

INFECTIONS CAUSED BY COMMUNITY-ASSOCIATED METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* EUROPEAN CLONE (ST80) IN SLOVENIA BETWEEN 2006 AND 2013

PRIKAZ PRIMEROV OKUŽB, POVZROČENIH S PROTI METICILINU ODPORNO BAKTERIJO *STAPHYLOCOCCUS AUREUS*, DOMAČEGA OKOLJA, KI PRIPADA EVROPSKEMU KLONU (ST80) V SLOVENIJI V OBDOBJU MED 2006 IN 2013

Urška DERMOTA^{1*}, Tomaž JURCA², Tatjana HARLANDER³, Marta KOŠIR⁴, Urška ZAJC⁵,
Majda GOLOB⁵, Irena ZDOVC⁵, Irena GRMEK KOŠNIK¹

¹National Laboratory for Health, Environment and Food (NLZOH), Department of Medical Microbiology, Gosposvetska ulica 12, 4000 Kranj, Slovenia

²University Medical Centre Ljubljana, Department of Infectious Diseases, Japljeva 2, 1000 Ljubljana, Slovenia

³National Laboratory for Health, Environment and Food (NLZOH), Department of Medical Microbiology, Mej vrti 5, 8000 Novo mesto, Slovenia

⁴National Institute of Public Health (NIJZ), Regional Unit Novo mesto, Mej vrti 5, 8000 Novo mesto, Slovenia

⁵Institute of Microbiology and Parasitology, University of Ljubljana, Veterinary Faculty, Gerbičeva 60, 1000 Ljubljana, Slovenia

Received: Sep 9, 2015
Accepted: Dec 9, 2015

Original scientific article

ABSTRACT

Introduction. According to the existing literature, a heterogeneous sequence type (ST) or clones of community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) circulate in Europe. In Europe, the European clone that belongs to sequence type ST80 is predominant.

Keywords:

CA-MRSA, ST80 (European clone), invasive infections, death, Slovenia

Methods. The aim of the study was to investigate the phenotypic and genotypic characteristics and epidemiological data of CA-MRSA ST80 and its occurrence in Slovenia. We retrospectively analyzed those CA-MRSA isolates that were isolated during microbiological procedures in microbiological laboratories between 2006 and 2013. Only CA-MRSA isolates from the national collection of CA-MRSA strains that belonged to ST80 (European clone) were analyzed. We determined the Pantone-Valentine leukocidin (PVL), *mecA* genes, exfoliative toxin genes and type of staphylococcal cassette chromosome (SCC*mec*) by polymerase chain reaction (PCR). We determined also *spa* type and sequence type.

Results. ST80 was confirmed in only 2 (0.5%) out of 385 CA-MRSA isolates, collected in a national collection of CA-MRSA. Both isolates were positive for the PVL genes, *mecA* gene, exfoliative toxin type D gene and SCC*mec* IV. One CA-MRSA isolate was confirmed in a wound swab taken from a 47-year-old male, and the second was isolated from blood cultures of a 69-year-old female. No epidemiological connections between them were found.

Conclusions. In Slovenia CA-MRSA infections caused by ST80 are rare. In the future, it is necessary that a surveillance study of CA-MRSA at the national level continues and CA-MRSA be considered as a public health threat.

IZVLEČEK

Izhodišča. Po podatkih iz literature kroži v Evropi zelo heterogena skupina sekvenčnih tipov (ST) in klonov proti meticilinu odporne bakterije *Staphylococcus aureus*, domačega okolja (CA-MRSA). V Evropi med CA-MRSA prevladuje evropski klon, ki pripada sekvenčnemu tipu ST80.

Gljučne besede:

CA-MRSA, ST80 (evropski klon), invazivne okužbe, smrt, Slovenija

Metode. V raziskavi smo želeli pridobiti informacijo o fenotipskih in genotipskih lastnostih ter epidemiološke podatke o CA-MRSA, ki pripadajo sekvenčnemu tipu ST80 in njihovo razširjenost v Sloveniji. Retrospektivno smo pregledali izolate CA-MRSA, ki so bili osamljeni med rutinsko mikrobiološko diagnostiko v mikrobioloških laboratorijih v obdobju od 2006 do 2013. Analizirali smo le izolate CA-MRSA, ki so bili vključeni v nacionalno zbirko izolatov CA-MRSA in so pripadali sekvenčnemu tipu ST80 (evropski klon). Za lažjo opredelitev CA-MRSA ST80 smo uporabili tudi verižno reakcijo s polimerazo (PCR), s katero smo določili tip kasete stafilokoknega kromosoma (SCC*mec*), gene *mecA*, levkocidina Pantone-Valentine (PVL) in tip stafilokoknega eksfoliativnega toksina. Izolatoma CA-MRSA smo določili tudi tip *spa* in sekvenčni tip.

Rezultati. ST80 smo potrdili pri dveh (0,5%) od 385 izolatov CA-MRSA, zbranih v nacionalni zbirki CA-MRSA. Pri obeh izolatih smo dokazali gene PVL, *mecA*, eksfoliativni toksin tipa D in tip SCC*mec* IV. En izolat CA-MRSA smo dokazali iz brisa rane 47-letnega bolnika, drug izolat pa pri 69-letni bolnici iz hemokulture. Oba primera nista bila epidemiološko povezana.

Zaključki. Okužbe, povzročene s CA-MRSA ST80 v Sloveniji, so redke. V prihodnosti je pomembno, da s sledenjem CA-MRSA na nacionalnem nivoju nadaljujemo, saj CA-MRSA po svetu predstavljajo novo grožnjo javnemu zdravju prebivalcev.

*Corresponding author: Tel: ++ 386 4 20 17 171; E-mail: urska.dermota@nlzoh.si

1 INTRODUCTION

Community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) is in Europe associated mainly with the European clone which belongs to the clonal complex (CC) / sequence type (ST) CC80 / ST80 (1-6). This clone was first recognized in Denmark in 1993 (7, 8) and now CA-MRSA ST80 is widely spread throughout Europe. Epidemiological data presume that this clone originated outside Europe, in the Middle East or in North Africa (1, 3, 5-6).

CA-MRSA ST80 is typically resistant to fusidic acid, kanamycin / amikacin and tetracycline (1). In Kuwait, a high-level mupirocin resistant CA-MRSA strain ST80 was found (1, 3, 5). The majority of CA-MRSA ST80 isolates carry Pantone-Valentine leukocidine (PVL) genes, staphylococcal cassette chromosome (SCCmec) IV and exfoliative toxin type D gene (*etd*) (3, 5-8). In France and in Croatia, a PVL-negative variant of CA-MRSA ST80 has been described (6). The European clone is associated mainly with skin and soft-tissue infections, but rarely causes other invasive infections such as bacteremia or meningitis (3, 4).

We have described the first PVL positive CA-MRSA ST80-IV isolates in Slovenia in four patients hospitalized in one Slovenian hospital in 2003 and 2004 (9). These CA-MRSA isolates were associated with skin and soft-tissue infections: one patient developed meningitis. All CA-MRSA isolates were typically resistant to beta-lactam antibiotics, kanamycin, tetracycline and fusidic acid and harboured PVL and *etd* genes (9).

In Slovenia, a national monitoring of CA-MRSA strains began in the year 2006. In a national collection obtained at medical microbiology department of the National Laboratory for Health, Environment and Food only CA-MRSA isolates that were resistant to oxacillin and cefoxitin and susceptible to at least two of the following four antibiotics, ciprofloxacin, erythromycin, clindamycin and gentamicin (screening definition of the presumptive CA-MRSA) were included (10-12). Dominant clones identified were ST5, ST45, ST22 and ST398. The authors, in their previous studies, confirmed that genetically heterogeneous CA-MRSA clones circulate in our country (11, 12).

To our knowledge, no case of death due to CA-MRSA ST80 has been reported in our country. Because ST80 is one of the predominant clones in Europe and because ST80 was confirmed in Slovenia in the years 2003 and 2004, the aim of the current study was to investigate the presence of the CA-MRSA ST80 strain among human samples in our country. The study describes the detailed characterization by epidemiological investigation, antimicrobial resistance pattern, toxin gene and molecular profiling.

2 METHODS

2.1 MRSA Surveillance in October 2013

In October 2013, a blood culture from a 69-year-old female yielded MRSA. She had a transsphenoidal biopsy of a large parasellar meningioma and was prior to surgery without health-care risk factors. Subsequent screening swabs from the nose and tracheal aspirate yielded CA-MRSA. In an attempt to identify the source of the CA-MRSA, an epidemiological investigation was started, and surveillance swabs (throat, nose, groin) were obtained from all her available household members, nose swabs from domestic animals (pigs, goats, poultry) and dust samples from the farm environment.

2.2 Retrospective Analyses of CA-MRSA Isolates Collected in a National Collection of Presumptive CA-MRSA Isolates

We reviewed presumptive CA-MRSA isolates in the strain collection database from 2006 to 2013. Only CA-MRSA that belonged to ST80 were included in further analyses.

2.3 Patients' Data and Case Definition

A trace back epidemiological investigation for each patient with CA-MRSA ST80 isolate was performed. Several features were collected from the medical report: patient characteristics (demographic data, clinical data), treatment and outcome. A CA-MRSA was defined as a strain isolated from ambulatory patients or from inpatients within 48 hours of hospital admission, with no risk factors for nosocomial acquisition in the previous year, such as colonisation or infection with MRSA, hospitalization or residence in long-term care facilities, surgery or use of an indwelling catheter. All other isolates were considered as HA-MRSA.

2.4 Bacterial Isolates and Antimicrobial Susceptibility Testing

All *S. aureus* isolates were identified by mass spectrometry (MALDI-TOF, Biotyper, Bruker Daltonic GmbH, Bremen, Germany). The susceptibility of CA-MRSA isolates was tested against 16 antimicrobial agents using the disk diffusion method according to the guidelines of the Clinical Laboratory Standard Institute (CLSI) (13). The antibiotics tested were penicillin, cefoxitin, vancomycin, gentamicin, tobramycin, kanamycin, erythromycin, clindamycin, tetracycline, ciprofloxacin, trimethoprim-sulfamethoxazole, chloramphenicol, rifampin, linezolid, mupirocin and fusidic acid (BD, Maryland, USA). Minimal inhibitory concentration (MIC) determination of oxacillin was performed using the E-test (bioMérieux, Marcy l'Etoile, France).

2.5 Molecular Characterization

MRSA isolates were screened by PCR (polymerase chain reaction) for the PVL encoding *lukS-PV* and *lukF-PV* genes and *mecA* gene using Genotype Staphylococcus (Hain Lifesciences, Germany). The identification of the *SCCmec* types and the presence of exfoliative genes (*eta*, *etb*, *etd*) was performed using primers as described previously (14-15). MRSA isolates with a positive phenotypic screening pattern were characterized by *spa* typing according to a method described previously (16). Multilocus sequence typing (MLST) was performed as described by Enright et al (17). ST designations were assigned by the MLST database (available from: <http://www.mlst.net>).

3 RESULTS

3.1 Isolate Characteristics

Between 2006 and 2013, among the 385 CA-MRSA isolates included in our national collections, only 2 (0.5%) were identified with resistance to penicillin, cefoxitin, kanamycin, tetracycline and fusidic acid and susceptibility to vancomycin, gentamicin, tobramycin, erythromycin, clindamycin, ciprofloxacin, trimethoprim-sulfamethoxazole, chloramphenicol, rifampin, linezolid and mupirocin.

Both CA-MRSA isolates had MIC of oxacillin between 16-64 mg/L and MIC of vancomycin 2 mg/L. PVL and *mecA* genes and *SCCmec* IV were confirmed in both CA-MRSA isolates. Both CA-MRSA isolates were associated to ST80.

3.2 Clinical Characteristics of MRSA Infection

One CA-MRSA isolate was isolated from a wound swab and one from blood culture. No CA-MRSA ST80 were found between 2006 and 2012. Epidemiological investigations showed that both CA-MRSA ST80 isolates were detected in the year 2013, in different regions of Slovenia, and no connection between them was found.

First CA-MRSA ST80 was confirmed in a 47-year-old male. The patient had no health-care associated risk factors for MRSA colonization or infection, and no surveillance swabs for MRSA were taken. He had clinical signs of a wound infection and bacterial sampling was performed in a surgical emergency department. Initial antibiotic therapy was inappropriate (amoxicillin/clavunate) and infection was cured with clindamycin.

Epidemiological investigation showed that the second CA-MRSA ST80 strain was isolated from a 69-year-old female. The patient was previously healthy with no known risk factors for MRSA infection. Upon admission for transsphenoidal biopsy of a large parasellar meningioma, no surveillance swabs for MRSA were taken. Infection of purulent meningitis occurred thirteen days after the first admission. A head CT scan showed inflammation in the

region where the biopsy had been performed and a lumbar puncture confirmed meningitis. Intravenous therapy with cefepime (2 g/6 hour) and vancomycin (3 g/day) was started. She was intubated and mechanically ventilated. The patient remained febrile despite the treatment and vancomycin was changed to daptomycin (10 mg/kg). Clindamycin was added, because she also developed pneumonia. Cerebrospinal fluid remained sterile but eubacterial PCR yielded *S. aureus*. Transesophageal Echo was negative. Despite several cleanings of the nose and paranasal sinuses the control CT scan revealed local deterioration. New ischaemic brain lesions were also seen. Ethmoidectomy and meatotomy of paranasal sinuses were performed. Patient slowly improved. Upon waking up, she started to bleed massively from both nostrils. Nasal tamponade was inserted and she received a transfusion of red blood cells. The massive bleeding repeated itself and she was unsuccessfully resuscitated. She died 25 days after the first surgery (Table 1).

3.3 MRSA Surveillance in October 2013

All human, animal and farm environmental samples tested in an association with 69-year-old female patient were MRSA negative.

4 DISCUSSION

The frequency of CA-MRSA ST80 varies from < 5% in Spain to 92% in Greece (1). A high frequency of ST80 strain is also documented in Kuwait, Lebanon, Israel, Egypt, Algeria and Tunisia, suggesting their clonal origin (1, 4, 6). According to the authors, CA-MRSA ST80 is also circulating in our neighboring countries (Italy, Austria, Croatia) (6), but surprisingly CA-MRSA ST80 is not very common in Slovenia. To date, infections caused by CA-MRSA ST80 seemed to be sporadic cases. In our previous study that lasted from 2003 to 2004, we confirmed CA-MRSA ST80 isolates in four hospitalized patients following necrotizing soft tissue infection, purulent abscesses and epidural catheter infection and meningitis (9). Between 2006 and 2013, we found only 2 (0.5%) CA-MRSA ST80 strains among 385 presumptive CA-MRSA isolates. Both CA-MRSA ST80 isolates were confirmed in the year 2013, in different regions of Slovenia, and no epidemiological connections between these two infections were found. According to our epidemiological investigation, both of the patients with CA-MRSA infection were in good health prior to their infections. The first patient had a wound infection, without health-care associated risk factors, and no surveillance samples for MRSA carriage were taken. The patient was treated in a surgical emergency department without hospital admission. However, it cannot be excluded that the patient was colonized with CA-MRSA prior to surgical procedure.

Table 1. Phenotypical, genotypical and epidemiological characteristics and clinical data from patients infected with CA-MRSA ST80 strain in Slovenia between 2006 and 2013.

Characteristic		
Gender	M	F
Age (years)	47	69
Diagnosis at hospital admission	Abscessus glutei	Meningeoma
Source of isolates	Wound swab	Blood cultures, tracheal aspirate
Type of infection	SSTI	Sepsis and meningitis
Outcome	recovered	death
Risk factors for MRSA colonization		
History of MRSA infection / colonization	No	No
Surgery in the past year	No	Yes
Hospitalization in the past year	No	Yes
Residence in a day care	No	No
Resistance pattern	P, OX, TE, FA, K	P, OX, TE, FA, K
MIC of oxacillin (mg/L)	16	64
Typing		
SCCmec type	IV	IV
spa type	t044	t044
MLST (ST)	80	80
Toxin gene profile		
PVL	+	+
etd	+	+

Legend:

M male, F female, SSTI skin and soft tissue infection, MIC minimal inhibitory concentration, PVL Panton-Valentine Leukocidin, MLST multi locus sequence typing, ST sequence type, SCCmec staphylococcal cassette chromosome *mec*, SCCmec type IV, *etd* exfoliative toxin gene type D, FA fusidic acid, K kanamycin, Te tetracycline, P penicillin, OX oxacillin (cefoxitin)

The second patient developed a serious infection caused by CA-MRSA ST80 after surgery and no surveillance samples for CA-MRSA carriage were taken prior to surgery. CA-MRSA was isolated from the patient's blood culture, tracheal aspirate, nose swab and cerebrospinal fluid. In an attempt to identify the source of the CA-MRSA, surveillance swabs were taken from the patient's husband. Because the patient was living on a farm, nasal swabs were also taken from clinically healthy piglets and dust samples from their environment. All samples tested were MRSA negative. The major limitation of our study was the lack of extensive staff and hospital environment screening. Therefore, the source of the patient's CA-MRSA isolate remained unclear. Based on clinical data, epidemiological investigation and lack of health-care risk factors, we predicted that CA-MRSA in the 69-year-old female was transmitted by the hands of the personnel temporarily colonized with bacteria or contaminated medical equipment during surgery, or the patient was colonized with CA-MRSA in the community. Upon confirmation of CA-MRSA isolate in the patient's specimens, the standard and contact precautions for preventing MRSA transmission in hospital were introduced. Despite appropriate antibiotic therapy, the patient died and, to our knowledge, this was the first documented death caused by CA-MRSA ST80 in Slovenia.

According to our results, we suspect that infections caused by CA-MRSA ST80 in Slovenia remain relatively low and are underestimated. Firstly, the epidemiological investigation of a patient's infections is performed only when atypical MRSA isolate is recovered from patient's specimens, such as resistance to tetracycline, which is associated with LA-MRSA (4). Secondly, phenotypically confirmed MRSA is not routinely tested for genotypic characteristics, such as spa type or ST. Thirdly, no information is available about the occurrence of CA-MRSA among healthy carriers that are a potential source of infections in the community. Finally, clinical samples are not routinely cultured, but only upon inappropriate therapy or progressing infection. In conclusion, we also speculate that migration will likely increase CA-MRSA carriage and infections in the near future also in our country.

In 2014, on behalf of the National Institute of Public Health of Slovenia, two surveillance programs were assigned to monitor CA-MRSA and LA-MRSA incidence on the national level. To date, CA-MRSA infections caused by the ST80 strain are rare in Slovenia, but molecular testing, epidemiological investigations and surveillance studies of CA-MRSA and LA-MRSA are needed to control and monitor these pathogens that are considered a public health threat all over the world.

ACKNOWLEDGEMENTS

The authors are grateful to Iztok Štrumbelj, Martina Kavčič, Tatjana Harlander, Tjaša Žohar-Čretnik, Barbara Zdolšek, Živa Petrovič, Slavica Robnik Lorenčič, Ljudmila Sarjanovič, Ingrid Berce (National Laboratory for Health, Environment and Food) and Jerneja Fišer, General Hospital dr. Franc Derganc, for providing the presumptive CA-MRSA isolates.

CONFLICTS OF INTEREST

The authors declare that no conflicts of interest exist.

FUNDING

None.

ETHICAL APPROVAL

All the data analysed in this study were collected at the National Laboratory for Health, Environment and Food without information about the identity of individuals diagnosed with CA-MRSA infections according to the Contagious Diseases Act, Health Care Databases Act and Communicable Diseases Reporting Regulation. The study was conducted in accordance with the code of Ethics of the World Medical Association (Declaration of Helsinki).

REFERENCES

- Chua K, Laurent F, Coombs G, Lindsay Grayson M, Howden BP. Not community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA)!: a clinician's guide to community MRSA - its evolving antimicrobial resistance and implications for therapy. *Clin Infect Dis* 2011; 52: 99-114.
- Deurenberg RH, Stobberingh EE. The evolution of *Staphylococcus aureus*. *Infect Genet Evol* 2008; 8: 747-63.
- Otter JA, French GL. Molecular epidemiology of community-associated methicillin-resistant *Staphylococcus aureus* in Europe. *Lancet Infect Dis* 2010; 10: 227-39.
- David MZ, Daum RS. Community-associated methicillin-resistant *Staphylococcus aureus*: epidemiology and clinical consequences of an emerging epidemic. *Clin Microbiol Rev* 2010; 23: 616-87.
- Mediavilla JR, Chen L, Mathema B, Kreiswirth BN. Global epidemiology of community-associated methicillin resistant *Staphylococcus aureus* (CA-MRSA). *Curr Opin Microbiol* 2012; 15: 588-95.
- Monecke S, Coombs G, Shore AC, Coleman DC, Akpaka P, Borg M. et al. A field guide to pandemic, epidemic and sporadic clones of methicillin-resistant *Staphylococcus aureus*. *PLoS ONE* 2010; 6: e17936.
- Faria NA, Oliveira DC, Westh H, Monnet DL, Larsen AR, Skov R. et al. Epidemiology of emerging methicillin-resistant *Staphylococcus aureus* (MRSA) in Denmark: a nationwide study in a country with low prevalence of MRSA infection. *J Clin Microbiol* 2005; 43: 1836-42.
- Stegger M, Wirth T, Andersen PS, Skov RL, De Grassi A, Simões PM. et al. Origin and evolution of European community-acquired methicillin-resistant *Staphylococcus aureus*. *mBio* 5: e01044-14.
- Dermota U, Grmek-Košnik I, Ravnik M, Budimir A, Ribič H, Cerkenik-Škafar A. First report of community-acquired methicillin-resistant *Staphylococcus aureus* from a Slovenian hospital. *J Hosp Infect* 2011; 79: 271-2.
- Grmek-Košnik I, Dermota U, Ribič H, Ravnik M, Kavčič M, Harlander T. et al. Analysis of Slovenian MRSA strains with susceptibility patterns suggestive of CA-MRSA. *Wien Klin Wochenschr* 2009; 121: 552-7.
- Grmek-Košnik I, Helena R, Rupnik M, Dermota U. Epidemiological surveillance of CA-MRSA infections in Slovenia between 2006 and 2013. In: 5th Southeast European Conference on Chemotherapy and Infection. Bled, 2014: 149.
- Dermota U, Mueller-Premru M, Švent-Kučina N, Petrovič Ž, Ribič H, Rupnik M. et al. Survey of community-associated-methicillin-resistant *Staphylococcus aureus* in Slovenia: identification of community-associated and livestock-associated clones. *Int J Med Microbiol* 2015; 305: 505-10.
- CLSI. Performance Standards for Antimicrobial Susceptibility Testing; Eighteenth Informational Supplement. CLSI document M100-S20. Wayne, PA: Clinical and Laboratory Standards Institute, 2010.
- Milheirico C, Oliveira DC, de Lencastre H. Update to the multiplex PCR strategy for assignment of *mec* element types in *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2007; 51: 3374-7.
- Monday SR, Bohach GA. Use of multiplex PCR to detect classical and newly described pyrogenic toxin genes in staphylococcal isolates. *J Clin Microbiol* 1999; 37: 3411-4.
- Harmsen D, Claus H, Witte W, Rothgänger J, Turnwald D, Vogel U. Typing of methicillin-resistant *Staphylococcus aureus* in a university hospital setting by using novel software for spa repeat determination and database management. *J Clin Microbiol* 2003; 41: 5442-8.
- Enright MC, Day NP, Davies CE, Peacock SJ, Spratt BG. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. *J Clin Microbiol* 2000; 38: 1008-15.