

MRSA DIVERSITY AND THE EMERGENCE OF LA-MRSA IN A LARGE TEACHING HOSPITAL IN SLOVENIA

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The methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the major causes of a variety of infections in hospitals and the community. One of the most prominent changes in the MRSA epidemiology is the emergence of livestock-associated MRSA (LA-MRSA) strains in the human population. The aim of this study was to follow the MRSA epidemiology in a large teaching hospital during an 8-year time period (2006–2013). Altogether 519 MRSA, cultured from screening or clinical samples, were distributed into 77 *spa* types, of which three (t003 and t001, associated with CC5; and t015; associated with CC45) were the most common. LA-MRSA-associated *spa* types (t011, t034, t108, t899; associated with CC398) started to emerge in the year 2009 and continued to be found annually at a frequency from 3.9% to 12.7% of all MRSA strains examined. Only 6 of 27 LA-MRSA strains were associated with infections.

Keywords: *spa* typing, LA-MRSA, *Staphylococcus aureus*, animals, colonization

Introduction

The methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most important multidrug resistant microorganisms and primarily causes healthcare-associated (HA-MRSA) infections [1, 2]. During the past two decades, MRSA was also increasingly reported in the community and this type was designated a community-associated MRSA (CA-MRSA). The CA-MRSA and HA-MRSA could be differentiated epidemiologically by association with nosocomial settings,

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as well as the time of onset in the hospital. They could also be differentiated pheno- and genotypically by attribution to clonal lineages (complexes), the type of SCC*mec* element, antibiotic resistance patterns, and the virulence potential [3, 4]. However, the distinction between both groups is disappearing [3]. The CA-MRSA is acquiring additional antibiotic resistances and both types are currently circulating in hospitals as well as in the community. Some successful clones have spread worldwide [5–7]. Since 2005, a group of MRSA associated with farm animals, termed as livestock-associated MRSA (LA-MRSA), started to emerge in the human population [8, 9]. The most common LA-MRSA group is ST398 with a large number of different *spa* types [7, 8, 10, 11]. Although LA-MRSA represents less than 10% of strains isolated from humans in the majority of countries, it is important to follow the national and local rates in order to adapt patient screening if necessary [11].

In Slovenia, the MRSA is well controlled and the MRSA colonization rate is used as a quality indicator [12, 13]. The hospitals have issued guidelines for the prevention of MRSA healthcare-associated transmission, including hand hygiene, which is the most important infection control measure, patient isolation, and surveillance of patients at risk [14]. However, despite good hospital controls, at present, a detailed MRSA epidemiology in Slovenia is less than thoroughly investigated, as the focus is mainly on the CA-MRSA [15–17]. Newly described *mecC*-positive isolates have been found in a large collection of 395 CA-MRSA, isolated nationwide from 2006 to 2013 [18]. In the same collection, a subset of MRSA isolated in 2010 were *spa*- and MLST-typed, and tested for presence of toxin genes [19]. Of 151 isolates, 9.9% belonged to ST398, representing the first report of LA-MRSA strains in Slovenia. Slovenian laboratories also participated in large international studies on *S. aureus* bloodstream infections [1, 20], MRSA in intensive care units (ICUs) [21], and an ECDC cross-sectional study on LA-MRSA for the year 2013 [11].

In this context, the aim of this study was to analyze the epidemiology of MRSA *spa* types in a single large teaching hospital during an extended (8-year) time interval, and to determine the possible presence and proportion of LA-MRSA.

Materials and Methods

Hospital setting

The University Clinical Centre Maribor (UCCM) is a tertiary teaching hospital, located in the North–East region of Slovenia, serving a population of

400,000. It has 1,300 beds with ca. 55,000 discharges (364,000 patient days) per year including the study period. The hospital had an active MRSA surveillance since 1999 and patients with at least one of the following risk factors are included in the screening: previous hospitalization within the last 12 months, transfer from another hospital or from a long-term care facility, previously known colonization with MRSA, patients with chronic wounds, all patient in critical care units, patients undergoing clean elective and implant surgery, patients on peritoneal dialysis, patients with more than 24 h room contact with a patient who was a confirmed as MRSA carrier, and healthcare workers at the wards with proven MRSA cases at the time of an epidemic.

Ethics

Ethical approval for this study was obtained from the National Medical Ethic Committee (no. KME84/08/12).

Isolation and characterization of MRSA

For the strain selection, isolation, and characterization, we followed the methods of Kotnik-Kevorkijan et al. [22]. *S. aureus* cultivated from clinically relevant samples were recognized as MRSA because of the resistance to ceftioxin, and were subsequently confirmed with *mecA* testing. For surveillance samples, the conventional culture media, including MRSA-screening plates (CHROMID[®] MRSA, bioMérieux, Marcy-l'Etoile, France) and trypticase soy broth containing NaCl, were used. MRSA strains were confirmed by PCR amplification of the *mecA* gene by a modification of previously published methods [23, 24]. An in-house method is established and is based on amplification of three different PCR products; two are *S. aureus*-specific and one targets *mecA* gene. The *mecC* testing was introduced in the second half of the year 2013 for strains that were resistant to ceftioxin and negative for *mecA*. No strains fulfilling these criteria were detected within the study interval. All isolates were frozen at $-70\text{ }^{\circ}\text{C}$ until further characterization. Only the first isolate of each patient was stored.

Antimicrobial susceptibility testing

Susceptibility testing, using a standard selection of antibiotics for *S. aureus*, was performed by disk diffusion according to the CLSI Performance Standards for Antimicrobial Susceptibility Testing (CLSI document M100;

<https://clsi.org/standards/products/microbiology/documents/m100/>). The antibiotics tested were tetracycline, clindamycin, erythromycin, ciprofloxacin, gentamycin, tobramycin, mupirocin, linezolid, trimethoprim–sulfamethoxazole, teicoplanin, vancomycin, and netilmicin. Since 2011, the susceptibility to vancomycin has been assessed by E-test.

Strain selection and spa typing

The MRSA, which were typed, were isolated between January 2006 and December 2013 from either surveillance or clinical samples. Only the first isolate of each patient was included in the analysis.

The isolates were thawed and cultured on blood agar. After DNA isolation using the QIAamp DNA Kit (QIAGEN, Venlo, Netherlands), the *spa* region was amplified using primers and conditions as described previously [25] and sequenced by a commercial service (MWG, Martinsried, Germany). The *spa* types were assigned using the Ridom StaphType software (Ridom GmbH, Würzburg, Germany) [25]. The Based Upon Repeat Pattern (BURP) algorithm of Ridom StaphType software was used to analyze the similarity of *spa* types and the grouping into clusters using the conditions described in the study of Mellmann et al. [26].

Results

Overall, 519 MRSA isolates from UCCM were *spa* typed between the years 2006 and 2013. The cultures were mostly obtained from surveillance swabs (nose, throat, skin, or wound) or, in rare cases, from other surveillance samples (urine or feces) and from patients with developed infections (blood culture, urine sample, swabs from wound, eye, ear, and oral mucosa). Altogether 77 *spa* types were found (Table I), three of which (t003, t001, and t015) were represented by more than 50 isolates each. Three further *spa* types (t041, t091, and t728) were also frequently isolated during this time period, whereas 40 *spa* types were represented only by a single isolate (2 LA-MRSA and 38 non-LA-MRSA *spa* types; Table I). Using the BURP algorithm, three large and several small clusters were obtained (Figure 1). Twelve *spa* types were singletons (t151, t316, t334, t791, t899, t1048, t1179, t1321, t3824, t4230, t4365, and t14168) and additional six were excluded from the analysis due to the low number of repeats (t026, t288, t390, t458, t808, and t1040).

The *spa* types associated with the LA-MRSA (t011, t034, t108, and t899) started to emerge in the year 2009 (Table I). These types represented 5.2%

Table I. Overview of *spa* types detected in a single hospital in 8-year period

<i>spa</i> type	CC ^a	2006	2007	2008	2009	2010	2011	2012	2013	Σ
t003	CC5	6	8	6	28	16	19	44	24	151
t001	CC5	38	20	6	4	1	–	–	–	69
t015	CC45	–	2	2	12	15	8	7	6	52
t041	CC5	2	–	12	6	2	3	1	–	26
t091	CC7	–	11	1	2	3	–	1	1	19
t728	nd	–	2	–	3	6	2	2	4	19
t011	CC398	–	–	–	3	6	1	1	6	17
t034	CC398	–	–	–	2	–	1	2	3	8
t108	CC398	–	–	–	–	1	–	–	–	1
t899	CC398	–	–	–	–	–	1	–	–	1
t002	CC5	–	2	–	3	2	1	–	2	10
t005	CC22	1	2	–	1	3	–	2	1	10
t288	nd	8	1	–	–	–	–	–	–	9
t006	nd	–	1	1	3	1	–	–	–	6
t355	CC152	1	–	–	1	2	1	–	1	6
t008	CC8	–	–	–	1	1	2	1	–	5
t026	nd	–	–	–	1	1	–	2	1	5
t127	CC1	–	–	–	1	2	2	–	–	5
t331	nd	–	–	–	–	–	2	2	1	5
t808	nd	–	–	–	–	–	2	1	2	5
t5605	nd	–	–	–	–	–	4	–	1	5
t201	nd	1	1	–	2	–	–	–	–	4
t3255	nd	–	4	–	–	–	–	–	–	4
t3824	nd	–	2	–	–	1	–	–	1	4
t030	ST239	3	–	–	–	–	–	–	–	3
t032	CC22	–	3	–	–	–	–	–	–	3
t037	ST239	–	–	2	–	–	–	1	–	3
t116	CC45	–	–	–	–	–	1	2	–	3
t919	nd	–	1	–	1	–	1	–	–	3
10 types ^b		1	1	2	2	1	3	2	8	20
38 types ^b		3	6	1	4	4	6	5	9	38
No. of typed MRSA strains		64	67	33	80	68	60	76	71	519
LA-MRSA		0	0	0	5	7	3	3	9	27

Note: *spa* types are grouped into most prevalent types, LA-MRSA types, types with up to three isolates, and sporadic types (one or two isolates).

^aCC deduced from *spa* type (based on data at Ridom server and Ref. [27]).

^b*spa* types present only by two strains (10) or by a single strain (38): t014, t020, t031, t044, t050, t062, t073, t102, t122, t133, t151, t310, t316, t334, t359, t360, t390, t449, t458, t542, t548, t550, t595, t685, t688, t709, t791, t830, t950, t1040, t1048, t1179, t1321, t2986, t3432, t3445, t4072, t4230, t4272, t4365, t5047, t5933, t7736, t8014, t10458, t10459, t14167, and t14168.

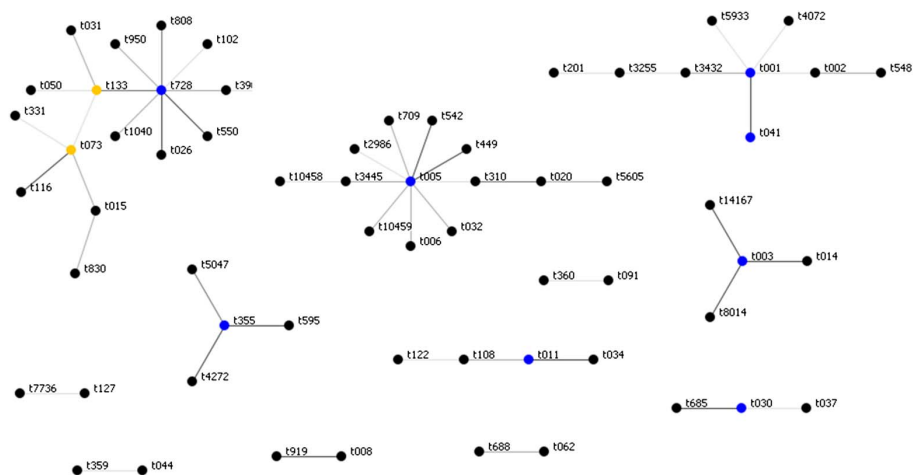


Figure 1. BURP clustering of *spa* types found in the hospital. The *spa* type with the highest founder-score is defined as the founder of the cluster (blue color); several *spa* types could have identically high founder-scores. The *spa* types with the second highest founder-score (subfounders) are marked with yellow

(27/591) of all characterized strains, and ranged from 3.9% to 12.7% of all isolates within an individual year. Only six of 27 LA-MRSA strains were associated with infections (Table II).

Although antibiotic resistance was routinely tested during the MRSA isolation, data are shown only for the LA-MRSA (Table III). All strains were resistant to tetracycline alone or together with other antibiotics. However, all strains were susceptible to mupirocin, linezolid, trimethoprim–sulfamethoxazole, teicoplanin, vancomycin, and netilmicin.

Table II. Summary of clinically relevant LA-MRSA strains showing the patient age and gender and of clinical presentations

Gender	Age (years)	Clinical diagnosis related to LA-MRSA	MRSA isolation year	<i>spa</i> type
Female	44	Pneumonia	2010	t108
Female	53	Otitis media	2011	t011
Male	52	Osteitis	2012	t011
Male	53	Pneumonia	2012	t034
Female	76	Conjunctivitis	2013	t011
Female	71	Stomatitis	2013	t011

Note: MRSA: methicillin-resistant *Staphylococcus aureus*; LA-MRSA: livestock-associated MRSA.

Table III. Antibiotic resistances in strains belonging to LA-MRSA-associated *spa* types isolated from surveillance or clinical samples (indicated by “infection”) in a single hospital from the year 2009 to 2013

Resistance	<i>spa</i> types (number of strains from surveillance samples)	<i>spa</i> types (number of strains from clinical samples)
Tetracycline	t011 (7) and t034 (2)	t011 (2)
Tetracycline and chloramphenicol	t011 (1)	t011 (1) and t108 (1)
Tetracycline and ciprofloxacin	t011 (2)	t899 (1)
Tetracycline, clindamycin, and erythromycin	t011 (2) and t034 (4)	–
Tetracycline, clindamycin, erythromycin, and ciprofloxacin	–	t034 (1)
Tetracycline, clindamycin, erythromycin, gentamycin, and tobramycin	t011 (2)	–

Note: LA-MRSA: livestock-associated MRSA.

Discussion

The reported rates for UCCM between 1998 and 2007 ranged from 53 to 114 patients colonized or infected with MRSA, and MRSA represented from 4.5% to 10% of all *S. aureus* isolates [22]. Information for the year 2008 is not available, but from 2009 to 2013, the number of MRSA patients and the percentage of MRSA among *S. aureus* isolates are comparable to the previous study (Table IV).

The *spa* types found in this study are associated with all main globally distributed clonal complexes (CC5, CC8, CC22, and CC45) except CC30 (Table I) [27]. Some *spa* types are associated with the clonal complex CC152, reported from West European countries and previously suggested to be present in the Balkan region [35]. Two *spa* types, associated with ST239, were also found in Asia [35, 36]. CC398 was continuously present since the year 2009. The three globally most widespread *spa* types (t002, t008, and t037) [28] were found, but in low proportions.

Table IV. Proportion of patients infected or colonized with MRSA during 5 out of 8 years of the study interval

	2009	2010	2011	2012	2013
All patients with MRSA	95	86	72	94	96
Infections with MRSA	7 (7.4%)	4 (4.6%)	1 (1.4%)	12 (12.8%)	5 (5.2%)
All patients with <i>S. aureus</i>	1,121	1,072	1,123	1,070	1,062
Percentage of MRSA among <i>S. aureus</i>	8.4	8.2	6.4	8.7	9.0
Number of days in bed	390,366	385,207	381,832	353,628	351,719
Number of discharges	61,218	62,689	60,535	52,497	56,752

Note: MRSA: methicillin-resistant *Staphylococcus aureus*.

Some of the most common *spa* types found (e.g., t001, t003, and t041) are frequently detected in several countries in Europe and worldwide. Type t001, which was predominant at the beginning of the study period, was previously reported for single Slovenian strain, isolated in 1999, and was also found in strains isolated from Poland and Germany during the same time period [29]. Similar to this study, t001 and t041 were among the predominant types reported from hospitals in Croatia, Bosnia and Herzegovina, and Serbia [30, 31]. Types t001, t041, and t003 were also commonly found in ICUs during the MOSAR study (2008–2011) in Slovenia, as well as in Italy, Greece, and Luxemburg [21].

Type t003, which replaced t001 at UCCM over time (Table I), is the second most common *spa* type in the Ridom server database (<http://spa.ridom.de/frequencies.shtml>). This *spa* type was also common in clinical and surveillance MRSA strains in one of the Canadian provinces [6], the second most common MRSA type found in the second European study on *S. aureus* bloodstream infections [20], and the most commonly isolated MRSA from cases of bacteremia in a large German federal state [32]. In Germany, type t003 also represented 64.3% of all MRSA strains in residents of long-term care facilities [33]. The high proportion of t003 strains at UCCM also appeared to have at least some relation to the hospitalization of residents from nursing homes for the elderly (data not shown).

Of the two other frequently isolated *spa* types, t015 was also very common in a previous national study, while t728 was limited only to UCCM [19].

Reports of LA-MRSA incidence vary considerably, depending on the country and patient population. For example, a European surveillance in 2007 reported a LA-MRSA frequency ranging from 0% to 4.7%, with the exception of the Netherlands (with 11.9% in the national reference laboratory and 25% in a single local laboratory) [8]. Ireland has reported low LA-MRSA levels (0.05%) during a time period of 2010–2014 [34]. Characterization of more than 2500 MRSA isolates from an Austrian region close to UCCM found CC398 strains represented 11.11% of isolates in 2007 and 8.17% in 2012 [35]. A study across 27 European countries has identified an overall proportion of 3.9% of LA-MRSA, but in five countries (including Slovenia), the proportion was higher than 10% [11]. The proportion of 3.9%–12.7% of LA-MRSA isolates within an individual year correlates well with national data reported for presumptive CA-MRSA analyzed in 2010 (9.9%; [19]).

As observed by others [21, 36, 37], the LA-MRSA was mostly isolated from surveillance cultures and rarely caused disease in the studied hospital.

Pigs and cattle are considered the main reservoir for the LA-MRSA, and people having professional contact with farm animals or living at industrial farms are considered to have increased risk for colonization [9]. As the study was carried out retrospectively, no further epidemiological data on possible animal contact are

available, but a high proportion of LA-MRSA is consistent with the location of UCCM in the more agriculturally oriented North–East region of Slovenia.

In summary, we found a broad diversity among MRSA strains in UCCM, with the predominance of *spa* types associated with CC5 and CC45. LA-MRSA represented up to 12.7% of all MRSA and started to emerge in the year 2009.

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Conflict of Interest

The authors report no conflict of interest.

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