ORIGINAL ARTICLE

Characterization and clonal representation of MRSA strains in Tuzla Canton, Bosnia and Herzegovina, from 2009 to 2017

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ABSTRACT

Aim To characterize methicillin-resistant *S. aureus* (MRSA) strains phenotypically and genotypically and to determine their clonal affiliation, representation and antibiotic resistance profile.

Methods A total of 62 randomly selected MRSA isolates of different clinical samples collected from 2009 to 2017 were phenotypically and genotypically analysed. Phenotypic analyses were performed by standard microbiological procedures, and using VITEK 2/AES instrument as well as MALDI-TOF (matrix-assisted laser desorption/ionization) technology. Genotypic characterization included *spa*, MLST (multilocus sequence typing) and SCC*mec* typing, and detection of the Panton-Valentine leukocidin (PVL) and other enterotoxin encoding genes.

Results The largest number of isolates, 21 (33.87%) belonged to ST228-MRSA-I, *spa* type t041, t1003 and t001. Other major clones were: ST239-MRSA-III, *spa* type t037 and t030 (27.41%); ST8-MRSA-IV, *spa* type t008 and t121 (12.9%); ST247-MRSA-I, *spa* type t051 (4.83%). PVL was detected in 10 isolates (SCC*mec* IV/V). During 2009 and 2010 the most frequent MRSA strain was South German clone, ST228-MRSA-I (80% and 90%, respectively), while in later years it was replaced with Brazilian-Hungarian clone ST239-MRSA-III (75% in 2015 and 2016). The South German clone, *spa* type t041 in 90.48% of cases was resistant to clindamycin, ciprofloxacin, erythromycin, cefoxitin, gentamicin, kanamycin, tobramycin and penicillin, while 70.58% samples of the Brazilian-Hungarian clone *spa* type t037 were additionally resistant to tetracycline and rifampicin.

Conclusion This research can supplement the existing knowledge about the clonal distribution of MRSA in Bosnia and Herzegovina and their sensitivity to antibiotics in order to improve the national control of these infections.

Keywords: antibiotic resistance, multi-locus sequence typing (MLST), *SCC*mec, *spa* typing

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INTRODUCTION

Staphylococcus aureus (*S. aureus*) is the causative agent of many human infections, including nosocomial ones (1). Antibiotic resistance increases the complexity in the treatment of *S. aureus* infections, especially infections caused by methicillin-resistant *S. aureus* (MRSA) that developed methicillin-resistance by the acquisition of *mecA* or *mecC* gene (2).

Initially, only hospital-associated MRSA (HA-MRSA) infections were present, but in the 1990s, community-associated MRSA (CA-MRSA) was found to disseminate among healthy individuals in Australia and the United States (3). Unlike HA-MRSA, CA-MRSA isolates were non multidrug resistant and genetically different from other MRSA strains present at that time (4). Generally, CA-MRSA is susceptible to narrow-spectrum non-beta lactams such as clindamycin, tetracyclines and trimethoprim sulfamethoxazole, while resistant to penicillin, oxacillin and erythromycin, where HA-MRSA is multiresistant (4,5).

Gene(s) responsible for resistance to methicillin and other beta-lactam antibiotics are situated on a mobile genetic element, the staphylococcal cassette chromosome *mec* (SCC*mec*). Seven major variants of SCC*mec* (I-VII) have been detected (6). Generally, HA-MRSA harbours SCC*mec* type I-III, while CA-MRSA carries SCC*mec* type IV, V or VII (7). In addition, CA-MRSA often contains the genes encoding Panton-Valentine leukocidin (PVL) toxin associated with skin and soft tissue infections, sometimes considered a marker of CA-MRSA infections (6,7). However, in recent years, this genetic diversity between HA-MRSA and CA-MRSA has started to fade, with no single distinguishing characteristic between them. PVL positive, multidrug-resistant HA-MRSA carrying SCC*mec* IV/V, as well as a portion of multiresistant CA-MRSA strains have been reported (6-8).

The global epidemiology of MRSA is heterogeneous, where the dominance and presence of certain clones differ between geographic regions (9). Numerous phenotypic and genotypic techniques are used for monitoring and typing of MRSA isolates, with the notable advantage of the later ones (10). Selecting the most appropriate typing tool in terms of cost, performance and interpretation can be challenging. Antibiogram typing is the main method used in most hospital outbreaks since it is highly available and standardized, although with a disadvantage in resistance variability (11). The most reliable typing methods are pulse-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST). PFGE, although the gold standard, is very demanding, expensive and difficult to reproduce between laboratories (5). MLST is a preferred technique because data can be exchanged between laboratories. First, MLST is used to group strains into sequence types (STs), and then closely related STs into clonal complexes (CCs). MLST is used in combination with PCR analysis of SCC*mec* element for the determination of MRSA clonal types (12).

Moreover, *spa* typing is one of the least laborious and inexpensive MRSA typing methods based on the DNA sequencing of the polymorphic X region of staphylococcal protein A (*spa*) (12). *Spa* typing enables more precise analysis of MRSA strains, allowing the evolution of the molecular epidemiology of MRSA to be examined (13).

The nomenclature of MRSA is currently based on the ST type and the type of SCC*mec* element. The majority of HA-MRSA clones belong to five phylogenetically distinct CCs: CC5 (ST5-I, -II, -IV, -VI; ST228-I), CC8 (ST 247-I, ST239-III, ST8-IV), CC22 (ST22-IV), CC30 (ST36-II) and CC45 (ST45-II, -IV) (6). During the last two decades, several CA-MRSA clones have emerged worldwide: CC80 (ST80-IV), CC30 (ST30-IV), CC8 (ST8-IV/USA300), CC1 (ST1-IV/USA400) and CC5 (ST59-IV/USA1000) (8,14).

In recent years, the major epidemiological changes have occurred to clonal replacement of MRSA strains, shifts over time have been observed in countries, small regions within one country, or single hospitals (15). Therefore, typing of MRSA isolates and the understanding of clonal distribution at the local and international level is of great importance for controlling and monitoring MRSA infections.

Since the molecular characterization of MRSA is already known in other cantons of Bosnia and Herzegovina (B&H), we intended to complete these data with the results from our canton.

The aim of this study was to phenotypically and genotypically characterize MRSA strains in Tuzla Canton, B&H, and to determine their clonal

affiliation, representation and antibiotic resistance profile, as well as to compare genotypically determined MRSA clones with their antibiotic sensitivity/resistance profile.

PATIENTS AND METHODS

Patients and study design

In this prospective study, 62 methicillin-resistant *Staphylococcus aureus* isolates were analysed phenotypically and genotypically, out of a total of 282 MRSA isolates obtained from patients hospitalized at the University Clinical Centre (UCC) Tuzla from January 2009 to December 2017. The phenotypic study was performed at the Institute of Microbiology, UCC Tuzla, while additional phenotypic and genotypic analysis was conducted at the National Laboratory for Health, Environment and FOOD (NLZOH), Centre for Medical Microbiology, Republic of Slovenia. Only the first isolate of each patient was taken. The MRSA isolates were obtained from patients hospitalized in the Intensive Care Unit-Surgical Block, Department of Lung and General Abdominal Surgery, Clinic for Orthopaedics and Trauma, Clinic for Internal Medicine, Clinic for Neurosurgery, Clinic for Children's Diseases, Clinic for Skin Diseases and Clinic for Cardiovascular Surgery. The youngest patient was a neonate and the oldest was 86 years old.

Ethics clearance and approval of the study were granted by the Ethical Committee of the University Clinical Centre Tuzla.

Methods

Isolation and identification of *S. aureus* were performed by standard microbiological methods (16). Different clinical samples (aspirate, swabs of wound, wound drainage, naval, skin, central venous catheter, throat, vagina, ear, cannula, and conjunctiva) were cultured on blood agar and glucose broth and incubated at 37 °C for 18-24 hours. Identification was performed by catalase and coagulase tests. For coagulase-positive *S. aureus*, antibiotic sensitivity/resistance by disk diffusion method was performed (3) to the following antibiotics (Liofilchem, Italy): clindamycin (2 µg), ciprofloxacin (5 µg), erythromycin (15 µg), fusidic acid (10 µg), cefoxitin (30 µg), gentamicin (10 µg), kanamycin (30 µg), linezolid (10 µg), penicillin (units), rifampicin (5 µg), trimethoprim/sulfamethoxazole (1.25/23.75 µg), tetracycline (30 µg), chloramphenicol (30 µg), mupirocin (200 µg) and tobramycin (10 µg). The zone of inhibition was read according to EUCAST Clinical Breakpoint Tables (2017) (3). Vancomycin sensitivity was performed by Vitek 2 antibiogram card 580 (Vitek 2 Compact, bioMérieux, France). Each cefoxitin-resistant isolate was tested for the presence of PBP2A protein using an agglutination test (bioMerieux, France), and Vitek 2 instrument (antibiogram card 580).

Phenotyping confirmation was performed at the National Laboratory for Health, Environment and FOOD, Centre for Medical Microbiology, Republic of Slovenia (NLZOH), using Matrix-assisted laser desorption/ionization) (MALDI-TOF) technology (MALDI-TOF MS, Biotyper, Bruker Daltonic GmBH, Bremen, Germany).

Molecular analysis of genes *mec*A, *mec*C, and PVL was performed by PCR using GenoType MRSA kit (Hain Lifescience, Germany). Genes encoding staphylococcal enterotoxins (*sea, seb, sec, sed* and *see*), toxic shock syndrome toxin (*tst*), locus enterotoxin gene cluster (*egc*) and staphylococcal exfoliative toxins (*eta, etb, etd*) were detected by multiplex PCR (17). SCC*mec* typing was performed using a multiplex PCR method as described previously (12). Amplification, sequencing and analysis of the polymorphic region of protein A (*spa* typing) were performed according to the method described previously, and analysed with Ridom SpaServer (18). STs were assigned based on *spa* types as published on http://spaserver.ridom.de (8,19,20).

Statistical analysis

Descriptive statistics, frequency, minimum and maximum values and percentages were used.

RESULTS

The MRSA was mostly isolated from the patients from the Intensive Care Unit-Surgical Block, 26 (41.23%), and all of them obtained from aspirates. MRSA was isolated in large numbers from wound swabs in 10 (16.12%) patients and most of them were hospitalized in the Clinic for Orthopaedics and Trauma, seven (70%). The smallest number of MRSA was isolated from swabs taken from throat, vagina, ear, cannula, conjunctiva and abscess, one of each, from patients hospitalized at other clinics/departments.

Table 1. Distribution of MRSA clones/types by Department/Clinic

Department / Clinic (No of isolates) (sample type)	No of isolates												
	South German *spa* type t041, t1003 and t001	**Hungarian / Brazilian** *spa* type t030 and t037	**USA 300** *spa* type t008 and t121	**North German / Iberian** *spa* type t051	**European clone** *spa* type t359	**Balkan clone** *spa* type t595	**Berlin clone** *spa* type t390 and 015	**USA 400** *spa* type 128	**UK-EMRSA-5** *spa* type t005 and t1895	**South German** clone variant *spa* type t892	**South German** clone variant *spa* type t1003	**MRSA- IV** *spa* type t11509	**Total**
Intensive Care Unit, Surgical Block (26) (aspirates)	16	4	2			1			1		1	1	26
Lung Surgery (8) (wound 3, wound drainage 5)		5	1					1	1				8
General Abdominal Surgery (2) (wound drainage)		1	1										2
Orthopaedics and Trauma (7) (wounds)	1	2	1		2		1						7
Internal Medicine (6) (wound 2, skin 2, other* 1)	3		1						1				5
Neurosurgery (3) (wounds)		2		1									3
Children's Diseases (naval 2, skin 2)			2	1		1							4
Skin Diseases (1) (skin)										1			1
Cardiovascular Surgery (2) (CVC)		2											2
Other Clinics† (4) (other)*	1	1		1			1						4
Total	21	17	8	3	2	2	2	1	3	1	1	1	62

*vagina, ear, cannula and conjunctiva; †Eye Clinic; Clinic for Gynaecology and Obstetrics; Ear Nose Throat Clinic, Clinic for Pulmonary Diseases; CVC, central venous catheter

Table 2. Distribution of MRSA clones by year

MRSA clone / spa type	No (%) of isolates during the year									
	2009	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**	**2016**	**2017**	**Total**
ST228-MRSA-I t041, t1003 and t001	4 (80)	9 (90)		1 (25)	3 (50)	2 (18.2)	1 (25%)	1 (12.5%)		21 (33.8)
ST239-MRSA-III t030 and t037		1 (10)	2 (25)	2 (50)	1 (16.6)	1 (9)	3 (75)	6 (75)	1 (16.6)	17 (27.4)
ST8-MRSA-IV t008 and t121			3 (37.5)			4 (36.4)		1 (12.5)		8 (12.9)
ST247-MRSA I t051			1 (12.5)						2 (33.3)	3 (4.8)
ST22-MRSA-IV t005 and t1895	1 (20)				2 (33.3)					3 (4.8)
ST152/377-MRSA-V t595				1 (25)		1 (9)				2 (3.2)
ST97-MRSA-IV t359			1 (12.5)						1 (16.6)	2 (3.2
ST45-MRSA-IV (NT) t390 and t015						2 (18.2)				2 (3.2)
ST1-MRSA-IV t128									1 (16.6)	1 (1.6)
ST111/228-MRSA-I t892			1 (12.5)							1 (1.6)
ST1481-MRSA-I t1003						1 (9)				1 (1.6)
MRSA-IV t11509									1 (16.6)	1 (1.6)
Total	**5**	**10**	**8**	**4**	**6**	**11**	**4**	**8**	**6**	**62**

NT, non-typeable;

Typing of 62 MRSA isolates carrying *mecA* gene showed that the greatest number of isolates, 21 (33.87%) belonged to ST228-MRSA-I, *spa* type t041, t1003 and t001 (South-German clone); 17 (27.41%) were identified as ST239-MRSA-III, *spa* type t037 and t030 (Brazilian-Hungarian clone), eight (12.9%) ST8-MRSA-IV, *spa* type t008 and t121 (USA300), three (4.83%) ST247-MRSA-I, *spa* type t051 (North German strain/Iberian clone), and three (4.83%) ST22-MRSA-IV, *spa* type t005 (UK-EMRSA-15) and t1895. Two (3.22%) isolates of each: ST152/377-MRSA-V, *spa* type t595 (Balkan clone), ST97-MRSA-IV, *spa* type t359 and ST45-MRSA-IV/NT (non-typeable), *spa* type t390 (Berlin clone) and t015 were found. There was a single (1.6%) isolate of each: ST1-MRSA-IV, *spa* type t128 (USA400); ST111/228-MRSA-I, *spa* type t892 (South-German clone variant); ST1481-MRSA-I, *spa* type t1003 (South German clone variant); and MRSA-IV, *spa* type t11509 (unclassified ST) (Figure 2).

Out of the total of 62 MRSA isolates, 26 (41.93%) were from the Intensive Care Unit-Surgical Block, of which 16 (61.53%) belonged to the South-German clone, 10 (16.12%) were from the Department of Lung Surgery and General Abdominal Surgery (of which six belonged to the Brazilian-Hungarian clone). There were seven isolates from the Clinic for Orthopaedics and Trauma that belonged to as many as 5 different clones. Out of the total of four MRSA obtained from patients from the Clinic for Children's Diseases, two (50%) belonged to the USA 300 clone (Table 1).

MRSA isolates for genotyping and phenotyping were selected from different years: five (out of 70) from 2009, 10 (out of 57) 2010, eight (out of 41) 2011, four (out of 20) 2012, six (out of 22) 2013, 11 (out of 30) 2014, four (out of 21) 2015, eight (out of 11) 2016 and six (out of 10) from 2017. ST228-MRSA-I clone was identified during 2009 and 2010 in four (out of five; 80%) and in nine (out of 10; 90%) cases, respectively (Table 2). This apparent predominance of the ST228-MRSA-I clone disappeared after 2010, with a variety of other strains emerging. In 2010, ST239-MRSA-III was detected for the first time, in one (out of 10) isolate, and in 2015 and 2016 it was the most frequent clone, in three (out of four; 75%) cases each year.

In 2011, ST8-MRSA-IV was detected in three (out of 8; 37.5%) isolates, and again in 2014 in four (out of 11; 36.36%) isolates. ST247-MRSA-I was also identified in 2011 in one (out of 8; 12.5%) isolate, and the second time in 2017 in two (out of 6; 33.3%) isolates (Table 2).

Antibiotic susceptibility testing showed that almost all 19 (out of 21; 90.48%) ST228-MRSA-I clones were resistant to clindamycin, ciprofloxacin, erythromycin, cefoxitin, gentamicin, kanamycin, tobramycin, and penicillin (Table 3). Two (out of 21; 9.52%) ST228-MRSA-I, *spa* type t1003 and one ST1481-MRSA-I were also resistant to chloramphenicol and intermediately sensitive to mupirocin. ST239-MRSA-III was mostly resistant to clindamycin, ciprofloxacin, erythromycin, cefoxitin, gentamicin, kanamycin, tobramycin, tetracycline, rifampicin, and penicillin, 12 (out of 17; 70.58%). However, five isolates had different antibiotic susceptibility profiles, with three of these (*spa* type 030) being resistant to trimethoprim/sulfamethoxazole and susceptible to erythromycin and clindamycin, and two (*spa* type 037) susceptible to tetracycline.

ST8-MRSA-IV, *spa* type t008, was resistant to erythromycin, cefoxitin, kanamycin and penicillin in six (out of 8; 75%) cases each, while two *spa* type t121 clones were also resistant to ciprofloxacin.

Table 3. Antibiotic susceptibility profile of genotyped MRSA clones

MRSA clone	Antibiotic														
	CC	CIP	ER	FA	FOX	GM	K	LZD	P	RA	SXT	Te†	C	MUP†	NN†
ST228-SCCmec I ST111/228 MRSA-I ST1481-MRSA-I	R	R	R	S	R	R	R	S	R	S	S	S	S/R	S/R	R
ST239-SCCmec III	S/R	R	S/R	S	R	S/R	S/R	S/R	R	S/R	S/R	S/R	S/R	S/R	S/R
ST8-SCCmec IV	S	S/R	R	S	R	S	R	S	R	S	S	S	S	S	S
ST247-MRSA	R	R	R	S	R	R	R	S	R	R	S	R	S	S	R
ST22-MRSA-IV	S	S/R§	S	S	R	S	S	S	R	S	S	S	S	S	S
ST152/377-SCCmec V	S	S	S	S	R	R	R	S	R	S	S	R	S	S	R
ST97-SCCmec IV	S	S	S	S	R	S	S	S	R	S	S	S	S	S	S
ST45-MRSA-IV/NT‡	S	S	S	S	R	S/R	S/R	S	R	S	S	S	S	S	S/R
ST1-SCCmec IV	R	S	R	S	R	S	R	S	R	S	S	R	S	S	S
MRSA-IV *spa* type t11509	R	S	R	S	R	S	S	S	R	S	S	S	S	S	S

†susceptibility testing to chloramphenicol, tobramycin and mupirocin was performed only in Slovenia;
‡ST45-MRSA-NT clone (*spa* type t015) of non-typeable SCC*mec* type had a more resistant antibiotic profile (S/R = t390/t015); §*spa* type t005 clone was susceptible to ciprofloxacin, while *spa* type t1895 clones were resistant;
CIP, ciprofloxacin (5 µg); E, erythromycin (15 µg); FA, fusidic acid (10 µg); FOX, cefoxitin (10 µg); GM, gentamycin (10 µg); K, kanamycin (30 µg); LZD, linezolid (10 µg); P, penicillin (1 µg); RA, rifampicin (5 µg); SXT, trimethroprim-sulfamethoxazole (5 µg); Te, tetracycline (30 µg); C, chloramphenicol (30 µg); MUP, mupirocin (200 µg); NN, tobramycin (10 µg); S, sensitive; R, resistant;

Out of 62 genotyped isolates, PVL was present in ten, eight ST8-MRSA-IV and two ST152/377-MRSA-V clones (Table 4). Enterotoxin A was found in 23 isolates, ten ST239-MRSA-III, nine ST228-MRSA-I, three ST247-MRSA-I and one ST1481-MRSA-I clone. Ten isolates were positive for enterotoxin gene cluster (egc) G, I, M, N, and O, while three of them were also positive for enterotoxin C. None of the isolates were positive for TSST or exfoliative toxins.

DISCUSSION

Although the prevalence of a reported MRSA decline in Europe, MRSA still remains an important pathogen, both in hospitals and the community due to a high level of resistance to multiple classes of antibiotics (3). Typing of MRSA strains is a valuable tool for understanding and controlling their transmission (21).

Table 4. Detection of toxins among MRSA strains

Toxin*	MRSA strain	Number of isolates	Total
PVL	ST-8-MRSA-IV	8	10
	ST-152/377-V	2	
Enterotoxin A	ST228-MRSA-I	9	23
	ST239-MRSA-III	10	
	ST247-MRSA-I	3	
	ST1481-MRSA-I	1	
egc (G, I, M, N, O)	ST228-MRSA-I	4	10
	ST45-MRSA-IV	1	
	ST22-MRSA-IV	3	
	ST1481-MRSA-I	1	
	MRSA-IV, spa type t11509	2	
Enterotoxin C	ST45-MRSA-NT	1	3
	ST22-MRSA-IV	2	

*No toxins were detected in 16 MRSA isolates; PVL, Panton-Valentine leukocidin; egc, enterotoxin gene cluster; NT, non-typeable

In the current study, phenotypic and genotypic analysis of 62 MRSA isolates showed that the most frequent type was ST228-MRSA-I (South-German clone) (predominantly *spa* type t041; 18/21, 85.71%). During the 1990s, a new multi-resistant MRSA clone (gentamicin resistant), ST228-MRSA-I, was discovered in Italian hospitals (22). It was also described in Germany among 1997-1998 (although rifampicin sensitive). It emerged elsewhere in Europe, and in Hungary it represented 28% of all MRSA isolates between 2001 and 2004 (11,23). Until 2008, this clone was constantly present but with some time and geographic variations. In Switzerland, an increasing number of infections caused by the ST228-MRSA-I was recorded from 2008-2010, but the prevalence dropped significantly from 2010 to 2014 (24). Our data showed a predominance of the ST228-MRSA-I clone from 2009-2010, while its presence dramatically declined after 2010. Two distinct antibiotic profiles of ST228-MRSA-I clone detected in our study were due to genetic differences in *spa* type t1003 that accounted for the resistance and decreased sensitivity to chloramphenicol and mupirocin, respectively. Increasing mupirocin resistance among MRSA isolates has been associated with an increased risk of staphylococcal infections and a failure to control MRSA transmission especially in a healthcare setting (25).

In accordance with our data, reports from neighbouring Slovenia showed a high prevalence of ST228-MRSA-I (*spa* type t041, t003 and t001) between 2006 and 2007 (26). In the Cantonal Hospital Zenica, out of the total of 23 MRSA isolates obtained from neonates and children up to one year of age,

87% belonged to spa type t355 (MLST CC152) (27). Also, *spa* type t041 was the most common *spa* type detected among isolates from the Clinical Hospital Centre Zagreb, Croatia, while t001 was the most common isolate in Mostar (B&H) (28).

The analysis of 248 MRSA isolates from 20 Croatian cities in 2004 showed that most isolates belonged to ST111-MRSA-I (ST228-MRSA-I variant) (29). We have identified one ST111-MRSA-I isolate in 2011, and a different variant in 2014 (ST1481-MRSA-I).

The second most frequent MRSA clone described in our study was ST239-MRSA-III *spa* type t037 and t030 (17/62, 27.4%). This clone, although present among isolates in 2010, became predominant only later, in 2015 and 2016. ST239-MRSA-III clone was at first described in the late 1970s and early 1980s in Australia, Great Britain and South America as resistant to gentamicin (30). ST239-MRSA-III clone at first emerged in Brazil, subsequently spread to South American and European countries, and became the most common MRSA clone between 1994 to 2008 (31). All isolates of this clone were resistant to ciprofloxacin, erythromycin, lincomycin, tetracycline, and trimethoprim/sulfamethoxazole. In China, isolates of ST239-MRSA-III clone were also resistant to clindamycin and gentamycin (32).

Studies showed that the ST239-MRSA-III clone had undergone significant changes; thus, in the pre-2000 period *spa* type t037 was dominant and was rapidly replaced by *spa* type t030. Unless control measures are taken, due to a faster growth rate of *spa* type 030, other countries or continents may also experience broad dissemination of this multi-antibiotic-resistant MRSA clone (33). Our data showed that, ST239-MRSA-III, *spa* type t037 clone was still the dominant type, while ST239-MRSA-III, *spa* type 030 was detected in only three isolates (in 2012 and 2014). As expected, the two *spa* types differed in antibiotic profiles, with *spa* type t030 exhibiting resistance or decreased sensitivity to both rifampicin and trimethoprim/sulfamethoxazole. In contrast to our study, no SCC*mec* III isolates were detected by PFGE analysis in isolates from Sarajevo, B&H (34).

ST247-MRSA-I is an ancient strain, the first recovered in the UK (also known as North German and Iberian strain; Archaic/Iberian clone). It is responsible for outbreaks in Barcelona in 1989, and more recently it had been detected in Australia, Croatia, Czech Republic and Italy (29,35,36). However, ST247-MRSA-I seems to be receding and it is becoming extremely rare in regions it was previously readily detected. Not surprisingly, only three isolates of this clone were detected in our study.

One of the most surprising moments in dealing with MRSA infections in recent years has been the emergence of CA-MRSA strains (6). Prior to 2001, the most common CA-MRSA was ST1-MRSA-IV (USA400 clone), the first known PVL-positive MRSA (5). Afterwards, a new CA-MRSA clone, ST8-MRSA-IV (USA300), has emerged (37). Since CA-MRSA classification cannot be based solely on genotype, without additional information none of our clones can be classified with certainty as CA-MRSA (4). Our major potential CA-MRSA isolate was ST8-MRSA-IV (*spa* type t008 and t121), while others belonged to ST152/377-MRSA-V and ST1-MRSA-IV. In recent years, ST8-MRSA-IV (*spa* type t008) was the predominant type of CA-MRSA in the United States (38). A large multicentre study from 16 European countries also showed the prevalence of ST8-MRSA-IV clone among CA-MRSA isolates (39).

ST8-MRSA-IV is a notable PVL-positive MRSA clone, with the most common *spa* types t008 and t002 (38,40). All eight of our ST8-MRSA-IV isolates harboured the PVL gene, while the remaining two PVL-positive isolates were ST152/377-MRSA-V. ST152 clone has often been associated with Balkan countries and reported to carry the PVL gene (41). It cannot be distinguished from clone ST377 using standard MLST primers, hence the ST152/377 classification. All other toxins tested (enterotoxin A and C, egc) were expressed by strains usually defined as HA-MRSA (17).

Interestingly, ST97-MRSA-IV detected in our study has usually been isolated from cattle and rarely from humans (8). However, without additional information, this clone cannot be classified as either community- or livestock-associated (LA-MRSA).

Due to economic reasons, only 62/282 of our isolates were randomly characterized genotypically. Although, somewhat ambiguous due to atypical and overlapping profiles, phenotypic methods can still be used for the classification of MRSA isolates with typical antibiograms (42).

One of the possible shortcomings of our research could be the small number of samples in relation to the examined period. We could not avoid this possible shortcoming due to the limited number of samples we could process genotypically.

In conclusion, by analysing our isolates over a long period of time, we confirmed that the clo-

nal distribution of MRSA is very variable. In Bosnia and Herzegovina different clonal types dominate depending on whether MRSA infection is acquired in a hospital or outpatient setting. The antibiotic profile of individual clonal types is largely uniform and as such is useful in epidemiological monitoring of MRSA and in smaller communities. In our study, we also demonstrated that the clonal types of MRSA that are potentially associated with outpatient setting are producers of different types of toxins suggesting even greater importance of characterizing MRSA through phenotypic and genotypic studies.

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