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BRIEF REPORT

Molecular epidemiology of invasive methicillin-susceptible *Staphylococcus aureus* strains circulating at a Swiss University Hospital

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Abstract In contrast to methicillin-resistant *Staphylococcus aureus*, little is known of the distribution of *spa* types among methicillin-susceptible *S. aureus* (MSSA). We have analyzed 101 nonrepetitive invasive MSSA isolates from infected patients, consecutively isolated during 14 months between 2006 and 2007 at University Hospital Basel. They were genetically characterized according to *S. aureus* protein A (*spa*) types and important virulence-associated genes. Sixty-five different *spa* types corresponding to nine different *spa* clonal complexes were observed. Analysis of different virulence genes showed a frequency of 17% for toxic-shock syndrome toxin and 5% for exfoliative toxin D. In conclusion, *spa* typing revealed a great genetic diversity without predominant *spa* type, not providing evidence for clonal spreading.

Report

Staphylococcus aureus is an important human pathogen and is isolated in up to 25% of nosocomial infections. *S. aureus* bacteraemia is associated with substantial excess morbidity and mortality worldwide [1]. A recent study from the United States showed a predominance of one clonal complex (ST8) among methicillin-susceptible *S. aureus* (MSSA) strains, whereas the remaining strains were genotypically heterogeneous [2]. Strain typing is an established tool for surveillance and determination of strain relatedness. Sequence-based methods such as protein A gene (*spa*) strain typing offer the opportunity of reproducible and comparable results and have been successfully used in epidemiological studies [3, 4]. To date, relatively little data exist on *spa* types and the molecular epidemiology of invasive MSSA strains in contrast to methicillin-resistant *S. aureus* (MRSA). In this study, our goal was to assess the frequency of circulating invasive MSSA *spa* types isolated from primarily sterile sites in patients at University Hospital Basel (UHBS), Switzerland, and to detect virulence associated genes such as Panton-Valentine leukocidin (PVL), toxic shock syndrome toxin (*tst*), exfoliative toxin A, B, and D (*eta*, *etb*, *etd*).

As standard operating procedure, invasive MSSA isolates from primarily sterile sites (cerebro-spinal fluid, sterile body fluids, biopsies, blood cultures) are routinely saved at −70°C at our laboratory. The isolates were identified as *S. aureus* on the basis of positive catalase, clumping factor (Slidex StaphPlus, bioMérieux, Marcy l'Etoile, France), and aurease (RAPIDECstaph, bioMérieux). Only one isolate per patient was included. DNA was extracted with LC MagnaPure system (Roche Diagnostics, Rotkreuz, Switzerland) according to manufacturer's instruction. The *spa* gene was amplified using primers spa-1113f and spa-1514r and sequencing was performed as previously described [4]. Sequences were analyzed with Ridom StaphType software version 1.4 (Ridom GmbH, Würzburg, Germany). Clustering analysis into *spa* clonal complexes (spa-CC) was done

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using default parameters (*spa* types are clustered if cost is less than or equal to 6 and *spa* types shorter than 5 repeats are excluded). PVL, *tst*, *eta*, *etb*, and *etd* genes were detected by PCR as described elsewhere [5–7]. Amplification of the 16S rRNA gene was used to confirm absence of PCR inhibitors. PCR products were visualized by electrophoresis on ethidium bromide-stained 3% agarose gels. Antibiotic susceptibility was tested applying a microbroth dilution procedure (MERLIN Diagnostika, Bornheim-Hersel, Germany) and interpreted in accordance with the Clinical and Laboratory Standards Institute (http://www.clsi.org).

A total of 105 patients with MSSA infection during a 14-month period in 2006–2007 were recorded. Isolates of 101 patients were analyzed; four isolates were not available. The median age of patients was 61 years (interquartile range 45–74) with 63.4% being males. The highest proportions of MSSA strains were isolated from the surgical department (42%), emergency department (31%), internal medicine (11%), and intensive care units (10%). Blood culture was the most common body site; the same organism was isolated from another source in 11.2% (9 isolates; not included to avoid double counting). Specimens from joint, bone, and skin/soft tissue each accounted for 5.9% (6 isolates). A total of 65 different *spa* types corresponding to nine different spa-CC were observed (Table 1), and 51 strains had a unique *spa* type. Eleven newly recognized *spa* types were found which have never been deposited before in the *spa* database (http://www.spaserver.ridom.de). The ten most frequent *spa* types

Table 1 Distribution of 101 invasive methicillin-susceptible *S. aureus* (MSSA) strains by *spa* clonal complex (spa-CC), presence of virulence-associated determinants, and antibiotic resistance profile

Spa-CC	*Spa* Type	Frequency	PVL	*etd*	*tst*	*eta/etb*	Penicillin R	Clarithromycin R	Quinolone R[b]	Site of Isolation	
										Blood	Other than blood
		n	*n*	*n*	*n*	*n*	*n* (%)	*n* (%)	*n* (%)	*n*	*n*
CC084/085	*t084* (8), t085 (1), *t091* (1), t094 (1), t254 (1), t279 (1), t346 (2), t803 (1), t2432 (1), t2434 (1)	18				1	16 (89)			15	3
CC012	*t012* (3), *t021* (2), t093 (1), t122 (2), t298 (1), t665 (1), t913 (1), t1306 (1), t1836(1), t2437 (1)	14	1		8		13 (93)		1 (7.1)	11	3
CC002	t002 (7), t062 (2), t548 (2)	11			5		5 (45.5)	3 (27.3)	1 (9)	10	1
CC376	t359 (3), t376 (1), t1198 (1), t1897 (2)	7	1	2			5 (71.4)			7	0
CC078	*t056* (4), *t078* (1), t258 (1)	6		2			1 (16.7)			4	2
CC005	*t005* (1), t223 (1), t712 (1), t790 (1), t902 (1), t2439 (1)	6					3 (50)	1 (16.7)		4	2
CC024	*t008* (2), *t024* (1), t190 (1)	4					1 (25)			4	0
CC888	*t127* (1), *t160* (1), t888 (1), t2433 (1)	4					2 (50)			2	2
CC230	t230 (1), t550 (1), t571 (1)	3			1		3 (100)	1 (33.3)		1	2
Others [a]	*t015* (6), t026 (4), t136 (1), t153 (1), t159 (1), t163 (1), t216 (1), t330 (1), t350 (1), t645 (1), t777 (1), t1662 (1), t1992 (1), t2435 (1), t2438 (1), t2441 (1), t2442 (1), t2443 (1), t2476 (1),	28		1	3	1	21 (75)			22	6
Total		101	2	5	17	2	70	5	2	80	21

Previously published MSSA *spa* types are in italics [3, 8]. Empty cells denote a negative result
eta/etb exfoliative toxin A/B, *etd* exfoliative toxin D, *PVL* Panton-Valentine leukocidin toxin, *R* resistant, *tst* toxic shock syndrome toxin
[a] Includes strains that were not typeable, singleton, no founder or excluded from analysis (shorter than five repeats)
[b] Resistant to at least one of these antibiotic compounds: levofloxacin, ciprofloxacin, norfloxacin

[t084 (8 strains), t002 (7), t015 (6), t026 (4), t056 (4), t012 (3), t359 (3), t008 (2), t021 (2), t062 (2)] accounted for 40.6% of all isolates. Comparing our MSSA *spa* types with those deposited in the *spa* server for MRSA and MSSA strains, t002 is the fourth most frequent *spa* type worldwide among MSSA and MRSA isolates. t008, t015, t024, and t084 correspond to the top ten *spa* types in this large database (http://www.spaserver.ridom.de). Some of our *spa* types are similar to those previously reported in MSSA strains (Table 1, *spa* types in italics) [3, 8]. Genetic diversity of our study isolates exceeded that of a previously published study where 14 different *spa* types among 84 MSSA strains were detected [3]. A recent study from France on MSSA bloodstream infections using pulsed-field gel electrophoresis (PFGE) for genetic analysis showed a small number of PFGE divisions among isolates and were associated with epidemic phenomena in healthcare institutions [9]. If *spa* typing or spa-CC data were analyzed by the type of ward, no cluster with the same MSSA *spa* type at the same time was observed. The findings of molecular typing were supported by epidemiological data from surveillance: there was no evidence for an outbreak situation during the study period.

S. aureus produces a variety of extracellular toxins. They are associated with abscesses, furunculosis, and necroticing pneumonia or toxic shock syndrome [5, 6]. The *tst* gene was detected in 17% of cases, *etd* in 5%, PVL and *eta/etb* each in 2%. One isolate carried both PVL and *etd* (*spa* type t1198). Every *etd*-positive strain was associated with a different *spa* type, 17 *tst*-positive strains with 13 different types (4 strains of *spa* t002). In our study, the percentage of *tst* gene-positive invasive MSSA strains was comparable with MSSA strains from bloodstream infections [9] but lower than in a collection of MSSA strains mainly from sites other than blood [3]. All toxin positive strains were isolated from blood cultures except for two *tst*-positive strains (from soft tissue and a joint biopsy).

In contrast to recent studies from France and Germany, the antibiotic resistance rates (Table 1) of quinolones and macrolides were low [3, 9]. However, 93% and 89% of spa-CC012 and spa-CC084/CC085 were penicillin-resistant, respectively. Only 5% of all strains were macrolide resistant, not allowing meaningful interpretation. The prevalence of methicillin-resistant *S. aureus* (MRSA) in our institution is low [10]; only two invasive isolates of *S. aureus* were found to be methicillin resistant and were therefore excluded.

Nine of 65 *spa* types representing 26.7% of MSSA strains were also represented in our local MRSA *spa* database (t002, t008, t012, t015, t024, t026, t163, t230, t548). This genetic similarity between MRSA and MSSA is consistent with other reports [3, 8, 11]. However, these *spa* types are also frequent types worldwide and therefore importation of MRSA strains seems more likely than acquisition of methicillin-resistance genes among the local MSSA strain pool as suggested previously [8, 11]. But the common genetic background among MSSA and MRSA strains indicates a certain selective advantage regardless of resistance and accessory virulence gene profile.

In conclusion, *spa* typing revealed a great genetic diversity among invasive MSSA strains consecutively isolated from primarily sterile sites without a predominant *spa* type or spa-CC. Besides worldwide common *spa* types and spa-CC, many rare and newly recognized *spa* types were observed. There was no evidence for MSSA cross-infections among patients. *tst* was the most frequent virulence gene detected among MSSA isolates. Further *spa* typing data on MSSA strains from other places would allow a more comprehensive understanding of the genetic background.

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