isolates resistant to gentamicin versus 30.3% in 2009. "Older" antibiotics, such as fusidic acid and SXT, have retained their activity against CA-MRSA.

The basis for the apparent increased virulence of CA-MRSA strains is incompletely understood. Because these strains usually contain PVL, which is usually absent in HA-MRSA strains, this protein is postulated by some researchers to be responsible for that 3. Highly virulent CA-MRSA strains USA400 and USA300 both harbor PVL operon and SCCmec IV. In our CA-MRSA genes encoding PVL were present in only two (4%) isolates. Typing of SCCmec region conformed on molecular level that these isolates belonged to CA-MRSA. In Austria, the percentage ranges from 3% to 7% 26, and in Portugal, among healthy children colonized with MRSA, PVL gene was detected in only 1% of isolates 27. In Canada, PVL positive CA-MRSA strains were detected in less than 5% of isolates 28. In contrast, in the study of Vandenesch et al. 9, methicillin resistance was conferred in all CA-MRSA isolates by the truncated SCCmec type IV element, and all the isolates contained the PVL locus.

In our country, the first finding of PVL-positive MRSA was reported in 2013 29. The presence of PVL genes was demonstrated in 2.5% (4 of 162) MRSA isolates from 26 hospitals in Serbia. The three of these isolates carried SCCmec type V element, and one carried SCCmec IV element.

**Conclusion**

The prevalence of MRSA among carriers in the District of Pomoravlje is 3.67%. Also, only 4% of CA-MRSA isolates are PVL-positive. Because of a low percentage, the presence of PVL gene cannot be used as a marker of PVL-MRSA.

**REFERENCES**

25. Petrović Jeremić LJ. Sensitivity of methicillin-resistant Staphylococcus aureus in hospital and non-hospital settings to the other groups of antibiotics. PONS 2009; 16: 18−25. (Serbian)