SHORT REPORT
Detection of methicillin-resistant Staphylococcus aureus carrying the mecC gene in human samples in Slovenia

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SUMMARY
Following the recognition of a mecC MRSA isolate from a patient hospitalized in the northeastern region of Slovenia, a national collection of 395 community-associated methicillin-resistant Staphylococcus aureus (CA-MRSA) isolates from 2006 to 2013 was screened. An additional six mecC MRSA strains were found and characterized as spa types t843, t9397 and t10009, and multilocus sequence type ST130. The low oxacillin minimum inhibitory concentrations and absence of the mecA gene make recognition of these MRSA strains problematical for diagnostic laboratories. In such strains the presence of mecC should be determined.

Key words: Human isolates, mecC-MRSA, Slovenia, spa types t843, t9397, t10009.

Methicillin-resistant Staphylococcus aureus (MRSA) is a major cause of hospital-associated (HA-MRSA), community-associated (CA-MRSA) and livestock-associated (LA-MRSA) infections [1]. In 2011, a novel divergent mecA gene homologue (mecaLGAlGAs), designated mecC, was discovered. This gene has less than 70% homology with the mecA gene and is associated with a novel staphylococcal cassette chromosome (SCCMec) type XI [2].

MRSA isolates harbouring the mecC gene have been reported in several European countries mainly from humans who had contact with livestock, and/or wild and domestic animals [2, 3]. Although the isolates with the mecC gene are associated with livestock, they differ from LA-MRSA [clonal complex (CC) 398] isolates related to pigs, which are highly resistant to tetracyclines used in pig production [2]. The range of infections caused by mecC-carrying MRSA is the same as seen in other S. aureus, including life-threatening diseases such as bacteraemia [1, 3].

MRSA is well-controlled in Slovenian hospitals. Some documented outbreaks include four cases of skin and soft tissue infections due to a CA-MRSA strain obtained from one hospital in 2003 and 2004 (spa type t044, sequence type (ST)80) [4] and in 2005, Panton–Valentine leukocidin (PVL)-positive CA-MRSA strains were identified in football players (spa type t002, ST5 and spa type t454, ST152) [5].
To date, mecC-positive MRSA isolates in animals, humans, or persons having direct contact with animals have not been reported or documented in Slovenia.

Case description and epidemiological investigation. The first mecC-positive MRSA was isolated from an 86-year-old female inpatient in a regional hospital in northeastern Slovenia, who was hospitalized after a stroke in April 2013. Although the patient had no risk factors for nosocomial acquisition in the previous year, namely hospitalization history or surgery, use of an indwelling catheter or other medical devices, and did not show any signs of infection, she was screened for MRSA upon admission. Nose and skin swabs taken within 48 h after admission were MRSA positive. A screening test for methicillin resistance (30-μg cefoxitin disk on Mueller–Hinton II agar; BD, USA) categorized the strain as resistant, but a slide agglutination assay for PBP2a/PBP2' (Oxoid) and PCR for the mecA gene [4] were negative. Due to these discrepancies, PCR for mecC gene was performed [2] which proved positive. The index patient lived on a farm and had contact with pigs and companion animals (cats, dog), but not with cattle and sheep. Two months after the MRSA isolation in the index patient an epidemiological investigation including sampling of the relatives, animals and farm environmental samples was performed. Throat and nose swab were taken again from the patient and environmental samples was performed using the E-test (bioMérieux, France). The strain from the index patient and mecC-positive MRSA strains from healthy carriers during routine surveillance for MRSA and four were recovered from clinical specimens from wound, skin and soft tissue infections. The oldest mecC-positive MRSA isolate was from 2007.

Susceptibility to antibiotics was tested using a standardized agar disk diffusion method according to Clinical Laboratory Standards Institute (CLSI) guidelines [8]. The mecC strain from the index patient and all six strains from the collection were resistant to penicillin and cefoxitin, but susceptible to vancomycin, gentamicin, tobramycin, kanamycin, erythromycin, clindamycin, tetracycline, ciprofloxacin, trimethoprim–sulfamethoxazole, chloramphenicol, rifampin, mupirocin and fusidic acid. The minimum inhibitory concentration (MIC) for oxacillin was performed using the E-test (bioMérieux, France). The strain from the index patient displayed an oxacillin MIC of 24 mg/l, and one strain from the collection was 2 mg/l; the remainder were between 4 and 16 mg/l.

The majority of mecC-positive MRSA strains originated from rural areas of northeastern and southern Slovenia, and only one strain originated from the western region of Slovenia. Epidemiological information about animal contact in these patients was lacking. The clustering of cases in rural areas and not in urban regions indicates that contact with livestock could be a risk factor [1, 3].
<table>
<thead>
<tr>
<th>Isolate no.</th>
<th>Isolate area in Slovenia</th>
<th>Year of isolation</th>
<th>Patient gender</th>
<th>Origin</th>
<th>MIC of oxacillin (mg/l)</th>
<th>PCR DNA microarray</th>
<th>Spa type</th>
<th>CC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>South</td>
<td>2007</td>
<td>F</td>
<td>Wound swab</td>
<td>8</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>East</td>
<td>2008</td>
<td>M</td>
<td>Wound swab</td>
<td>8</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>East</td>
<td>2008</td>
<td>M</td>
<td>Wound swab</td>
<td>2</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>South</td>
<td>2010</td>
<td>M</td>
<td>Screening swab (throat, nose, skin)</td>
<td>12</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>West</td>
<td>2012</td>
<td>M</td>
<td>Screening swab (nose)</td>
<td>16</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>East</td>
<td>2013</td>
<td>M</td>
<td>Wound swab</td>
<td>4</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7*</td>
<td>East</td>
<td>2013</td>
<td>F</td>
<td>Screening swab (nose, skin)</td>
<td>24</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

* Index patient.

F, female; M, male; MIC, minimum inhibitory concentration; PCR, polymerase chain reaction; PVL, Panton–Valentine leukocidin; lukM, leukocidin M; lukF, leukocidin F; lukS, leukocidin S; lukD, leukocidin D; lukY, leukocidin Y; hlg, haemolysin gamma; hla, haemolysin alpha; hlb, haemolysin beta; hld, haemolysin delta; edinB, epidermal cell differentiation inhibitor B; sak, staphylokinase; chp, chemotaxis inhibitory protein; scn, staphylococcal complement inhibitor; ACME, arginine deaminase; cap8, capsule type 8; icaA, intracellular adhesion protein A; icaC, intracellular adhesion protein C; icaD, biofilm PIA synthesis protein D; clfA, clumping factor A; clfB, clumping factor B; bbp, bone sialoprotein-binding protein; agr, accessory gene regulator; CC clonal complex.
Molecular characterization of mecC MRSA strains. All mecC MRSA strains were investigated by DNA microarray using StaphyType kit 2.0 (Alere Technologies GmbH, Germany) to detect genes encoding species markers, antimicrobial resistance genes, virulence genes and typing markers (SCCmec, capsule, agr) at the French National Reference Centre for Staphylococci in Lyon [9], and were spa-typed [10]. The characteristics of all seven mecC strains are shown in Table 1. The genes encoding PVL and lukM, toxic shock syndrome toxin, exfoliative toxins, ACME, enterotoxins and genes sak, cph, scn were absent. The lack of the latter genes which are involved in human immune evasion, in all seven strains could indicate their possible adaptation to animals rather than humans [2, 3]. None of the mecC strains carried genes for resistance to other antibiotics. A range of sequence types and clonal complexes have been identified in mecC strains from humans and a diverse range of animal species throughout Europe (CC49, CC130, CC425, CC599, CC1943) [2, 3]. All our strains belonged to CC130 and to three different spa types, t843 (n=5), t9397 (n=1) and t10009 (n=1). Similar results have been observed in other studies [1, 3].

In conclusion, MRSA strains positive for mecC have been present in Slovenia since 2007, but were only recognized in routine laboratory testing for MRSA in 2013. Because the oxacillin MIC can be below the cut-off point for resistance, and in the susceptible range of the CLSI breakpoint (≤ 2 mg/l), these MRSA strains may be overlooked. All microbiology laboratories should be aware of the possibility of mecC S. aureus and isolates resistant or intermediate resistant to oxacillin or cefoxitin and mecA-negative should be tested with an appropriate PCR for the mecC gene. Failure to detect these MRSA strains could have serious consequences on public health. Finally, ongoing surveillance for mecC MRSA in humans, animals, food and persons in close contact with animals is required to detect changes in MRSA epidemiology in Slovenia.

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DECLARATION OF INTEREST

None.

REFERENCES