

Validation of Pulsed-Field Gel Electrophoresis and *spa* Typing for Long-Term, Nationwide Epidemiological Surveillance Studies of *Staphylococcus aureus* Infections[∇]

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Pulsed-field gel electrophoresis (PFGE) of genomic macrorestriction fragments has been used by the Belgian Reference Laboratory for Staphylococci for national hospital surveys of methicillin-resistant *Staphylococcus aureus* since 1992. The sequencing of the polymorphic X region of the protein A gene (*spa* typing) offers significant advantages over PFGE in terms of speed, ease of interpretation, and exportability. To validate its potential use for national surveillance, we evaluated the robustness of *spa* typing compared with that of PFGE based on a collection of 217 *S. aureus* strains representative of the Belgian *S. aureus* epidemiology during the last 13 years. *spa* typing and PFGE both showed high discriminatory power (discriminatory indexes of 0.98 and 0.96, respectively) and achieved high concordance (95.9%) in type classification. Both methods also showed good concordance with multilocus sequence typing (MLST) (95.5%). However, we observed occasional “violations” of MLST clonal complex assignment by *spa* typing. Our results suggest that both PFGE and *spa* typing are reliable methods for long-term, nationwide epidemiological surveillance studies. We suggest that *spa* typing, which is a single-locus-based method, should preferably be used in combination with additional markers, such as staphylococcal cassette chromosome *mec* typing or resistance or virulence gene detection.

Staphylococcus aureus is a leading human pathogen, responsible for a wide range of diseases, from superficial skin infections to life-threatening conditions, such as endocarditis, pneumonia, and toxic shock syndrome (24). Strains of *S. aureus* resistant to methicillin (methicillin-resistant *S. aureus* [MRSA]) and to other β -lactams first emerged in 1961, shortly after the introduction of methicillin into clinical practice (21). Since then, MRSA has spread worldwide, causing outbreaks in the hospital setting (hospital-acquired [HA]-MRSA) as well as in the community (community-acquired [CA]-MRSA), becoming a major health issue (1).

During the last few decades, diverse typing methods, first phenotypic, then genotypic, have been used for monitoring and tracking MRSA spread. Among these, pulsed-field gel electrophoresis (PFGE) of genomic macrorestriction fragments has emerged as the gold standard method for MRSA epidemiology (36). PFGE proved to be a highly discriminatory and sensitive technique in microepidemiological (local or short term) and macroepidemiological (national, continental, or long term) surveys (2, 25, 28, 31, 37, 40). Nevertheless, some authors have argued that the stabilities of PFGE markers may be insufficient for the reliable application of PFGE to long-term or macroepidemiological studies (3). In addition, PFGE remains suboptimal regarding other aspects: it is a technically demanding and labor-intensive method, its interpretation leaves room for subjectivity (40), and interlaboratory result comparison remains difficult and dependent on strict adherence to standardized protocols and interpretation criteria (4, 11, 26).

In recent years, sequence-based methods, which provide fast, unambiguous, and exportable typing data, have been developed. Multilocus sequence typing (MLST) is a method based on the sequence polymorphism of \sim 500-bp-long fragments of seven housekeeping genes (13). Initially designed for the study of bacterial population genetic structures, this technique has proved to be adequate for the long-term global epidemiology and study of the recent evolution of *S. aureus* (13, 14). A second technique, called staphylococcal cassette chromosome *mec* (SCC*mec*) typing, is based on the molecular characterization by multiplex PCR of the mobile genetic element carrying the methicillin resistance gene (*mecA*) (29). The combination of the MLST type and the SCC*mec* type, defined as the “clonal type,” is now used for the international nomenclature of MRSA clones (14). However, MLST typing remains too expensive and labor-intensive for its application to outbreak investigations and routine surveillance at a national level (14, 30).

The sequencing of the polymorphic X region of the protein A gene (*spa*), which contains a variable number of 24-bp-repeat regions flanked by well-conserved regions, is called *spa* typing (15). This single-locus-sequence-based typing method combines a number of technical advantages, such as rapidity, reproducibility, and portability (35, 36). Moreover, due to its repeat structure, the X region simultaneously indexes micro- and macrovariations, enabling the use of *spa* typing in both local and global epidemiological studies (22).

PFGE has been used by the Belgian Reference Laboratory for Staphylococci for both local outbreak investigations and national hospital surveys of MRSA since 1992 (6–9, 37, 39). *spa* typing offers significant advantages over PFGE in terms of speed, workflow capacity, ease of interpretation, and exportability. We performed this study to (i) validate the use of

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TABLE 1. Discriminatory power and concordance of PFGE and *spa* typing based on a collection of 217 *S. aureus* strains

Method	Classification level	Typeability (%)	Value for classification level		DI (95% CI)	Concordance (%) with:	
			No.	Most frequent (%)		PFGE type	PFGE group
PFGE	Type	100	83	28	0.961 (0.949–0.973)		
	Group	100	52	28	0.935 (0.922–0.948)		
<i>spa</i> typing	Type	100	95	18	0.976 (0.969–0.983)	95.9	94.7
	CC	97 ^a	23	43	0.892 (0.874–0.911)	92.1	93.4

^a Six *spa* types, shorter than five repeats, were excluded.

PFGE for long-term, nationwide epidemiology by the Reference Laboratory for Staphylococci, (ii) evaluate the robustness of *spa* typing and its concordance with PFGE and MLST, and (iii) establish, if possible, a “permutation nomenclature” from PFGE to *spa* typing to validate its potential use for the national surveillance of MRSA.

MATERIALS AND METHODS

Strain collection. A group of 217 strains (93 MRSA and 124 methicillin-susceptible *S. aureus* [MSSA] strains) were selected from the Belgian Reference Laboratory for Staphylococci collection based on their PFGE profiles. The goal was to select strains with various degrees of genetic relatedness, collected all over the country during a 13-year period as part of national surveillance studies and outbreak investigations. Strains representative of all major epidemic Belgian HA PFGE types (6–9) as well as minor or sporadic PFGE types were selected from national surveillances in 1992, 1995, 1997, 2001, and 2003 (6–8, 12) and outbreaks (10). CA-MRSA strains (described in reference 5) as well as MSSA strains collected during a Belgian national survey in 2003 (M. Hallin, O. Denis, A. Deplano, R. De Mendonça, R. De Ryck, S. Rottiers, and M. J. Struelens, submitted for publication) were included, as they presented a large genotypic diversity.

DNA extraction. DNA was extracted as described by Ünal et al. (41). Briefly, isolates cultured for 24 h on Columbia agar with 5% sheep blood were successively incubated with lysostaphin and proteinase K, boiled, and finally centrifuged. This lysate was used as a DNA template in all PCRs described below.

Identification and characterization of oxacillin resistance. The presence of *mecA* and *nuc* genes was tested by PCR as previously described (17).

Molecular typing methods. (i) **PFGE.** SmaI restriction fragments of genomic DNA were separated by PFGE with a CHEF mapper system (Bio-Rad Laboratories, Nazareth, Belgium) as previously described (12). Similarities among macrorestriction patterns were determined both by visual comparison and by computer matching with BioNumerics 4.0 software (Applied Maths, Ghent, Belgium). Bands in the size range between 36 and 700 kb were analyzed by the Dice similarity coefficient, with the position tolerance set to 0.8%, and dendrograms for similarity were built using the unweighted-pair group method using arithmetic averages. Patterns differing by less than seven fragments are considered to belong to the same PFGE group (represented by a capital letter), and those differing by zero to three fragments are considered to belong to the same PFGE type (represented by a number) (6).

(ii) ***spa* typing.** *spa* typing was performed as described by Harmsen et al. (18), and *spa* types were determined with Ridom StaphType software version 1.4 (Ridom GmbH, Würzburg, Germany) and analyzed by the BURP algorithm, with the following default parameters: *spa* types shorter than five repeats were considered nongroupable, and *spa* types belonged to the same group (clonal complex [CC]) if the cost was less than or equal to six (according to the excision, duplication, substitution, and insertion/deletion model) (34).

(iii) **MLST.** MLST was performed as described by Enright et al. (13) on all MRSA strains as well as on a subsample of 32 MSSA strains representative of every PFGE group. Sequence types (STs) were determined with the MLST database, accessible via <http://www.mlst.net>.

SCC*mec* typing. SCC*mec* typing was performed by multiplex PCR as described by Oliveira and de Lencastre (29).

Typing system evaluation. (i) **Typeability.** Typeability, defined as the proportion of strains that are assigned a type by a typing system, was calculated as described by Struelens (38).

(ii) **DI.** Discriminatory index (DI), defined as the average probability that the typing system will assign a different type to two unrelated strains, was calculated as described by Hunter and Gaston (19) and the confidence intervals as described by Grundmann et al. (16).

(iii) **Concordance.** Concordance between methods was calculated as described by Robinson et al. (33). Briefly, all possible pairs of strains are examined, and their types are classified as “identical” or “different” by the two methods and then cross-classified in a two-by-two table. Concordance, expressed in percentages, corresponds to the proportion of pairs for which the two methods are in agreement.

RESULTS

PFGE and *spa* typing: discriminatory power and concordance. The PFGE patterns of the 217 strains belonged to 52 groups and 85 types. Sixty-one types belonging to 33 groups were represented by a single strain.

Ninety-five *spa* types were found (62 were represented by a single strain). Eighty-nine of them were distributed by the BURP algorithm in 13 groups (CCs) and 10 singletons (groups represented by a single type), while 6 *spa* types shorter than five repeats were excluded from the clustering (Table 1).

The two methods showed both a high discriminatory power (DIs above 0.95 for both methods) and excellent concordance (95.9%) (Table 1).

Discrepancies between the two methods were mainly due to strains classified as singletons by one method but presenting the same type as epidemic strains by the other method. For example, a large majority of strains that we considered sporadic based on their PFGE patterns (having a difference of more than six fragments compared to any other strain in the database) belonged to *spa* CC002 or *spa* CC008, harboring the same *spa* type as epidemic strains (Fig. 1). Moreover, strains belonging to several distinct epidemic PFGE types were also undistinguishable by *spa* type: PFGE type A20 and A21 strains were mainly t008 (6 of 8 A20 strains and 3 of 5 A21 strains), while PFGE type G10 strains (all 3) and certain C3 strains (11 of 22) shared *spa* type t002.

Concordance with MLST. To assess the performance of PFGE and *spa* typing compared to that of MLST, we worked on a 125-strain subset (93 MRSA and 32 MSSA strains) representative of all the PFGE groups present in the 217-strain collection. MLST showed a DI of 0.93, identifying 33 different STs, of which 23 were represented by a single strain. The 33 STs belonged to 20 CCs.

The performances of PFGE and *spa* typing in terms of concordance with MLST were equally good (95.5 and 95.4%, respectively).

No	Year	Category	SCCmec	MLST type	PFGE-type	spa type	MLST CC	PFGE group	spaCC																							
1	2003	HA-MRSA	II	225	C1	t003	5	C	2																							
2	2003	HA-MRSA	IV	5	C2	t002																										
3	2003	MSSA	5		C3					t447																						
4	2001	HA-MRSA									IV	5	t045																			
5	1999	HA-MRSA									I			5	t001																	
6	2003	HA-MRSA									IV					5	t769															
7	1997	HA-MRSA									III							5	I													
8	2003	MSSA									5									G10	t002											
9	1992	HA-MRSA																				III	5	G12								
10	1992	HA-MRSA																				I			5	I						
11	1992	HA-MRSA																				I					5	I				
12	2001	HA-MRSA																				IV							5	I		
13	2003	HA-MRSA																				II									5	I
14	2001	HA-MRSA																				II										
15	1999	HA-MRSA					II	5	I																							
16	2003	HA-MRSA		II		5	I																									
17	2001	HA-MRSA	I	5	I																											
18	2001	HA-MRSA	I							5		I																				
19	2001	HA-MRSA	I										5	I																		
20	2001	HA-MRSA	I												5	I																
21	2001	HA-MRSA	I														5	I														
22	2003	HA-MRSA	I								5								I													
23	1997	HA-MRSA	I																	5	I											
24	2003	HA-MRSA	I																			5	I									
25	1999	HA-MRSA	I																					5	I							
26	2003	HA-MRSA	I																							5	I					
27	2003	HA-MRSA	I																									5	I			
28	2001	HA-MRSA	I																											5	I	
29	2003	HA-MRSA	IV					5	I																							
30	1992	HA-MRSA	I			5	I																									
31	1992	HA-MRSA	I	5	I																											
32	1999	HA-MRSA	I							5		I																				
33	1992	HA-MRSA	I										5	I																		
34	1995	HA-MRSA	I												5	I																
35	1997	HA-MRSA	I														5	I														
36	2003	HA-MRSA	I								5								I													
37	1992	HA-MRSA	I																	5	I											
38	1999	HA-MRSA	I																			5	I									
39	2001	HA-MRSA	I																					5	I							
40	1997	HA-MRSA	I																							5	I					
41	2001	HA-MRSA	I																									5	I			
42	2003	HA-MRSA	I																											5	I	
43	1999	HA-MRSA	I					5	I																							
44	2003	HA-MRSA	I			5	I																									
45	2003	HA-MRSA	IV	5	I																											
46	2002	CA-MRSA	IV							5		I																				
47	2004	CA-MRSA	IV										5	I																		
48	2004	CA-MRSA	IV												5	I																
49	2003	MSSA	IV														5	I														
50	1999	HA-MRSA	IV								5								I													
51	2001	HA-MRSA	IV																	5	I											
52	2001	HA-MRSA	IV																			5	I									
53	2003	HA-MRSA	IV																					5	I							
54	2003	MSSA	IV																							5	I					
55	2003	MSSA	IV																									5	I			
56	2003	HA-MRSA	IV																											5	I	
57	1999	HA-MRSA	IV					5	I																							
58	2001	HA-MRSA	I			5	I																									
59	2001	HA-MRSA	I	5	I																											
60	1992	HA-MRSA	III							5		I																				
61	1997	HA-MRSA	IV										5	I																		
62	2003	MSSA	IV												5	I																
63	2001	HA-MRSA	IV														5	I														
64	2003	HA-MRSA	IV								5								I													
65	1999	HA-MRSA	IV																	5	I											
66	2004	MSSA	IV																			5	I									
67	2005	MSSA	IV																					5	I							
68	1992	MSSA	IV																							5	I					
69	2003	MSSA	IV																									5	I			
70	2001	HA-MRSA	IV																											5	I	
71	2003	MSSA	IV					5	I																							
72	2001	HA-MRSA	IV			5	I																									
73	2001	MSSA	IV	5	I																											
74	2003	CA-MRSA	IV							5		I																				
75	2002	CA-MRSA	IV										5	I																		
76	2004	MSSA	IV												5	I																
77	2003	HA-MRSA	IV														5	I														
78	2003	MSSA	IV								5								I													
79	2003	HA-MRSA	IV																	5	I											
80	2003	MSSA	IV																			5	I									
81	1997	HA-MRSA	IV																					5	I							
82	1999	HA-MRSA	IV																							5	I					
83	2000	HA-MRSA	IV																									5	I			
84	2000	HA-MRSA	IV																											5	I	
85	2000	HA-MRSA	IV					5	I																							
86	2001	HA-MRSA	IV			5	I																									
87	2003	HA-MRSA	IV	5	I																											
88	1995	HA-MRSA	IV							5		I																				
89	1997	HA-MRSA	IV										5	I																		
90	1999	HA-MRSA	IV												5	I																
91	2001	HA-MRSA	IV														5	I														
92	2001	HA-MRSA	IV								5								I													
93	2001	HA-MRSA	IV																	5	I											
94	2003	HA-MRSA	IV																			5	I									
95	2003	CA-MRSA	IV																					5	I							
96	2004	CA-MRSA	IV																							5	I					
97	2004	CA-MRSA	IV																									5	I			
98	2004	CA-MRSA	IV																											5	I	
99	2004	CA-MRSA	IV					5	I																							
100	2004	CA-MRSA	IV			5	I																									
101	2004	CA-MRSA	IV	5	I																											
102	2004	CA-MRSA	IV							5		I																				
103	2004	CA-MRSA	IV										5	I																		
104	2004	CA-MRSA	IV												5	I																
105	2005	CA-MRSA	IV														5	I														
106	2004	CA-MRSA	IV								5								I													
107	2001	HA-MRSA	IV																	5	I											
108	2003	MSSA	IV																			5	I									
109	2003	MSSA	IV																					5	I							
110	2003	MSSA	IV																							5	I					
111	2003	MSSA	IV																									5	I			
112	2005	MSSA	IV																											5	I	
113	2005	MSSA	IV					5	I																							
114	2004	MSSA	IV			5	I																									
115	2004	MSSA	IV	5	I																											
116	2003	MSSA	IV							5		I																				
117	2003	MSSA	IV										5	I																		
118	2001	MSSA	IV												5	I																
119	2001	HA-MRSA	V*														5	I														
120	2005	MSSA	IV								5								I													
121	2003	MSSA	IV																	5	I											
122	2003	MSSA	IV																			5	I									
123	2003	MSSA	IV																					5	I							
124	2003	MSSA	IV																							5	I					
125	2005	CA-MRSA	IV																									5	I			
228																														D4	t744	8
885								A1	t051																							
247						A6	t008																									
683				A23	t054																											
8				A24	t770																											
8				A9	t008																											
8				A20	t008																											
8				A21	t008																											
250				F2	t121																											
250				F3	t008																											
22				L1	t032																											
25				U1	t078																											
25				U7	t081																											
25				M	t168																											
886				P2	t318																											
36				J1	t018																											
642				J2	t019																											
30				J7	t046																											
30				J8	t433																											
30				J4	t021																											
30				J4	t019																											
34				J5	t773																											
887				Spor	t655																											
NEW																																
45				B2	t038	45	B	38																								
45				B2	t247																											
45				B2	t598																											
45				B2	t739																											
45				B2	t740																											
80				X1	t131				80	X	131																					
80				X1	t044																											
80				X1	t044																											
80				X1	t044																											
80				X1	t044																											
88				Spor	t186							78	R	SGT																		
88				Spor	t186																											
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88				Spor	t186																											
88				Spor	t186																											
121				N2	t645	121	N	645																								
121				N2	t645																											
121				N2	t645																											
121				N2	t645																											
121				N2	t645																											
182				E1	t493				12	O	160																					
377				Y	t355																											
425				Z	t747																											
101				O2	t056																											
7				O1	t091																											
12				O3	t160																											
15				T1	t084																											
59				W1	t216																											
78				Spor								78	H																			
78				Spor																												
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FIG. 1. Years of isolation and typing results for 125 *S. aureus* strains obtained by SCCmec typing, MLST, spa typing, and PFGE. Clustering results are indicated as follows: congruent groups or CCs obtained by MLST, spa typing, and PFGE are represented by shaded blocks, and discordant results are represented in separated, colorless boxes. SGT, singleton; Spor, sporadic; NF, no founder assigned; NEW, MLST profile containing one new allele, with no ST assigned yet; *, not typeable by the Oliveira and de Lencastre method (29).

TABLE 2. Typing characteristics of 16 strains belonging to *spa* CC131

No. of strains	Category	<i>spa</i> profile											<i>spa</i> type	MLST profile	ST	CC	SCC <i>mec</i> type	PFGE type	
1	CA-MRSA	07	23	12	--	--	--	--	--	34	33	34	t131	1-3-1-14-11-51-10	ST80	CC80	IV	X1	
10	CA-MRSA	07	23	12	--	--	--	--	--	34	34	33	34	t044	1-3-1-14-11-51-10	ST80	CC80	IV	X1/X2
2	CA-MRSA, HA-MRSA	07	--	12	21	17	13	13	34	34	33	34	t186	22-1-14-23-12-4-31	ST88	CC78	IV	Sporadic/H	
1	MSSA	07	23	--	21	16	--	--	--	34	33	13	t127	1-1-1-1-1-1-1	ST1	CC1		S1	
1	MSSA	07	23	12	21	17	--	--	--	34	34	33	34	t267	3-1-1-1-1-5-3	ST97	CC97		V2
1	MSSA	07	16	12	--	23	--	--	--	--	--	34	t209	3-27-1-1-1-1-10	ST109	CC9		R2	

PFGE and MLST. MLST CC22, CC9, CC80, and CC121 corresponded to one PFGE group each (Fig. 1). CC30, CC45, CC78, and CC25 each contained two distinct PFGE groups. Conversely, two strains belonging to distinct CCs (ST101 and ST7) fell into the same PFGE group.

A more complex picture was observed with strains of CC5 and CC8 lineages: PFGE subdivided them into 9 and 12 distinct groups, respectively (Fig. 1). Most of those groups corresponded to sporadic PFGE patterns but also included PFGE groups C, G, and D (containing epidemic HA-MRSA PFGE types C3, G10, and D4), all of which belonged to CC5 and some of which (PFGE types C3 and G10) belonged to the same ST (ST5). In the same way, strains of PFGE types A20 and A21 (considered two distinct epidemic HA-MRSA clones) both belonged to ST 8.

***spa* typing and MLST.** As for PFGE, each MLST CC corresponded to one *spa* CC, as was the case for CC5, CC8, CC45, CC22, and CC25 (Fig. 1). Exceptions included CC30 and CC121, both of which corresponded to one *spa* CC and one *spa* singleton.

All strains belonging to CC80 shared the same *spa* CC (CC131). However, *spa* CC131 also included strains belonging to four other MLST CCs (two ST88, one ST97, one ST1, and one ST109 strain) (Table 2).

As for PFGE types, several *spa* types corresponded to the same ST. Conversely, in CC5 and CC8 as well as in CC30, *spa* typing was not able to distinguish STs. Strains belonging to distinct STs which harbored the exact same *spa* type were as follows: t008 was associated with ST683, ST8, and a new ST (a ST885; t002 with ST5 and ST231; t001 with ST5 and ST228; t038 with ST45 and a new ST (a single-locus variant of ST45); and t019 with ST30 and ST642. However, such a violation of MLST ST "assignment" also happened occasionally for PFGE typing: PFGE J5 was associated with ST642 and ST30, J2 with ST30 and ST34, and B2 with ST45 and a new ST (a single-locus variant of ST45).

In summary, despite concordance percentages similar to these seen with MLST, PFGE classification in groups and types rarely violated the MLST assignment of CCs and STs, while this type of violation was much more frequent for the *spa* classification into types and CCs. To quantify this problem, we examined the concordance tables for each method with MLST and compared the percentages of discordant pairs of strains that belonged to distinct STs or CCs by MLST but were clumped into the same type, group, or CC by either PFGE or *spa* typing (Table 3). Up to 12% of the discrepancies between *spa* type and MLST ST and 61% of those between *spa* CC and MLST CC fell into this category, compared with only 5% of the

discrepancies between PFGE type and MLST ST and 0.2% of those between PFGE group and MLST CC.

SCC*mec* typing and clonal type assignment. Given the clustering of several PFGE types and groups into common MLST STs and the discrepancies observed between *spa* types and PFGE types as described above, the SCC*mec* types of MRSA strains were determined to examine whether the combination of SCC*mec* with the different typing methods could resolve conflicting assignments.

The most frequent SCC*mec* type among the 93 MRSA strains was type IV ($n = 53$), followed by type I ($n = 30$). Following the method described by Oliveira and de Lencastre (29), six strains were of SCC*mec* type II, three strains were of SCC*mec* type III, and one was nontypeable (SCC*mec* type V) (20) (Fig. 1). The combination of SCC*mec* typing with the three methods allowed an increase of concordance from 95.5% between PFGE type and MLST ST to 96.1% between PFGE type plus SCC*mec* type and clonal type (Table 3) and from 95.3% between PFGE and *spa* typing to 95.6% between PFGE plus SCC*mec* typing and *spa* typing plus SCC*mec* typing. This minor improvement in intermethod agreement for PFGE was due to the fact that PFGE G10/ST5/*spa* CC002 strains harbored SCC*mec* type II, while PFGE C3/ST5/*spa* CC002 strains harbored SCC*mec* I, III, or IV. However, SCC*mec* typing did not help to resolve the conflicts within CC8 and CC30 lineages, since all ST30/*spa* CC021/CC012 strains harbored SCC*mec* type IV, all ST247/*spa* CC008 strains (epidemic-PFGE A1 and PFGE-sporadic types) harbored SCC*mec* type I, and all except

TABLE 3. Concordance of *spa* typing and PFGE with MLST and clonal type based on a 125-strain subset

Classification level	No. of strains	Concordance (%) with:			% Violation of assignment to ^b :	
		MLST ST	MLST CC	Clonal type	MLST ST	MLST CC
<i>spa</i> type	125	95.4			12	0
<i>spa</i> CC	124 ^a		98.6			61
<i>spa</i> type plus SCC <i>mec</i> type	93			94.8		
PFGE type	125	95.5			5	0
PFGE group	125		91.5			0
PFGE type plus SCC <i>mec</i> type	93			96.1		

^a One *spa* type, shorter than five repeats, was excluded.

^b Calculated as the proportion of discordant pairs of strains in the two-by-two concordance table that were considered different by MLST but similar by the other typing method.

one ST8/*spa* CC008 strain (PFGE-epidemic A20 and A21 as well as PFGE-sporadic types) harbored SCC*mec* type IV (Fig. 1).

DISCUSSION

PFGE has been used by the Belgian National Reference Laboratory for Staphylococci since 1992 for both local and national investigations (6–9, 37). It is considered the “gold standard” for typing *S. aureus* strains (36). However, *spa* typing possesses significant advantages over PFGE in terms of speed, ease of interpretation, and exportability (35, 36). The goal of this study was to evaluate the robustness of *spa* typing and its concordance with PFGE and MLST based on a strain collection representative of the Belgian *S. aureus* population.

Ideally, the test population for evaluating a typing systems should (i) be of large size ($n > 100$), (ii) reflect as much as possible the diversity expected in the microbial population to which the typing system will be applied, (iii) be constituted by strains that are epidemiologically unrelated, and (iv) not be selected on the basis of type characteristics (38). The strain collection described here fulfils most of these criteria, as it was large and diverse but was, however, selected on the basis of PFGE results to specifically cover the range of patterns identified over a decade in a particular region. Regarding the epidemiological relatedness of the strains studied, we selected strains coming from different locations if from the same year and from distinct years if from the same location.

PFGE is known to be a highly discriminatory and valuable technique for outbreak investigation (31, 37, 40). However, it has been argued that the stability of PFGE may be insufficient for its reliable application to long-term epidemiological studies: high degrees of genetic variation leading to multiple PFGE profiles have been observed for pandemic clones with “long” evolutionary histories. This was attributed to the heterogeneous selective pressure exerted in different hospital ecosystems (1). This has also been attributed to the fact that large chromosome fragments undergo faster variations than smaller ones (3), leading to a progressive drift of PFGE patterns over time. In this study, we showed the excellent concordance between PFGE type and MLST ST and between PFGE type plus SCC*mec* type and clonal type based on a nationwide strain collection covering a 13-year period. Our results not only validate the use of PFGE for long-term, nationwide epidemiological studies but also provide a good basis for a nomenclature permutation table between MLST ST (with or without SCC*mec* type) and PFGE type (with or without SCC*mec* type). However, PFGE is a labor-intensive method and requires technical skills, and despite the efforts made to standardize protocols and interpretation criteria, interlaboratory result comparison remains difficult (11, 26).

spa typing is a single-polymorphic-locus-sequence-based typing method. This technique possesses a significant number of practical advantages over PFGE. It is a rapid, reproducible, and easy-to-perform technique, providing unambiguous and exportable data (35, 36). In addition, an extensive study has demonstrated that it is applicable to both local and global epidemiological studies (22). Using a large national collection, *spa* typing was confirmed in the present study to be as discriminatory and concordant with MLST as PFGE. However, we

observed occasional “violations” of MLST ST assignment by *spa* typing with strains belonging to the same CC but to different STs which harbored the same *spa* type. Furthermore, strains belonging to distant MLST CCs (CC80, CC78, CC1, CC9, and CC97) had very similar *spa* profiles which clustered in a unique *spa* CC (*spa* CC131). We suspect that this phenomenon could be due to intergenomic recombination involving the *spa* locus. Although this type of event is presumed to be rare (22), it should have occurred, in this case, at least four times from one lineage to other lineages. Such a phenomenon was recently described by Robinson and Enright for another lineage (MLST ST239). The authors described a large chromosomal replacement, encompassing the *spa* locus, from ST30 to ST8 which generated ST239 (32). They speculated that this type of large replacement may be lineage specific. However, our findings suggest that this type of event may be more extensive than previously appreciated. Therefore, the extent and range of genetic lineages of *S. aureus* involved in large horizontal transfer in this chromosome region warrant further investigation.

spa typing was also highly concordant with PFGE. The majority of discrepancies observed between the two methods were encountered in MLST CC8 and CC5 and were of two different kinds. First, the large majority of MRSA strains which were classified as sporadic based on their PFGE profiles harbored the same *spa* type as epidemic MRSA belonging to *spa* CC008 (MLST CC8) and *spa* CC002 (MLST CC5). This could be due to the fact that PFGE may be in this case too discriminating as a result of the patterns of these strains drifting over time, as discussed above. This could also reflect genetic events such as the insertion/deletion of different mobile genetic elements that may be, to some extent, involved in the epidemic behavior of *S. aureus* and lead to different PFGE patterns while *spa* and MLST types remain indistinguishable.

The clustering of several epidemic HA-MRSA PFGE types in common *spa* CCs and *spa* types (as well as in common MLST ST) also deserves comment. This was the case for the clustering of G10 and C3 PFGE types into *spa* CC002/MLST ST5 and for the clustering of A20 and A21 PFGE types into *spa* CC008/MLST ST8. Again, one can speculate that all these discrepancies are due to the fact that PFGE may be too discriminating. However, these epidemic clones are known to possess, besides their specific PFGE profiles, several other distinctive features that are of potential epidemiological relevance. As described previously (7, 9), PFGE G10 and C3 strains have completely different susceptibility profiles for macrolides and tetracyclines: G10 strains usually harbor the *tetM* and *ermC* genes (conferring a constitutive resistance to tetracycline/minocycline and an inducible resistance to macrolides/lincosamides/streptogramins [MLS], respectively), while C3 isolates are susceptible to those drugs. G10 strains also harbor SCC*mec* type II, while C3 strains harbor SCC*mec* types I, III, and IV. Furthermore, G10 strains frequently carry the TSST-1 gene (7). In the same way, A20 strains possess the *ermA* gene (conferring a constitutive resistance to MLS), while A21 strains either are susceptible to MLS or harbor the *ermC* gene. All these observations suggest, as mentioned by others (8, 25), a parallel evolution of strains belonging to the same successful lineage but possessing different resistance and/or virulence genes acquired by insertion of large mobile elements, like

staphylococcal pathogenicity island 1 for TSST-1 and transposon Tn544 for *ermA* (23, 27). The same mechanisms could explain the differences observed in their PFGE patterns.

In conclusion, the excellent concordance we found between PFGE and MLST in our collection validates the use of PFGE by the Belgian Reference Laboratory for Staphylococci for long-term, nationwide epidemiological surveillance studies. We also have shown that *spa* typing can be used for the same purpose but with some caution. Since *spa* typing is a single-locus-based method, we suggest that additional markers, such as SCCmec type and resistance or virulence genes, should be determined, especially in the rapidly evolving CC8 and CC5 lineages. Furthermore, the occasional "violation" of MLST CC assignment by *spa* typing, which is presumably due to recombination events involving the *spa* locus, underlines the need for the confirmation of the MLST profiles of a subset of representative strains.

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