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

U. DERMOTA[1]*, I. ZDOVC[2], I. STRUMBELJ[1], I. GRMEK-KOSNIK[1], H. RIBIC[1], M. RUPNIK[1,3,4], M. GOLOB[2], U. ZAJC[2], M. BES[5], F. LAURENT[5] AND M. MUELLER-PREMRU[6]

[1] *National Laboratory for Health, Environment and Food, Maribor, Slovenia*
[2] *University of Ljubljana, Veterinary Faculty, Institute of Microbiology and Parasitology, Ljubljana, Slovenia*
[3] *Faculty of Medicine, University of Maribor, Maribor, Slovenia*
[4] *Centre of Excellence for Integrated Approaches in Chemistry and Biology of Proteins, Ljubljana, Slovenia*
[5] *French National Reference Centre for Staphylococci, Lyon, France and Hospices Civils de Lyon, Lyon, France*
[6] *Institute of Microbiology and Immunology, University of Ljubljana, Faculty of Medicine, Ljubljana, Slovenia*

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SUMMARY

Following the recognition of a *mec*C 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 *mec*C 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 *mec*A gene make recognition of these MRSA strains problematical for diagnostic laboratories. In such strains the presence of *mec*C 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 *mec*A gene homologue (*mec*A_{LGA251}), designated *mec*C, was discovered. This gene has less than 70% homology with the *mec*A gene and is associated with a novel staphylococcal cassette chromosome (SCC*mec*) type XI [2].

MRSA isolates harbouring the *mec*C 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 *mec*C 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 *mec*C-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].

* Author for correspondence: Mrs U. Dermota, National Laboratory for Health, Environment and Food, Department of Medical Microbiology Kranj, Gosposvetska ulica 12, SI-4000 Kranj, Slovenia.
(Email: urska.dermota@nlzoh.si)

To date, *mec*C-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 *mec*C-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 *mec*A gene [4] were negative. Due to these discrepancies, PCR for *mec*C 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 from all members of the family (husband, daughter, son-in-law). Nasal swabs were taken from seven clinically healthy piglets and dust samples from their environment. All the samples were analysed according to the protocol used in a baseline study on MRSA in holdings of breeding pigs to assess the prevalence and diversity of MRSA in pig primary production [6].
Only the index patient was again positive for *mec*C MRSA, while all other samples from humans, animals and the environment were negative. Therefore the source and origin of the index patient's *mec*C-positive MRSA isolate remained unclear.

Screening for mecC-positive strains in a CA-MRSA collection. Among MRSA carrying the *mec*C gene, antimicrobial susceptibility patterns are most similar to CA-MRSA, in being classically susceptible to the majority of non-β-lactam antibiotics [7]. Clinical data also indicate that *mec*C MRSA are primarily community-associated. As we do not routinely screen for *mec* genes in phenotypically confirmed MRSA in Slovenia, we therefore sought to identify additional *mec*C strains through a retrospective screen survey of *mec* genes in the Slovenian national collection of presumptive CA-MRSA isolates. This collection is maintained at the National Laboratory for Health, Environment and Food (NLZOH) and currently contains 395 isolates recovered since 2006. Inclusion criteria for the presumptive CA-MRSA collection were based on phenotypic definition of being resistant to cefoxitin and oxacillin, and susceptible to at least *two* of the following four antibiotics: ciprofloxacin, erythromycin, clindamycin or gentamicin. All isolates in the collection were further genotypically characterized by SCC*mec* type, presence of PVL, and *spa* type. The majority of isolates met the latest relevant molecular definition of CA-MRSA [1]. No replicate MRSA isolates from the same patient were included. The *mec*A gene was detected in 385 (97·5%) isolates while 10 (2·5%) were *mec*A-negative. Six of these 10 were positive for the *mec*C gene. The remainder remain under investigation of the genetic basis of β-lactam resistance. Two of the *mec*C-positive MRSA strains were isolated from healthy carriers during routine surveillance for MRSA and four were recovered from clinical specimens from wound, skin and soft tissue infections. The oldest *mec*C-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 *mec*C 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].

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Table 1. *Characterization of seven* mec*C-positive MRSA isolated from humans in Slovenia, 2006–2013*

Isolate no.	Isolate area in Slovenia	Year of isolation	Patient gender	Origin	MIC of oxacillin (mg/l)	PCR		DNA microarray												
						***mec*A**	***mec*C**	PVL *luk*M	*luk*F/*luk*S/ *luk*D/*luk*Y/ *hlg*A	*hla* *hlb* *hld*	*edinB*	*sak* *chp* *scn*	ACME	*cap8*	*icaA* *icaC* *icaD*	*clfA* *clfB* *bbp*	*agr*	*Spa* type	CC
1	South	2007	F	Wound swab	8	–	+	–	+	+	+	–	–	+	+	+	III	t843	130
2	East	2008	M	Wound swab	8	–	+	–	+	+	+	–	–	+	+	+	III	t10009	130
3	East	2008	M	Wound swab	2	–	+	–	+	+	+	–	–	+	+	+	III	t843	130
4	South	2010	M	Screening swab (throat, nose, skin)	12	–	+	–	+	+	+	–	–	+	+	+	III	t843	130
5	West	2012	F	Screening swab (nose)	16	–	+	–	+	+	+	–	–	+	+	+	III	t9397	130
6	East	2013	M	Wound swab	4	–	+	–	+	+	+	–	–	+	+	+	III	t843	130
7*	East	2013	F	Screening swab (nose, skin)	24	–	+	–	+	+	+	–	–	+	+	+	III	t843	130

* Index patient.

F, female; M, male; MIC, minimum inhibitory concentration; PCR, polymerase chain reaction; PVL, Panton–Valentine leukocidin; *luk*M, leukocidin M; *luk*F, leukocidin F; *luk*S, leukocidin S; *luk*D, leukocidin D; *luk*Y, 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 *mec*C 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 (SCC*mec*, capsule, *agr*) at the French National Reference Centre for Staphylococci in Lyon [9], and were s*pa*-typed [10]. The characteristics of all seven *mec*C strains are shown in Table 1. The genes encoding PVL and *luk*M, toxic shock syndrome toxin, exfoliative toxins, ACME, enterotoxins and genes *sak, chp, 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 *mec*C strains carried genes for resistance to other antibiotics.

A range of sequence types and clonal complexes have been identified in *mec*C 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 *mec*C 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 ($\leqslant 2$ mg/l), these MRSA strains may be overlooked. All microbiology laboratories should be aware of the possibility of *mec*C *S. aureus* and isolates resistant or intermediately resistant to oxacillin or cefoxitin and *mec*A-negative should be tested with an appropriate PCR for the *mec*C gene. Failure to detect these MRSA strains could have serious consequences on public health. Finally, ongoing surveillance for *mec*C 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.

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