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Molecular characterisation of methicillin-susceptible and methicillin-resistant *Staphylococcus aureus* in inpatients and outpatients in Bosnia and Herzegovina

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Molekulare Charakterisierung Methacillin-sensibler und Methacillin-resistenter Isolate von *Staphylococcus aureus* bei stationären und ambulant behandelten Patienten in Bosnien und Herzegovina

Zusammenfassung Das Ziel der vorliegenden Arbeit war es, den genetischen Hintergrund von Methacillin-sensiblen (MSSA) und Methacillin-resistenten *Staphylococcus aureus* (MRSA), die aus verschiedenen klinischen Proben bei stationär und ambulant behandelten Patienten gewonnen wurden, zu erforschen. Die Methacillin-Resistenz wurde durch die Anwesendheit von Meca-Genen PCR-DM bestätigt. Die genetische Charakterisierung erfolgte mittels SPA Typisierung und BURP. *Staphylococcus aureus* wurde aus 68 und 79 Proben der stationär bzw. ambulant behandelten Patienten isoliert, 31 (46 %) bzw. 14 (18 %) davon waren MRSA. Bei 37 stationären und 65 ambulanten Patienten mit MSSA wurden 22 bzw. 38 SPA-Typen in sieben bzw. acht Haupt-SPA-Clonal Complexes (CC) klassifiziert. Haupt-MSSA SPA-CC war CC015 (MLST CC45), bei 16 % der stationären bzw. 21 % der ambulanten Patienten. Die meisten der MRSA Isolate waren SPA-CC 355/595 (MLST CC152). Bei 32 % der stationären und 43 % der ambulanten Patienten wiesen die MSSA Isolate einen MRSA-Hintergrund auf; dies weist darauf hin, dass die MRSA nicht aus den dominanten MSSA-Klonen hervorgegangen sind.

Schlüsselwörter: MSSA, MRSA, Sequenzielle Typisierung, Molekulare Epidemiologie, Antimikrobielle Resistenz

Summary The aim of this study was to investigate the genetic background of methicillin-susceptible (MSSA) and methicillin-resistant *Staphylococcus aureus* (MRSA) obtained from clinical specimens of inpatients and outpatients. Methicillin resistance was confirmed by the presence of the *mec*A gene by PCR. The genetic characterisation was performed using *spa* typing and the algorithm based upon repeat pattern (BURP). *Staphylococcus aureus* was isolated from 68 and 79 inpatient and outpatient samples, 31 (46 %) and 14 (18 %) of which were MRSA, respectively. Among 37 inpatients and 65 outpatients with MSSA, 22 and 38 *spa* types were clustered into seven and eight *spa*-CCs, respectively. The main MSSA *spa*-CC of inpatients and outpatients was *spa*-CC015 (multilocus sequence typing (MLST) CC45). Most MRSA were associated with *spa*-CC355/595 (MLST CC152). MRSA-related background was found in 32 % of inpatients and 43 % of outpatients with MSSA, suggesting that MRSA did not arise from predominant MSSA clones.

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Keywords: MSSA, MRSA, Sequence typing, Molecular epidemiology, Antimicrobial resistance

Introduction

The importance of *Staphylococcus aureus* as a human pathogen, apart from its ability to cause a diverse range of life-threatening infections, is its extraordinary potential to develop antimicrobial resistance [1]. Resistance to beta-lactam antibiotics is coded by the *mec*A gene, which is situated on the mobile genetic element staphylococcal cassette chromosome *mec* (SCC*mec*). Accordingly, methicillin-resistant *S. aureus* (MRSA) originated through the transfer of SCC*mec* element from MRSA into methicillin-susceptible *S. aureus* (MSSA) and the genetic background determines the stability of the new MRSA clone [2].

It has been previously shown that the classification of *spa* types into *spa*-clonal complexes (*spa*-CCs) is the best way to improve the interpretation of *spa* typing, as well as *spa* typing along with the algorithm based upon repeat pattern (BURP) in accordance with typing results obtained by multilocus sequence typing (MLST) [3].

In Bosnia and Herzegovina, there are very few reports about the MSSA/MRSA prevalence obtained from hospitalised/outpatient and healthy individuals [4, 5] and there is no information on the MSSA/MRSA population structure including the relation between MSSA/MRSA lineages. The aim of this study was to investigate the genetic background of MSSA/MRSA isolates obtained from various clinical specimens of inpatients and outpatients in Zenica-Doboj Canton (Bosnia and Herzegovina).

Patients and methods

Settings, bacterial isolates and study design

In the period of December 2009 to May 2010, 68 and 79 consecutive, non-duplicate *S. aureus* strains isolated from various clinical specimens in various hospital departments (inpatients) as well as outpatient departments (outpatients), respectively, of the Cantonal Hospital Zenica were prospectively included in the analysis. The hospital is a 849-bed tertiary-level hospital admitting about 25,000 patients per year, with 240,000 patient days. The population covered by this institution is 331,229 in Zenica-Doboj Canton (ten municipalities), Bosnia and Herzegovina.

Staphylococcus aureus isolates were identified according to standard microbiological methods [6]. Coagulase-positive organisms (*S. aureus*) were tested for oxacillin and cefoxitin sensitivity/resistance by disk-diffusion method at Mueller–Hinton (MH) agar (Oxoid, Basingstoke, UK; growth zone inhibition around 1 and 30 µg oxacillin and cefoxitin disk, respectively) in accordance with Clinical Laboratory Standards (CLSI) [7]. The isolates that were identified as *S. aureus* were stored in deep agar at −20 °C for further analyses. The genetic characterisation of 68 inpatient and 78 outpatient isolates (one outpatient isolate was contaminated) was performed at the department of Medical Microbiology of the Maastricht University Medical Centre (MUMC). All *S. aureus* isolates were analysed for the presence of the *S. aureus*-specific *fem*A gene as well as the MRSA-specific *mec*A gene using a multiplex real-time PCR assay [8].

The disk-diffusion method using Mueller–Hinton agar (Oxoid, Besingstoke, UK) was used to test against ten antimicrobials (Oxoid, Basingstoke, UK). Inducible clindamycin resistance was identified as a D-shaped inhibition zone by the clindamycin–erythromycin double-disk test. The applied susceptibility criteria were according to CLSI [7]. *Staphylococcus aureus* ATCC 25923 control strain was used. Multidrug resistance (MDR) was defined as resistance to three or more groups of antibiotics.

The information record for study patients admitted to the hospital (clinical) included: age, gender, occupation, place of residence, length of hospital stay (admission and discharge date), place of residence at the time of admission to the hospital (e.g. at home, other hospital, nursing home), prior hospital stay (past 12 months), hospital department and surgery at the previous stay, contact with person(s) with history of hospitalisation in the past 12 months, hospital department at present stay in the hospital, health status at the admission to the hospital (diagnosis, other chronic diseases, e.g. diabetes, cardiovascular diseases, malignancy), surgery at the present stay, antibiotic usage in the past 4 weeks, corticosteroid therapy, type of infection or origin of the isolate and causative agent isolated (MSSA or MRSA).

An institutional review board approval was obtained from the Ethics Committee of the Zenica Cantonal Hospital prior to the initiation of the study, and all participants read and signed informed consents about the purpose of the study (participation was voluntary and anonymous) as well.

Typing of the spa locus

Real-time amplification of the *spa* locus, followed by sequencing, was performed as described before [9, 10]. The *spa* types were clustered into *spa*-CCs using the algorithm BURP with the Ridom Staph Type, version 1.5, software package (http://www.ridom.de) [11]. The default settings recommended by the manufacturer were used. Since it was shown that *spa* typing, together with the algorithm BURP, yielded results consistent with typing results obtained by MLST [11–15], the associated CCs, as determined with MLST, were allocated through the Ridom SpaServer (http://spaserver.ridom.de).

Statistical analysis

Descriptive and analytical statistical methods according to the data obtained and their distribution (Kolmogov-

Table 1. Demographic and clinical characteristics of the inpatients and outpatients with infection due to MSSA and MRSA

Number (%) of patients[a]								
	Inpatients				Outpatients			
	Infection due to MSSA	Infection due to MRSA	Risk ratio (95 % CI)	p value	Infection due to MSSA	Infection due to MRSA	Risk ratio (95 % CI)	p value
Causative agent				0.002[b]				
	37 (54.4)	31 (45.6)			65 (82.3)	14 (17.7)		
Age, median (range), years	22.82 (1–76)	9.46 (1–60)		0.001[b]	26.41 (1–65)	10.6 (1.79)		0.000[b]
Contact with person(s) with history of hospitalisation in the past 12 months				0.004[b]				0.615
Yes	19 (51.4)	25 (86.2)			1 (1.6)	0		
No	18 (48.6)	4 (13.8)			63 (98.4)	14		
Type of infection or origin of isolate				NV				NV
Surgical wound	7 (18.9)	2 (6.5)			9 (13.8)	0		
Skin and soft tissue infections	11 (29.7)	16 (51.6)			9 (13.8)	4 (28.6)		
Respiratory tract	16 (43.2)	7 (22.6)			35 (53.8)	6 (42.9)		
Ear, eyes	1 (2.7)	3 (9.7)			12 (18.5)	4 (28.6)		
Genital tract	2 (5.4)	0						
Blood culture	0	3 (9.7)						
Department at present stay in the hospital[c]				0.000[b]				NA
Paediatrics, paediatrics TBC	19 (51.4)	23 (74.2)						
Surgery[d]	16 (43.2)	4 (12.9)						
Other[e]	8 (21.6)	4 (12.9)						

MSSA methicillin-susceptible *Staphylococcus aureus, MRSA* methicillin-resistant *Staphylococcus aureus, NV* test is not valid, *NA* not applicable, *CI* confidence interval
[a]Not all patients responded to all questions
[b]Statistically significant difference
[c]Some patients were stayed in several hospital departments (percentage of 31 and 37 patients, respectively)
[d]Including ICU surgery, paediatrics surgery, gynaecology, orthopaedics and trauma, urology, ORL/maxillofacial surgery
[e]Including internal medicine (gastroenterology, diabetology, cardiology, nephrology, pulmology), dermatology, infectious diseases, psychiatry

Smirnov test, χ^2 test, Student *t*-test, parametric and non-parametric correlation rank, ANOVA) were performed using SPSS version 15.0. All tests were two-tailed, and $p<0.05$ was considered to be statistically significant. Data analysis was performed using SPSS, version 15.0. Categorical variables were compared using the χ^2 or Fisher exact test, as appropriate. Continuous variables were compared using the Wilcox rank-sum test. All *p* values were two-tailed, and a *p*-value of less than 0.05 was considered statistically significant. Adjusted odds ratios (aORs) and 95 % confidence intervals (CIs) were computed. To identify risk factors for infection due to MSSA/MRSA isolates, the variables that were associated with more than 10 % of the patients who had this type of infection (by the use of bi-variable analysis, $p<0.05$ or that had a prior clinical significance) were entered into backward, stepwise logistic regression models. Significant variables were grouped if they were closely correlated, and only one variable per group was chosen for entry into a model. The final model was chosen on the basis of plausibility and on the basis of having the lowest −2 log-likelihood function.

Results

MSSA/MRSA prevalence

Of 68 and 79 inpatient and outpatient *S. aureus* isolates 31 (45.6 %) and 14 (17.7 %) were MRSA, respectively (Table 1).

The MRSA prevalence among *S. aureus* isolates was higher in hospitalised patients than in outpatients (45.6 and 17.7 %, respectively; $p=0.002$). The mean age for patients with MRSA infection was lower than for patients with MSSA infection in inpatients as well as in outpatients, 9.46 years ($p=0.001$) and 10.60 years ($p<0.000$), respectively.

There was no statistically significant difference in MSSA/MRSA infections according to gender in both hospitalised patients and outpatients (RR = 1.147 and 1.111, $p=0.629$ and 0.812, respectively). Duration of hospitalisation ($p=0.097$), prior hospital stay, surgery at a previous hospital stay and surgery at the present hospital stay (RR = 1.294, $p=0.288$; RR = 1.833, $p=0.556$; RR = 0.631, $p=0.257$, respectively), as well as corticosteroid and antibiotic usage (RR = 1.461, $p=0.222$, and RR = 0.422, respectively) were not identified as risk factors in hospitalised

patients. Hospitalised patients with MRSA infection had more frequent contacts with person(s) with the history of hospitalisation in the past 12 months than those with MSSA infection ($p=0.004$).

The prevalence of MRSA compared with MSSA infection was significantly higher at the Paediatric Department than the prevalence at other hospital departments (23 MRSA out of 38 *S. aureus* infections, 60.5 %; $p=0.000$). According to the isolate origin, the highest MRSA prevalence in both hospitalised and outpatients was noted in the samples obtained from skin and soft tissue infections (SSTI), 16 (out of 27 samples, 59.3 %), and 4 (out of 13 samples, 30.8 %), respectively (51.6 and 28.6 % of all samples, respectively; Table 1).

Genetic lineages

Among 37 inpatient MSSA isolates, 25 (67.6 %) were clustered into eight main *spa*-CCs (16 *spa* types), the two isolates into no founder clusters (with one strain in each), four (10.8 %) strains were singletons, and five (13.5 %) MSSA strains were excluded from the BURP analysis because they belonged to *spa* types smaller than five repeats. One inpatient MSSA strain was non-typeable with *spa* typing. The two main MSSA *spa*-CCs were *spa*-CC015 and *spa*-CC159 associated with MLST CC45 and CC121, respectively, which harboured six (16.2 %) isolates each. The most common inpatient MSSA *spa* types were t091 (four isolates, 18.2 %), and t728 (three isolates, 13.6 %; Table 2).

Among 31 inpatient MRSA isolates, 25 (80.6 %) were clustered into *spa*-CC355/595 associated MLST CC152 (Table 3).

Among 65 outpatient MSSA isolates, 38 *spa* types were identified, clustered into eight main *spa*-CCs (45 strains, 24 *spa* types). The main *spa*-CC among MSSA outpatient isolates was *spa*-CC015, associated with MLST CC45, which harboured 14 (21.5 %) isolates (Table 2).

Seven (50 %) outpatient MRSA isolates were clustered into *spa*-CC355/595 associated with MLST CC152, which contained six strains with *spa*-type t355 and one strain with *spa*-type t595 (Table 3).

Eight (four in each, inpatient and outpatient MSSA) out of ten MSSA isolates that were excluded from BURP analyses were associated with MLST CC45 (t026; Table 2).

Total 32 and 43 % of the inpatient and outpatient MSSA, respectively, had a genetic background common to MRSA lineages MLST CC45, CC5, CC8, CC22, and CC30. The genetic background of 32.4 and 23.1 % of the inpatient and outpatient MSSA isolates, respectively, was not associated with MRSA-associated lineages, i.e. MLST CC7, CC15 and CC121. Of the remaining 35 and 34 % of the inpatient and outpatient MSSA, respectively, MLST CC could not be determined in 5.4 and 7.5 %, respectively.

Antimicrobial resistance

No resistance to vancomycin and rifampicin was detected. Overall, *S. aureus* resistance rate to imipenem, gentamicin, clindamycin, trimethoprim/sulfamethoxazole and chloramphenicol was higher in hospitalised patients compared with outpatients, but only in case of imipenem and gentamicin it was statistically significant ($p<0.05$) (5.6 %, 0 %, and 38.2 %, 12.5 %, respectively; Table 4).

Inducible clindamycin-resistance phenotype showed twoout of four and 18 erythromycin-resistant/clindamycin-sensitive MSSA in outpatients, and none out of two of inpatient erythromycin-resistant/clindamycin-sensitive MRSA.

There were 30 and 62 (46.8 and 96.9 %) inpatient and outpatient MSSA isolates, respectively, sensitive to all antibiotics tested (excluding of beta-lactams), and corresponding rates for MRSA were seven and four (22.6 and 28.6 %), respectively.

None of the MSSA isolates from inpatient and outpatients were resistant to more than three antibiotics. Only one MRSA in both inpatients and outpatients (2.6 and 7.1 %, respectively) was resistant to more than three antibiotics.

Discussion

This paper represents the first investigation of the MSSA and MRSA population structure of inpatient and outpatient MSSA and MRSA isolated at the Cantonal Hospital Zenica in the period between December 2009 and May 2010 using *spa* typing and the BURP algorithm.

MSSA isolates in our study were clustered into 14 clusters (and singletons), whereas MRSA only into four clusters. In this study, 33.9 and 44.5 % of MSSA isolates from inpatients and outpatients had a previously described MRSA-related background. Most MRSA isolates obtained in this study were associated with *spa*-CC355/595, associated MLST CC152 which were not found in MSSA isolates.

The genetic background of MSSA isolates observed in this study was heterogeneous and has shown distinct clusters, as previously described in other studies [16, 17]. The MLST CC45. which was predominant in both studied settings, widely spread MSSA and MRSA in many countries of Western Europe, Scandinavia, Canada and the United States [16–19]. The most predominant MRSA in a Netherlands hospital and outpatient settings were MLST CC5 and CC8, respectively [8]. MLST CC5, which has been identified as a minor clone in our MSSA isolates, has been found in the other countries too, with more or less similar frequencies [18, 19].

The prevalence of antibiotic resistance to MSSA as well MRSA isolates from our collection was lower (except for gentamicin) than in some other studies [19]. Only one MRSA isolate from an inpatient and one from an outpatient (*spa*-type 355 and non-typable, respectively; both strains were from skin and soft tissue infections) was

Table 2. Distribution of *spa* types and *spa*-CCs among clinical (inpatients/outpatients) MSSA

Spa-CC type	Associated MLST CC	Isolation setting	No of strains (%)	No of *spa* types (%)	Spa types[a]
Spa-CC015	45	Inpatients	6 (16.2)	3 (13.7)	t015 (2), t630 (1), t728 (3)
		Outpatients	14 (21.5)	8 (21.1)	t015 (1), t065 (1), t728 (6), t1469 (1), t1574 (1), t1726 (2), t2223 (1), t4460 (1)
Spa-CC192	22	Inpatients	3 (8.1)	2 (9.1)	t005 (2), t016 (1)
		Outpatients	9 (13.8)	2 (5.3)	t005 (8), *t7131* (1)
Spa-CC159	121	Inpatients	6 (16.2)	3 (13.7)	t159 (2), t284 (2), t435 (2)
		Outpatients	4 (6.1)	3 (7.9)	t159 (2), t272 (1), *t7244* (1)
Spa-CC002	5	Inpatients	2 (5.4)	2 (9.1)	t010 (1), t045 (1)
		Outpatients	3 (4.6)	2 (5.3)	t002 (2), t010 (1)
Spa-CC008/024	8	Inpatients	1 (2.7)	1 (4.5)	t068 (1)
		Outpatients	1 (1.5)	1 (2.6)	t008 (1)
Spa-CC084	15	Inpatients	2 (5.4)	2 (9.1)	t085 (1), t346 (1)
		Outpatients	5 (7.7)	4 (10.5)	t084 (1), t085 (1), t094 (2), *t7111* (1)
Spa-CC021/012	30	Inpatients	0	0	
		Outpatients	1 (1.5)	1 (2.6)	t012 (1)
Spa-CC1211		Inpatients	1 (2.7)	1 (4.5)	t2375 (1)
		Outpatients	2 (3.0)	2 (5.3)	t2375 (1), *t7133* (1)
Spa-CC091	7	Inpatients	4 (10.8)	1 (4.5)	t091 (4)
		Outpatients	6 (9.2)	1 (2.6)	t091 (6)
7146/7161 (No founder)		Inpatients	1 (2.7)	1 (4.5)	t7161 (1)
		Outpatients	1 (1.5)	1 (2.6)	t7146 (1)
688/7144 (No founder)	5	Inpatients	0	0	
		Outpatients	1 (1.5)	1 (2.6)	t688 (1)
267/521 (No founder)	1	Inpatients	1 (2.7)	1 (4.5)	t521 (1)
		Outpatients	0	0	
3277/3589 (No founder)		Inpatients	0	0	
		Outpatients	1 (1.5)	1 (2.6)	t3589 (1)
Singletons		Inpatients	4 (10.8)	3 (13.6)	t056 (2), t189 (1), *t7250* (1)
		Outpatients	9 (13.8)	9 (23.7)	t089 (1), t156 (1), t488 (1), t1537 (1), t1825 (1), t3011 (1), *t7139* (1), *t7148* (1), *7249* (1)
Excluded[b]	45	Inpatients	5 (13.5)	2 (9.1)	t026 (4), t870 (1)
		Outpatients	5 (7.7)	2 (5.3)	t026 (4), t2172 (1)
Non typeable		Inpatients	1 (2.7)		
		Outpatients	3 (4.6)		
Total		Inpatients	37 (99.9)	22 (22.2)	
		Outpatients	65 (100.2)	38 (38.4)	

CC as determined with MLST
CC clonal complex, *MSSA* methicillin-susceptible *Staphylococcus aureus, MLST* multilocus sequence typing
[a]Italicised *spa* types are new *spa* types
[b]*Spa* types smaller than five *spa* repeats

resistant to ciprofloxacin (3.4 and 7.1 %, respectively); in other geographical regions, ciprofloxacin resistance up to 80 % was noted [19].

Although the prevalence of MSSA with the genetic background common to MRSA (CC45, CC5, CC8, CC22) in the hospital was lower than in other studies [20–23], the MRSA prevalence among inpatient *S. aureus* was relatively high. At the same time, the number of MSSA with the genetic background common to MRSA (CC45, CC5, CC8, CC22, and CC30) in outpatients was higher than in hospitalised patients, but the prevalence of MRSA was much lower in this setting when compared with the hospital. Moreover, among our MSSA isolates several MSSA lineages not associated with the MRSA background were found (CC7, CC15, CC121), an observation previously described in other countries too [28–30]. This observation might suggest that only the genetic environment of MSSA strains is not sufficient for the stable integration of SEC*mec* elements [15, 21].

Table 3. Distribution of *spa* types and *spa*-CCs among clinical (inpatients/outpatients) and colonising MRSA

Spa-CC type	Associated MLST CCs	Isolation setting	No of strains (%)	No of *Spa* types (%)	*Spa* types[a]
Spa-CC192	22	Inpatients	1 (3.2)	1 (12.5)	*t7152* (1)
		Outpatients	0	0	
Spa-CC002	5	Inpatients	2 (6.5)	2 (25.0)	t002 (1), t041 (1)
		Outpatients	2 (14.3)	1 (20.0)	t548 (2)
Spa-CC008/024	8	Inpatients	1 (3.2)	1 (12.5)	t919 (1)
		Outpatients	2 (14.3)	2 (40.0)	t451 (1), t919 (1)
355/595 (No founder)	152	Inpatients	25 (80.6)	2 (25.0)	t355 (24), t595 (1)
		Outpatients	7 (50.0)	2 (40.0)	t355 (6), t595 (1)
Singletons		Inpatients	2 (6.5)	2 (25.0)	t1179 (1), t1855 (1)
		Outpatients	0	0	
Non typaeble		Inpatients	0		
		Outpatients	3 (21.4)		
Total		Inpatients	31 (100.0)	8 (80)	
		Outpatients	14 (100.0)	5 (50)	

CC as determined with MLST
CC clonal complex, *MRSA* methicillin-resistant *Staphylococcus aureus*, *MLST* multilocus sequence typing
[a]Italicised *spa* types are new *spa* types

Table 4. Antimicrobial resistance of *S. aureus* isolates

		Percentages of resistance to antimicrobial agent									
Setting	MRSA/MSSA	IMP	ERY	VAN	GEN	AMK	CIP	CLI	SXT	CHL	RIF
Inpatients	MSSA (37)	0	0	0	10.5	0	0	2.6	4.5	4.8	0
	MRSA (31)	14.3	6.9	0	75.9[a]	0	3.2	3.4	18.6	7.7	0
Total (68)		5.6[a]	2.9	0	38.2[a]	0	1.5	2.9	10.3	5.7	0
Outpatients	MSSA (65)	0	6.3	0	0	2.6	0	0	0	3.6	0
	MRSA (14)	0	12.5	0	62.5[a]	0	7.1[a]	0	33.3[a]	0	0
Total (79)		0	9.4	0	12.5	2.0	1.3	0	5.6	3.1	0

MSSA methicillin-susceptible *Staphylococcus aureus*, *MRSA* methicillin-resistant *Staphylococcus aureus*, *IMP* imipenem (10 µg), *ERY* erythromycin (15 µg), *VAN* vancomycin (30 µg), *GEN* gentamicin (10 µg), *AMK* amikacin (30 µg), *CIP* ciprofloxacin (5 µg), *CLI* clindamycin (2 µg), *SXT* trimethoprim/sulfamethoxazole (25 µg), *CHL* chloramphenicol (30 µg), *RIF* rifampicin (5 µg), *NT* not tested
[a]Statistically significant difference

MDR characterises hospital-associated (HA) MRSA isolated from patients with identified risk factors [26]. The results of this study have shown a very low MDR prevalence of MRSA isolates and the absence of risk factors usually connected with MRSA infections [2], with exceptions related to contacts with previously hospitalised patient and the patients' age. The large majority of MRSA in this study (MLST CC152) were isolated at the paediatric hospital department, obtained from children up to 1 year of age with SSTIs. In addition, the same MRSA lineage was found in four other hospital departments (dermatology, internal medicine, ICU and surgery) even in another building, as well as in outpatients during the study period (December 2009 to May 2010), and in healthy food handlers during 2009. It is well known that skin infections occur predominantly in children and young adults without risk factors. With intrafamilial spread, family members can serve as a reservoir of CA-MRSA); therefore, the epidemic MRSA clone might be extended in the community [2, 27, 28]. This suggests the probable predominance of MLST CC152 in the community in this region and its spread by cross-transmission into the hospital [28–30].

In conclusion, the results of this study indicate that more MSSA CCs compared with MRSA CCs were found and suggest that MSSA is more heterogeneous (and similar in both studied settings). Yet, the finding that a large proportion of MSSA had genetic background uncommon to MRSA (only the CCs 22, 5 and 8 were common to both MSSA and MRSA) suggests that these genetic backgrounds do not provide a genomic environment for the integration of SCC*mec* elements

There are certain limitations to this study.

The investigation of clinical MSSA/MRSA cultures was prospective, but epidemiological data were mainly collected retrospectively resulting in an overestimation of MRSA infections at least for samples from the respiratory tract (specific diagnoses were missing, and it was unknown whether the patients were infected or colonised with MRSA). Secondly, our observation should be interpreted with caution, because it was performed over

a short time frame, and there were no MLST data from Bosnia and Herzegovina for comparison. Moreover, this research was initiated without any knowledge about a MRSA outbreak at the hospital at that time (it was not registered), which substantially limits conclusions about the molecular epidemiology in this region.

Nevertheless, this study outlines the importance of MRSA screening at hospital admission of patients in order to prevent pathogen access to a hospital. Further studies are needed for a better evaluation of the epidemiology of *S. aureus* in order to develop effective prevention strategies aimed to control MRSA spread.

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Conflict of interest
The authors declare no conflict of interest related to this article.

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