Distribution of *spa* types among methicillin-resistant *Staphylococcus aureus* isolates during a 6 year period at a low-prevalence university hospital

Lukas Fenner,¹ Andreas F. Widmer,² Marc Dangel² and Reno Frei¹

Correspondence
Reno Frei
rfrei@uhbs.ch

¹Microbiology Laboratory, University Hospital Basel, Petersgraben 4, CH-4031 Basel, Switzerland

²Division of Infectious Diseases and Hospital Epidemiology, University Hospital Basel, Petersgraben 4, CH-4031 Basel, Switzerland

This study describes the distribution and frequencies of strain types by protein A-encoding gene (*spa*) typing among a total of 200 methicillin-resistant *Staphylococcus aureus* (MRSA) single-patient isolates collected between 2000 and 2005 at the University Hospital Basel, Switzerland. Nine frequent *spa* types accounted for 49.5 % of MRSA isolates, whereas *spa* type t041 (15 % of all isolates) belonged to a local epidemic strain that is also a common strain type in southern Germany. Successful control of the outbreak strain was documented by epidemiological data and confirmed by *spa* typing results. The *spa* type t044 (3.5 %), corresponding to a widely disseminated European community-acquired MRSA (CA-MRSA), was first observed in 2002. The well-known CA-MRSA USA300 clone was detected in four patients (2 %). Sporadic strains occurring less than four times (32 different *spa* types) accounted for 23 % of isolates. No predominant *spa* type was seen, indicating a great genetic diversity. Only 34.5 % of patient isolates were acquired nosocomially. The presence of one or more of ten common virulence genes was shown in 79 % of strains. It was demonstrated that the sequence-based *spa* typing method allows analysis of local MRSA epidemiology in relation to other regions and countries over time.

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INTRODUCTION

Staphylococcus aureus is a frequent cause of human infections and is one of the most important nosocomial pathogens. The emergence and spread of both healthcare- and community-associated methicillin-resistant *S. aureus* (MRSA) are challenging infection control interventions and limiting treatment options. An epidemiological work-up of clusters requires typing of potentially related strains. Analysis of chromosomal DNA restriction patterns by PFGE is still regarded as the gold standard for strain typing, but lacks an appropriate interlaboratory comparability (Van Belkum *et al.*, 1998). Currently, sequence-based methods, such as protein A-encoding gene (*spa*) typing, offer excellent intra- and inter-laboratory reproducibility, and the opportunity to compare results internationally (Harmsen *et al.*, 2003; Strommenger *et al.*, 2006). *spa* typing has also been validated for long-term nationwide surveillance studies (Hallin *et al.*, 2007). Recently, virulence genes such as those encoding Panton–Valentine leukocidin (*pvl*) or exfoliative toxin D (*etd*) have been associated with

emerging MRSA clones and specific clinical presentation (Vandenesch *et al.*, 2003; Yamasaki *et al.*, 2006).

The prevalence of MRSA at University Hospital Basel (UHBS), Switzerland, is low, as shown previously (Mertz *et al.*, 2007). Little is known about *spa* type distribution in low-prevalence hospital settings. In the present study, we evaluated the distribution and frequency of *spa* types among non-replicate isolates obtained from patients with MRSA admitted to UHBS from 2000 to 2005 and compared them with the global *spa* type database. Furthermore, a representative selection of strains was characterized by the presence or absence of important virulence-associated genes.

METHODS

Isolates, cultivation and DNA extraction. At UHBS, each non-repetitive clinical MRSA isolate has been typed by PFGE since 1994. Single-patient isolates obtained from patients between 2000 and 2005 at UHBS differing by at least one band in the PFGE pattern were stored at –70 °C in our laboratory. The isolates were identified as *S. aureus* on the basis of a positive catalase reaction, agglutination with Slidex Staph Plus (bioMérieux), aurease detection (RAPIDEC Staph; bioMérieux) and the presence of the *femA* gene by PCR (Mehrotra *et al.*, 2000). The methicillin resistance gene (*mecA*) was detected by

Abbreviations: CA-MRSA, community-acquired MRSA; MLST, multilocus sequence typing; MRSA, methicillin-resistant *Staphylococcus aureus*; *spa*-CC, *spa* clonal complex; UHBS, University Hospital Basel.

PCR (Murakami *et al.*, 1991). Isolates were thawed from frozen storage, subcultured on sheep blood agar (bioMérieux) and DNA was extracted using an LC MagnaPure system (Roche Diagnostic) according to the manufacturer's instructions. If more than one isolate per band pattern was available, only the first isolate was chosen.

PFGE and *spa* typing. PFGE was carried out as described previously (Stranden *et al.*, 2003). For *spa* typing, the polymorphic X region of the *spa* gene was amplified using primers *spa*-1113f and *spa*-1514r, and sequenced as described previously (Harmsen *et al.*, 2003; Aires de Sousa *et al.*, 2005). Sequences were analysed using Ridom StaphType software version 1.4 (Ridom GmbH) and synchronized using SpaServer (www.spaserver.ridom.de). To test the assumption that isolates with an identical PFGE pattern had the same *spa* type, ten randomly chosen isolates were subsequently typed; all ten of these isolates were of the expected *spa* type. Clustering analysis into *spa* clonal complexes (*spa*-CCs) was carried out using the clustering algorithm BURP (based upon repeat pattern) using default parameters (*spa* types were clustered if calculated costs were less than or equal to four and *spa* types shorter than five repeats were excluded) (Strommenger *et al.*, 2006).

Detection of virulence-associated genes by PCR. PCR was used to detect the following genes: *pvl*, toxic shock syndrome toxin-encoding gene (*tst*), exfoliative toxin A, B and D encoding genes (*eta*, *etb* and *etd*), and staphylococcal enterotoxin A–E encoding genes (*sea*–*see*) as described elsewhere (Lina *et al.*, 1999; Mehrotra *et al.*, 2000; Yamasaki *et al.*, 2006). Amplification of the 16S rRNA gene was used to confirm the absence of PCR inhibitors. PCR products were visualized on ethidium bromide-stained 3 % agarose electrophoresis gels.

Clinical data. Data from all MRSA patients were collected prospectively, stored in a case-report form, and categorized into nosocomial and non-nosocomial cases according to guidelines issued by the Centers for Disease Control and Prevention (Garner *et al.*, 1988).

RESULTS AND DISCUSSION

General description of patients and MRSA strains

Between 2000 and 2005, a total of 200 MRSA patients was identified, with a median age of 64 years (interquartile range 40–79 years). Among these, 69 patients (34.5 %) had acquired their MRSA strain nosocomially. The most common site of isolation was the nasopharynx (56 %), followed by wounds (12.5 %), the respiratory tract (10.0 %), urine (7.5 %), vagina/cervix (6.5 %), biopsies (4.5 %) and blood (2.5 %). Of the 200 isolates, 126 were recovered from hospitalized patients (surgery 21.5 %, geriatrics 11.5 %, gynaecology 11.5 %, internal medicine 10.5 % and intensive care unit 8.0 %) and 74 isolates from patients in the emergency department (14.0 %), outpatient clinics (14.5 %) and dermatology (8.5 %).

Among these 200 isolates, 102 strains showed a unique pattern by PFGE (at least one band difference) of which 86 were available for testing. *spa* typing discriminated 42 different *spa* types, whilst 1 strain was not typable. When PFGE bands were analysed using the Dice similarity coefficient and a dendrogram for similarity was built using the unweighted pair-group method, PFGE clustering

showed good concordance with *spa*-CC analysis (data not shown).

Distribution and frequency of *spa* types

Over the 6 year period, 23 % of MRSA isolates corresponding to 32 *spa* types were observed less than four times (Table 1). The ten most common *spa* types are listed in Table 1. *spa* type t002 and t041 correspond to strains present in southern Germany situated close to Basel. *spa* type t008 (13 % of all isolates) is represented in most European countries and comprises the Archaic/Iberian/Irish clone, as well as more recently the USA300 MRSA and other clones (Deurenberg *et al.*, 2007). *spa* types t002 and t008 were constantly present throughout the observed period (Fig. 1). Four of the twenty-six isolates with *spa* t008 showed the typical PFGE pattern of the community-acquired MRSA (CA-MRSA) USA300 clone recently imported from the USA to Switzerland (Tietz *et al.*, 2005). Another well-known CA-MRSA (*spa* type t044), widely disseminated in European countries and positive for the *pvl* and *etd* genes, was present at UHBS from 2002 (Vandenesch *et al.*, 2003; Yamasaki *et al.*, 2006; Deurenberg *et al.*, 2007). Three of the fifteen globally most frequent *spa* types in the Ridom database (t011, t012 and t084) were not detected at UHBS. Other globally frequent *spa* types such as t001, t004, t015, t024, t032 and t037 were only isolated occasionally (Table 1). An outbreak of MRSA *spa* type t041 was experienced, affecting 20 geriatric patients in 2002 (Fig. 1). Further transmission was stopped and successful control of the MRSA clone at the hospital was documented by *spa* typing. A cluster of nine patients with *spa* type t400 was seen in 2004: five of these belonged to a defined outbreak. Looking at the distribution among the various clinical departments revealed that CA-MRSA of *spa* type t044 was detected only in the emergency department, dermatology, outpatient clinics and the surgical department. In the department of internal medicine, the intensive care unit and the geriatric clinic, *spa* type t008 was less common (0–5 % vs 18–28 %). Based upon BURP *spa* clustering, the most frequent *spa*-CCs were *spa*-CC002 (29.5 %) and *spa*-CC008 (22 %), followed by *spa*-CC037 (4.5 %), *spa*-CC1510 (3.5 %) and three other unnamed clusters (12.5 %). Nosocomially acquired MRSA corresponded mainly to *spa* types t041, t400 and t030 (Table 2).

By using *spa* typing, Harmsen *et al.* (2003) identified *spa* types t001, t003 and t023 as the predominant types at a German university hospital and demonstrated the dynamics of MRSA clone circulation over two different study periods. Typing results of non-replicate MRSA strains from another German university hospital in 154 patients and 21 staff members over 1 year revealed 34 different *spa* types (Mellmann *et al.*, 2005). In a study from Austria, the most common *spa* types among 382 MRSA isolates found were t001 (28.8 % of all isolates), t190 (27.0 %), t008 (14.1 %), and t041 (11.3 %) (Ruppitsch *et al.*, 2006), whereas the 42 remaining *spa* types accounted for less than or equal to 2.4 % each. However, the strain

Table 1. Frequency of the ten most common *spa* types among MRSA single-patient isolates recovered between 2000 and 2005 at UHBS compared with relative global frequency and geographical spread

<i>spa</i> type	No. of isolates (%)	Relative global frequency (%)*	Geographical spread*†	<i>spa</i> -CC	No. of nosocomial isolates (%)‡	Comments*
t041	30 (15)	1.1	Hun, Bel, Nor, Ita, Aus, Den, Swe, Swi, Ger, Net, Cro	<i>spa</i> -CC002	22 (73.3)	Southern German MRSA
t008	26 (13)	6.7	Bel, Bul, Ita, Ice, Isr, Est, UK, Fra, Aus, USA, Swi, Jap, Cro, Swe, Net, Den, Nor, Ger	<i>spa</i> -CC008	9 (34.6)	Northern German MRSA (sub-clone); USA300 ORSA IV (CA-MRSA in the USA); Archaic/Iberian/Irish clone, ST250 ORSA I
t002	22 (11)	5.1	Hun, Bel, Chn, Cyp, Isr, Fra, Est, UK, Aus, USA, Swe, Swi, Fin, Net, Den, Nor, Jap, Ger	<i>spa</i> -CC002	7 (31.8)	Rhine Hesse MRSA (prototype); EMRSA-3; New York clone; Japanese clone; paediatric clone; USA100 ORSA II; USA800 ORSA IV; ST5 ORSA I
t400	13 (6.5)	NA	UK, Den, Swi, Fra, Net	<i>spa</i> -CC008	6 (46.2)	
t040	9 (4.5)	0.5	Swe, Den, Nor, Swi, Net, Ger	NF	2 (22.2)	
t595	8 (4)	NA	Fra, Swi, Swe, Den	NF	0 (0)	
t044	7 (3.5)	3.0	Bel, Hun, Ita, Net, Bul, Fra, Swi, UK, Swe, Ger, Nor, Cro, Den, Aus, Cyp	SGL	0 (0)	CA-MRSA ST80 (<i>pvl</i> ⁺), widely disseminated in Europe
t003	5 (2.5)	15.2	Bel, Swe, Aus, Swi, USA, Fra, Nor, Den, Net, Cro, Ger	NF	1 (20)	Rhine Hesse MRSA (subclone); EMRSA-3; New York clone
t1510	5 (2.5)	NA	Ger, Nor, Swi	<i>spa</i> -CC1510	0 (0)	
t030	4 (2)	0.7	Chn, Net, Aus, Bul, Swe, Cyp, Fra, Nor, Swi, Cro, Den, Ger	<i>spa</i> -CC037	4 (100)	
Other <i>spa</i> types	46 (23)				10 (21.7)	<i>spa</i> types: detected only once – t004, t015, t016, t024, t032, t045, t051, t064, t067, t121, t355, t370, t391, t548, t1251, t1473, t1512, t1522, t1523, t1524, t1525, t1594, t1709, NT; detected twice – t007, t018, t038, t074, t586; detected three times – t001, t019, t026, t037
NA	25 (12.5)				8 (32)	
Total	200 (100)				200 (100)	

EMRSA, epidemic MRSA in UK; NA, not applicable; NF, no founder; NT, not typable; ORSA, oxacillin-resistant *S. aureus*; SGL, singleton; ST, multilocus sequence type.

*According to SpaServer (www.spaserver.ridom.de).

†Aus, Austria; Bel, Belgium; Bul, Bulgaria; Chn, China; Cro, Croatia; Cyp, Cyprus; Den, Denmark; Est, Estonia; Fra, France; Ger, Germany; Hun, Hungary; Ice, Iceland; Isr, Israel; Ita, Italy; Jap, Japan; Lat, Latvia; Net, The Netherlands; Nor, Norway; Pol, Poland; Sou, South Africa; Swe, Sweden; Swi, Switzerland; UK, United Kingdom; USA, United States of America.

‡Percentage among isolates with the same *spa* type.

collection consisted of arbitrarily chosen isolates from different origins.

The method of *spa* typing is impressive for its speed, reproducibility, ease of interpretation and suitability for international comparison, despite its limits as a single-locus-based method (Hallin *et al.*, 2007). Multilocus sequence typing (MLST) is another excellent typing tool based on sequence analysis of seven *S. aureus* housekeeping genes and is part of the current nomenclature of MRSA strains (Deurenberg *et al.*, 2007). MLST is

especially suitable for investigating the clonal evolution of MRSA, but it is laborious and less discriminatory than *spa* typing (Deurenberg *et al.*, 2007).

Frequencies of virulence-associated genes

Out of 86 representative MRSA strains with a unique PFGE pattern, 68 isolates (79%) were positive for one or more of the virulence-associated genes tested: 13 (15.1%) were positive for *pvl*, 5 (5.8%) for *tsst*, 3 (3.5%) for *etd* and 57 (66%) for one of the staphylococcal enterotoxin A–D

Fig. 1. Distribution of the most frequent *spa* types among single-patient MRSA isolates between 2000 and 2005 at UHBS. Only *spa* types occurring at least four times are included. O, Other *spa* types; NA, strain not available.

encoding genes, but no *eta*, *etb* or *see* was detected (Table 2). All *tsst*-positive strains were also positive for the *sec* gene. Strains that were positive for *pvl* were associated with 7 different *spa* types, *tsst*-positive strains with 4, *sea*-*sed*-positive strains with 30 different *spa* types. Only *etd* was

exclusively associated with a single *spa* type, t044, consistent with the findings of Vandenesch *et al.* (2003). Within a group of the same *spa* type, strains harboured different virulence genes, suggesting parallel evolution by acquisition of additional mobile elements as observed previously (Hallin

Table 2. Gene profiles of virulence-associated determinants and *spa* types among 86 MRSA isolates representing a unique pattern in PFGE

Columns do not add up to 100 % as several gene profile configurations are possible.

Gene profile	No. of isolates (%)	<i>spa</i> type(s)*
<i>pvl</i> ⁺ , <i>etd</i> ⁻	10 (11.6)	t002 (2), t008 (4), t016 (1), t019 (1), t355 (1), t595 (1)
<i>tsst</i> ⁺ , <i>sec</i> ⁺	5 (5.8)	t002 (1), t007 (2), t026 (1), t030 (1)
<i>pvl</i> ⁺ , <i>etd</i> ⁺	3 (3.5)	t044
<i>eta</i> ⁺ , <i>etb</i> ⁺	0 (0)	—
<i>sea</i> ⁺	27 (31.4)	t001 (1), t008 (7), t018 (1), t024 (1), t030 (4), t037 (1), t041 (6), t051 (1), t074 (1), t121 (1), t400 (1), t1512 (1), t1525 (1)
<i>sed</i> ⁺	21 (24.4)	t002 (10), t003 (2), t008 (3), t024 (1), t045 (1), t400 (1), t548 (1), t586 (1), t1709 (1)
<i>sec</i> ⁺	16 (18.6)	t002 (1), t007 (2), t015 (1), t026 (2), t030 (1), t032 (1), t038 (1), t1510 (2), t1522 (1), t1523 (1), t1524 (1), t1709 (1), NT (1)
<i>seb</i> ⁺	2 (2.3)	t064 (1), t1512 (1)
<i>see</i> ⁺	0 (0)	—
<i>sea</i> ⁺ / <i>seb</i> ⁺ / <i>sec</i> ⁺ / <i>sed</i> ⁺ †	57 (66.3)	
Positive for any toxin	68 (79)	
Total	86 (100)	

NT, Not typable.

*Numbers in parentheses indicate the number of occurrences.

†Positive for at least one of the four staphylococcal toxins

et al., 2007). Overall, toxin expression in our MRSA collection was comparable with other reports. The frequency of *pvl*-positive strains was higher (15 vs 6%) than in a previous study (Ghebremedhin *et al.*, 2005) and the proportion of strains positive for one or more staphylococcal enterotoxins was lower (66 vs 89%). The frequencies of virulence gene-positive strains in our study could be biased by the selection of the tested strains, as only isolates differing by at least one band in the PFGE pattern were included.

Conclusion

Among a total of 200 MRSA isolates obtained from patients admitted to UHBS between 2000 and 2005, some of the frequent *spa* types corresponded to those frequently represented in the global *spa* database and corresponded to frequent types in the neighbouring country of Germany. Nine frequent *spa* types accounted for 49.5% of MRSA isolates and can be considered to be endemic at UHBS, whereas *spa* type t041 (15% of all isolates) belonged to a local epidemic strain. Two well-known international CA-MRSA clones (corresponding to USA300 and MLST ST80 clones, 5.5% of the total strains) were also present at our university hospital. Another 32 *spa* types occurring less than four times accounted for 23%. These were considered to be sporadic strains, possibly indicating the introduction of new MRSA clones from outside. Genetic diversity was considerable and there was no dominant 'UHBS' *spa* type throughout the study period except for outbreak strains.

We demonstrated that strain types and strain type frequencies can easily be compared with other countries using *spa* typing and that clusters as well as the control of outbreak strains can be clearly documented. Based on our results, we cannot draw any conclusion regarding implementation of infection control measures by *spa* typing as has been suggested by Mellmann *et al.* (2005). Thus, further studies should focus on the usefulness of *spa* typing with regard to efficient infection control measures. The impact of *spa* clustering analysis also needs to be addressed in order to be able to detect variations in endemic *spa* types.

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