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Staphylococcal Interspersed Repeat Unit Typing of *Staphylococcus aureus*: Evaluation of a New Multilocus Variable-Number Tandem-Repeat Analysis Typing Method[▽]

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The present study evaluates the performance of the staphylococcal interspersed repeat unit (SIRU) method applied to a diverse collection of 104 *Staphylococcus aureus* isolates previously characterized by pulsed-field gel electrophoresis (PFGE), *spa* typing, multilocus sequence typing (MLST), and staphylococcal cassette chromosome *mec* typing for methicillin-resistant *S. aureus*. The SIRU method distributed the 104 strains into 81 SIRU profiles that could be clustered into 12 groups and 29 singletons. The discriminatory power of the method at the profile level, translated by Simpson's index of diversity (SID), was similar to that of PFGE subtyping (SID = 99.23% versus 99.85%) and slightly higher than that of *spa* typing (SID = 97.61%). At the group level, the SIRU SID (93.24%) was lower than that of PFGE typing (95.41%) but higher than that of MLST (SID = 91.77%). The adjusted Rand (AR) coefficient showed that SIRU typing at the group level had the highest congruence with MLST (AR = 0.5736) and with clonal complex (CC) (AR = 0.4963) but the lowest congruence with PFGE subtype (AR = 0.0242). The Wallace coefficient indicated that in the present collection, two strains with the same SIRU profile have a 100% probability of belonging to the same CC, a 90% probability of sharing the same *spa* type, and an 83% probability of being classified in the same sequence type. The high discriminatory power of the SIRU method, along with its apparent concordance with MLST results, makes it potentially valuable for *S. aureus* short-term epidemiological investigations and population dynamics as well.

Staphylococcus aureus, especially methicillin-resistant *S. aureus* (MRSA), continues to be a major cause of health care-associated and, more recently, community-associated infections (40, 62). It is critical to have access to an accurate typing method to design cost-effective intervention and prevention strategies (45, 48, 61). A large number of molecular typing methods have been developed to assess strain relatedness for outbreak control, surveillance programs, and population structure and evolution studies (58, 61). The three most used typing methods for *S. aureus* have advantages and disadvantages, as follows. (i) Pulsed-field gel electrophoresis (PFGE), which is the "gold standard" typing method, has high discriminatory power and accuracy, but it is time-consuming and expensive, and the interlaboratory exchange of results is challenging. (ii) Sequence-based multilocus sequence typing (MLST) is easy to perform, and the results, given as an allelic profile, are portable and easy to exchange due to a public database available on the Internet (http://www.mlst.net), but it is expensive and not useful for local outbreak investigations. MLST is frequently combined with staphylococcal cassette chromosome *mec* (SCC*mec*) typing in order to define clonal types of MRSA (24). (iii) *spa* typing, a single-locus sequence typing method, is being used more frequently for *S. aureus* typing, and the development of a public database on the Internet (http://spaserver.ridom.de), as with MLST, ensured an international typing nomenclature and thus a great facility in exchanging typing data. By calculation of Simpson's index of diversity (SID), it was shown that *spa* typing is nearly as discriminatory as PFGE (1, 25), although it takes into account a single variable region of the protein A gene.

In choosing a new method, it is worth taking into consideration that PCR-based methods are commonly used in typing laboratories because of their accuracy, ease of use, low cost, and speed in retrieving results (in a few hours).

Many bacterial genomes carry loci of repetitive DNA, which may contain variable repeated units among strains (43, 60). Systems based on a multilocus variable-number tandem-repeat (VNTR) analysis (MLVA) have been used extensively for typing of clinical isolates of several bacterial species and were shown to perform well compared to other genotyping methods (43, 61). *S. aureus* harbors a diverse population of DNA repeats, which allowed the design of various MLVA schemes (28, 30, 39, 53, 60). Hardy et al. (36, 38) developed a MLVA scheme for *S. aureus* where seven novel multiple tandem repeats with a high degree of similarity in the flanking regions were identified based on the alignment of seven *S. aureus* sequenced genomes (strains N315, MW2, Mu50, MSSA476, MRSA252, NCTC8325, and COL). Six of these seven loci were located on intergenic regions scattered around the *S. aureus* genome; the remaining locus corresponds to the protein A gene, *spa*. The method, designated staphylococcal interspersed repeat unit (SIRU) typing, relies on PCR amplification of the seven loci of repetitive DNA, using primers specific for

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the flanking regions of each locus, and on the determination of the size of each amplicon, which reflects the number of repeated units present on the targeted SIRU. To each of the seven loci is attributed the respective number of DNA repeats, generating a combination of seven numbers that characterizes each strain and corresponds to the allelic profile. This allelic profile makes the SIRU method amenable to interlaboratory comparisons and database management, comparable to MLST. So far, the SIRU method has been applied to *S. aureus* isolates from nosocomial outbreaks in the United Kingdom and Germany, mainly MRSA isolates, and therefore to highly related strains (29, 35–37). Very recently, a single study evaluated a MLVA scheme including the SIRU typing loci and the *sspA* gene, using a European collection of contemporary *S. aureus* isolates (39).

The aim of the present study was to evaluate the SIRU method with a more diverse collection of *S. aureus* isolates, including MRSA and methicillin-susceptible *S. aureus* (MSSA) isolates, from different continents, isolated throughout several decades and previously characterized by well-established typing methods (PFGE, *spa* typing, MLST, and SCC*mec* for MRSA).

MATERIALS AND METHODS

Bacterial isolates. A collection of 104 strains (78 MRSA and 26 MSSA strains), previously characterized by PFGE (16), *spa* typing (1, 56), MLST (3), and SCC*mec* typing (for MRSA strains) (46), was selected from the Laboratory of Molecular Genetics collection at Instituto de Tecnologia Química e Biológica, Oeiras, Portugal (Table 1). The selected collection included hospital- and community-related strains isolated during a period of over 50 years (from 1943 to 2006) from 17 countries distributed over four continents. Efforts were made to select strains with various degrees of genetic relatedness. Therefore, the collection included (i) strains belonging to the five main clonal complexes (CCs) of *S. aureus*—CC5 (n = 23), CC8 (n = 32), CC22 (n = 4), CC30 (n = 7), and CC45 (n = 9); (ii) isolates belonging to minor CCs—CC1 (n = 7), CC509 (n = 3), CC50, CC59, CC97, CC101, CC228, CC398, and CC1021 (one isolate of each); (iii) two isolates belonging to CC80, ST80, and SCC*mec* type IV, identified as community-acquired MRSA; (iv) six single CC isolates, referred to as singletons (S1, sequence type 157 [ST157]; S2, ST447; S3, ST668; S4, ST707; S5, ST580; S6, ST445); and (v) four strains belonging to nondefined CC groups (CC assignments assessed by eBURST v3) and designated ND1 to ND4.

Strains N315, NCTC8325, COL, and MW2 were included for reproducibility and methodology control, since their genomes are fully sequenced and were used in the theoretical design of the SIRU method (38).

PFGE analysis. PFGE patterns were analyzed in BioNumerics, version 4.61, software (Applied Maths, Sint-Martens-Latem, Belgium) as previously described (25), with minor modifications, including an optimization setting of 1.0% for band pattern comparisons and a 98% Dice coefficient similarity cutoff for PFGE subtypes.

***spa* typing and MLST analysis.** *spa* types were assigned through the Ridom web server (http://spaserver.ridom.de). Additionally, for one isolate previously characterized as nontypeable, the *spa* type was determined through sequencing of SIRU21 (see below). MLST alleles and STs were identified through the MLST database (http://www.mlst.net), and CCs were defined using the eBURST v3 algorithm (http://eburst.mlst.net).

SIRU typing. DNA was extracted as previously reported (3). The SIRU method was performed as previously described (36, 38), with the following minor modifications: (i) 0.5 U of AmpliTaq DNA polymerase (Applied Biosystems, CA) was used per PCR in a final volume of 25 μl, (ii) an annealing temperature of 59°C was used with primer SIRU05R2 (see below), and (iii) PCR products (10 μl) were resolved in a 2.5% Seakem LE (Cambrex, Rockland, ME) agarose gel in 0.5% Tris-borate-EDTA buffer (Bio-Rad, Hercules, CA) at 5 V/cm for 2.5 h. The size of each amplicon was determined by visual inspection by comparison with a 50-bp ladder size marker and by computer analysis using Bionumerics, version 4.61, software, which facilitates marker-based normalization of the migration distances and therefore guarantees accurate length measurements. The number of repeats was calculated, taking into account the combined size of the repeat unit and the flanking regions of each locus, as follows: (i) for SIRU01, repeat unit of 55 bp + flanking regions of 184 bp = 239 bp for one-repeat-length amplicon; (ii) for SIRU05, 60 bp + 146 bp = 206 bp; for SIRU05 with primer SIRU05R2, 60 bp + 156 bp = 216 bp; (iii) for SIRU07, 56 bp + 191 bp = 247 bp; (iv) for SIRU13, 64 bp + 148 bp = 212 bp; (v) for SIRU15 131 bp + 212 bp = 343 bp; (vi) for SIRU16, 159 bp + 162 bp = 321 bp; and (vii) for SIRU21, 24 bp + 96 bp = 120 bp.

The primers used to amplify each of the seven loci were previously published (36), except for those for locus 16 (SIRU16) and an additional reverse primer for locus 5 (SIRU05). New SIRU16 primers, SIRU16_2F (5′-TGGTGTTAATTTA GCTTGC-3′) and SIRU16_2R (5′-AAACGCAACTTGAAGAAACG-3′), were designed through sequence alignments of the SIRU16 loci of the seven *S. aureus* genomes previously considered for the design of the primers for the remaining loci (38). The new SIRU05 locus reverse primer was designed specifically for strains for which there was no amplification with the previously published primers (SIRU05L and SIRU05R), namely, for strains belonging to STs 1, 22, 45, and 80. Therefore, primer SIRU05R2 (5′-AGTTGTAGTCATCTTACTGC-3′) was designed through sequence alignments of the available SIRU05 loci of MW2, MSSA476 (both ST1), and EMRSA-15 (ST22) (sequence from the EMRSA-15 genome sequencing project at the Wellcome Trust Sanger Institute [http://www.sanger.ac.uk/sequencing/Staphylococcus/aureus/EMRSA15]). All sequence alignments were performed using ClustalW2 (http://www.ebi.ac.uk/Tools/clustalw2/index.html), with default parameters.

eBURST v3 software was used to cluster SIRU profiles. Isolates sharing six of seven loci with at least one isolate (single-locus variants) were included in the same SIRU group. Singletons represent profiles that appear once and have no related profiles in the collection.

Comparison of typing methods. SID and the respective confidence intervals were calculated as described previously (33, 57). The quantitative level of congruence between typing methods was assessed by a framework proposed by Carriço et al. (15), based on the adjusted Rand (AR) and Wallace (W) coefficients, available at http://www.comparingpartitions.info/. The AR coefficient quantifies the global agreement between two methods, whereas the W coefficient indicates the probability that two isolates classified as the same type by one method are also classified as the same type by another method (15).

RESULTS

SIRU typeability. In a first approach, we tested the method with four completely sequenced strains (N315 [ST5-SCC*mec* II], NCTC8325 [ST8-MSSA], COL [ST250-SCC*mec* I], and MW2 [ST1-SCC*mec* IV]) for which theoretical SIRU profiles had previously been published (36, 38). Five of the seven loci were amplified from the four tested strains; SIRU16 was amplified from strain MW2 only, whereas SIRU05 was amplified from all strains except MW2. A BLAST search showed that the published SIRU16 forward primer (SIRU16_L) (36) has similarity with strain MW2 but not with the three remaining tested strains. The SIRU16_L primer was found to have similarity with strain MSSA476, which belongs to ST1, like MW2, and with strain MRSA252 (ST36-SCC*mec* II). The primer was tested on additional ST36 isolates, and it performed well. These preliminary results led us to design a new SIRU16 primer that anneals with all available genomes of *S. aureus* strains (see Materials and Methods). The SIRU typing method using the new SIRU16 primers was applied to the whole collection. A seven-digit profile was obtained for only 70 of 104 strains, indicating a typeability of 67% when isolates with at least one nonamplified locus were considered nontypeable.

Considering the typeability of each locus separately, SIRU07 was the unique locus that showed 100% typeability. SIRU05 showed the lowest typeability (72%), followed by SIRU15 (95%), while the remaining SIRUs showed a typeability of 99% (one isolate was nontypeable). The consistent nonamplification of SIRU05 from all strains belonging to particular CCs, i.e., CC22, CC45, and CC80, led us to perform a BLAST

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TABLE 1. Characteristics of the 104 *S. aureus* isolates and SIRU typing results

Strain	Isolation date (yr)	Country	No. of SIRU repeats[a]							SIRU profile[b]	SIRU group[c]	PFGE type[d]	*spa* type	ST	CC[e]	SCC*mec* type[f]	Reference(s)
			SIRU01	SIRU13	SIRU15	SIRU16	SIRU21	SIRU05	SIRU07								
IPOP38	2001	Portugal	1	1	1	3	7	5[g]	3	1	G1	T2	t127	1	1	MSSA	5
CV73	1997	Cape Verde	1	1	2	3	7	5[g]	3	2	G1	T1	t127	1	1	MSSA	11
HU332	2003	Hungary	1	1	2	3	7	5[g]	3	2	G1	F11	t127	1	1	II	17
HGSA240	2003	Portugal	1	1	2	3	7	2	3	3	G1	T7	t127	81	1	MSSA	13
HSA49	1993	Portugal	2	2	1	3	10	3	1	4	G2	P1	t002	5	5	IV	7
122MEXU	1998	Mexico	2	3	1	3	10	3	1	5	G2	M2	t002	5	5	II	63
78MEXC	1997	Mexico	2	3	1	3	10	3	1	5	G2	A2	t002	5	5	II	63
79MEXC	1997	Mexico	2	3	1	3	10	3	1	5	G2	N3	t002	5	5	II	63
ARG229	1995	Argentina	2	3	1	3	10	3	1	5	G2	A4	t002	100	5	II	7, 9, 18
BK2464	1990	United States	2	3	1	3	10	3	1	5	G2	A1	t002	5	5	II	52
BM18	1989	United States	2	3	1	3	10	3	1	5	G2	B2	t002	5	5	IV	22, 51
N315	1982	Japan	2	3	1	3	10	3	1	5	G2	Q2	t002	5	5	II	41
47MEXU	1997	Mexico	2	3	1	3	8	3	1	6	G2	A3	t895	5	5	II	63
COB3	1996	Colombia	2	3	1	3	7	3	1	7	G2	B3	t045	5	5	IV	31
HU245	2001	Hungary	2	3	1	3	10	5	1	8	G2	A1	t002	5	5	II	17
HU317	2003	Hungary	2	3	1	3	6	3	1	9	G2	T5	t062	5	5	II	17
HU363	2004	Hungary	2	3	1	3	6	3	1	9	G2	T6	t062	5	5	II	17
JP1	1997	Japan	2	3	1	3	10	1	1	10	G2	M1	t002	5	5	II	8
JP26	1997	Japan	2	3	1	2	10	2	1	11	G2	ZF	t002	5	5	II	7, 8
PL72	1991	Poland	2	3	1	3	10	7	1	12	G2	P2	t053	5	5	IV	42
POL3	1992	Poland	2	3	1	3	10	8	1	13	G2	B4	t053	5	5	IV	42
ARG33	1996	Argentina	2	3	1	3	10	99	1	14	G2	Q1	t001	85	5	IIIA	7, 9, 18
ARG64	1996	Argentina	2	3	1	3	10	99	1	14	G2	S1	t001	85	5	I	7, 9, 18
HAR41	1998	Germany	2	3	1	3	10	2	1	15	G2	I2	t001	228	228	I	47
HU2	1996	Hungary	3	0	1	3	12	2	2	16	G3	D2	t989	239	8	III	23
HUSA304	1993	United States	3	0	1	3	12	2	2	16	G3	D1	t1053	239	8	III	23
HU270	2002	Hungary	3	0	1	2	6	2	2	17	G3	E4	t030	239	8	III	17
HU294	2003	Hungary	3	0	1	3	13	2	2	18	G3	D5	t538	239	8	IIIA	17
TUR1	1996	Turkey	3	0	1	3	6	2	2	19	G3	E7	t030	239	8	III	7
TUR27	1996	Turkey	3	0	1	3	6	2	2	19	G3	E8	t030	239	8	III	7
PL46	1995	Poland	4	0	1	2	6	2	2	20	G3	E6	t030	157	S1	III	42
CPS22	1985	Portugal	4	0	1	3	6	1	2	21	G4	E1	t421	239	8	IIIvar	21
CPS68	1985	Portugal	4	0	1	3	6	1	2	21	G4	E3	t421	239	8	IIIvar	21
HSA10	1992	Portugal	4	0	1	3	6	1	2	21	G4	E2	t421	239	8	IIIvar	7
GRE108	1998	Greece	4	0	1	3	7	2	2	22	G4	C5	t461	239	8	III	4, 7
HGSA142	2003	Portugal	4	0	1	3	7	0	2	23	G4	D3	t037	239	8	IIIA	13
HGSA339	2003	Portugal	4	0	1	3	7	0	2	23	G4	C3	t037	239	8	IIIA	13
HGSA57	1995	Portugal	4	0	1	3	7	0	2	23	G4	C6	t037	239	8	IIIA	12
HSJ216	1997	Portugal	4	0	1	3	7	99	2	24	G4	C1	t037	239	8	IIIA	10
HU25	1993	Brazil	4	0	1	3	6	0	2	25	G4	C1	t138	239	8	IIIA	59
HU272	2002	Hungary	4	0	1	3	10	2	2	26	G4	R2	t787	239	8	III	17
TAW166	1998	Taiwan	4	0	1	3	10	8	2	27	G4	N2	t036	254	8	IV	6, 7
TAW97	1998	Taiwan	4	0	1	3	7	3	2	28	G4	C2	t037	239	8	IIIA	6, 7
BK1953	1995	United States	4	0	1	4	11	4	2	29	G5	F1	t051	247	8	IA	52
HGSA13	1998	Portugal	4	0	1	4	11	4	2	29	G5	N1	t051	247	8	IA	12
HUR97	1998	Hungary	4	0	1	4	11	4	2	29	G5	F8	t051	247	8	IA	50
COL	1965	United Kingdom	4	0	1	4	10	6	2	30	G5	F6	t008	250	8	I	51
E2213	1965	Denmark	4	0	1	4	11	6	2	31	G5	F4	t051	247	8	I	19
E2453	1965	Denmark	4	0	1	4	11	6	2	31	G5	F2	t051	247	8	I	19
HPV107	1992	Portugal	4	0	1	3	11	4	2	32	G5	F1	t051	247	8	IA	55
GRE18	1998	Greece	4	0	99	2	7	2	2	33	G6	F5	t037	239	8	III	7
HGSA15	1994	Portugal	4	0	99	2	7	99	2	34	G6	C4	t037	239	8	IIIA	12
HAR22	1991	United Kingdom	2	3	0	3	15	12[g]	2	35	G7	J1	t022	22	22	IV	47
HGSA128	2000	Portugal	2	3	0	3	16	12[g]	2	36	G7	J2	t032	79	22	IV	7, 12
HU303	2003	Portugal	2	3	0	3	16	12[g]	2	36	G7	J3	t032	22	22	IV	17
IPOP2	2001	Portugal	2	3	99	3	16	12[g]	2	37	G7	J1	t032	22	22	IV	7
DEN4415	2001	Denmark	2	1	2	2	9	3	2	38	G8	K1	t021	36	30	II	26
HAR24	1993	United Kingdom	2	1	2	2	11	2	2	39	G8	K1	t018	36	30	II	47
HGSA202	2003	Portugal	2	1	2	2	11	3	2	40	G8	K3	t018	30	30	MSSA	13
DEN4358	2001	Denmark	0	0	0	3	10	1[g]	2	41	G9	H8	t116	45	45	V	26

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PLN49	1997	Poland	0	0	0	3	10	1[g]	2	41	G9	H9	t015	45	45	IV	7, 42
CA04	1998	United States	0	0	1	3	8	1[g]	2	42	G9	H6	t124	45	45	IV	20
CV81	1997	Cape Verde	0	0	1	3	11	1[g]	2	43	G9	H3	t861	508	45	MSSA	11
HAR38	1996	Germany	0	0	1	3	9	1[g]	2	44	G9	H1	t004	45	45	IV	47
HU281	2002	Hungary	0	0	1	3	9	1[g]	2	44	G9	H5	t038	45	45	IV	17
HSA19	1992	Portugal	0	0	1	3	7	1[g]	2	45	G9	H4	t1072	45	45	MSSA	5
IPO516	2006	Portugal	0	0	1	3	10	1[g]	2	46	G9	H7	t2429	45	45	V	2
IPOP56	2001	Portugal	0	0	1	3	10	1[g]	2	46	G9	H2	t1538	45	45	MSSA	5
HU109	1996	Hungary	1	0	1	3	8	1	2	47	G9	E5	t984	239	8	III	50
GRE14	1998	Greece	4	2	6	3	7	1[g]	1	48	G10	L1	t044	80	80	IV	4, 7
HFF189	2005	Portugal	4	2	6	3	7	1[g]	1	48	G10	L2	t044	80	80	IV	This study
CHL5	1997	Chile	2	0	1	3	3	2	1	49	G11	I1	t535	83	5	I	7, 9
JP82	1997	Japan	2	0	1	3	8	2[g]	1	50	G11	G2	t375	92	509	IVA	7, 8
COB111	1998	Colombia	2	1	2	3	7	3	1	51	G12	G1	t1572	84	509	IV	7, 31
HFF202	2005	Portugal	2	1	2	3	7	2	1	52	G12	X	t1537	707	S4	MSSA	This study
MW2	1998	United States	1	2	2	3	8	5[g]	3	53	S1	T3	t128	1	1	IV	14
IPOP58	2001	Portugal	1	2	3	3	6	2	3	54	S2	U1	t189	188	1	MSSA	5
HSJ109	1995	Portugal	4	2	4	2	11	9	3	55	S3	ZC	t1897	573	1	MSSA	5
HDE1	1996	Portugal	2	3	99	3	9	12	1	56	S4	B1	t311	5	5	IV	54
HBA3	2006	Portugal	2	3	1	2	3	3	1	57	S5	A1	t535	5	5	IV	2
HDE288	1996	Portugal	2	3	1	3	9	11	1	58	S6	B1	t311	5	5	VI	54
NCTC8325	1943	United Kingdom	3	0	1	4	10	6	3	59	S7	F3	t211	8	8	MSSA	http://www.nctc.org.uk
ARG199	1996	Argentina	4	99	7	3	11	9	1	60	S8	D4	t148	86	8	II	7, 9, 18
GRE4	1998	Greece	99	0	99	99	7	3	2	61	S9	R1	t037	239	8	IIIA	7
HAR36	1993	Germany	4	0	1	3	13	6	2	62	S10	F7	t009	254	8	IV	47
DEN2946	2001	Denmark	2	1	1	2	7	5	2	63	S11	K4	t975	30	30	IV	26
CV11	1997	Cape Verde	2	1	2	2	7	4	2	64	S12	K2	t942	30	30	MSSA	11
HGSA256	2003	Portugal	2	1	2	2	12	1	1	65	S13	K5	t166	34	30	MSSA	13
IPOP24	2001	Portugal	2	1	2	2	5	3	1	66	S14	K6	t1076	34	30	MSSA	5
E1114	1960	Denmark	0	1	1	3	8	2	1	67	S15	W	t518	50	50	MSSA	32
TAW214	1998	Taiwan	2	1	4	2	7	11[g]	4	68	S16	ZG	t437	59	59	IV	6, 7
IPOP50	2001	Portugal	2	2	1	3	9	1[g]	3	69	S17	T4	t359	97	97	MSSA	5
IPOP51	2001	Portugal	2	4	2	2	9	99	2	70	S18	U2	t1075	106	101	MSSA	5
CV55	1997	Cape Verde	3	1	1	3	8	2	2	71	S19	ZB	t937	398	398	MSSA	11
JP87	1997	Japan	2	1	1	3	8	9[g]	1	72	S20	V	t375	89	509	IIvar	7, 8
E260	1957	Denmark	2	1	1	0	10	2	3	73	S21	ZE	t1194	446	1021	MSSA	32
E3373	1967	Denmark	3	1	4	3	8	10	2	74	S22	F10	t164	447	S2	MSSA	32
CV87	1997	Cape Verde	3	2	2	2	99	9	4	75	S23	S2	t941	668	S3	MSSA	11
DCC1185	1997	Portugal	2	2	2	3	7	2	2	76	S24	ZA	t1065	580	S5	MSSA	5
E216	1957	Denmark	1	2	7	2	11	11	1	77	S25	ZH	t1191	445	S6	MSSA	32
E691	1959	Denmark	2	1	1	4	13	2	2	78	S26	ZD	t1207	49	ND1	MSSA	32
DCC300	1996	Portugal	2	1	1	2	12	2	1	79	S27	Y	t166	10	ND2	MSSA	5
DEN2230	2001	Denmark	3	0	2	3	10	2	2	80	S28	Z	t355	152	ND3	V	26
CV161	1997	Cape Verde	3	4	2	3	9	1[g]	3	81	S29	F9	t359	669	ND4	MSSA	11

[a] 0, the size of the amplicon obtained was shorter than that of a complete repeat; 99, no amplification was obtained.
[b] Numerical nomenclature of SIRU profiles. New numbers are attributed to profiles that differ in at least one allele.
[c] S, singletons, i.e., SIRU profiles that appear once and have no related profiles.
[d] PFGE types and subtypes were determined using thresholds of 80% and 98% similarity, respectively.
[e] CC assignments were assessed by eBURST v3 on 9 June 2008. S, singletons; ND, not determined. The sequence types are included in groups for which no founder could be determined.
[f] Strains that do not have SCC*mec* are labeled as MSSA strains.
[g] SIRU05 amplification using the SIRU05R2 reverse primer.

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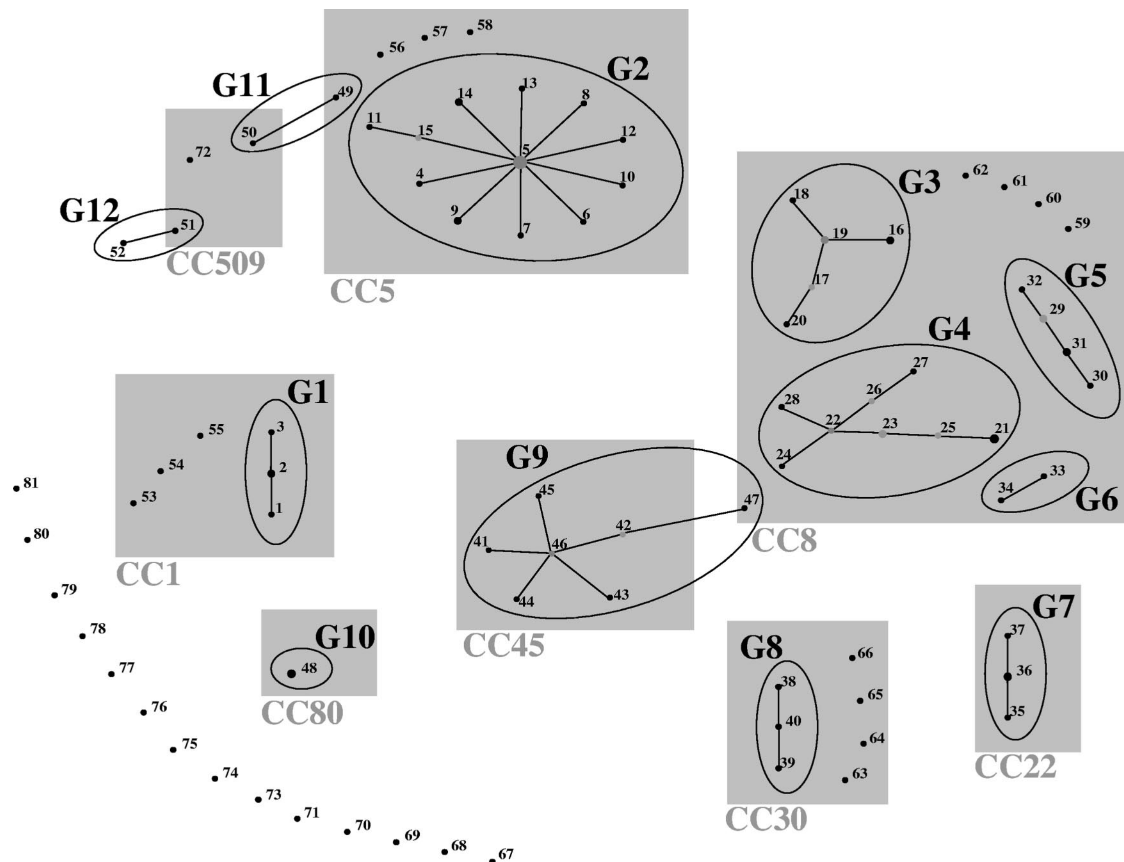

FIG. 1. Schematic representation of the SIRU groups identified by eBURST v3 analysis. The size of the dots is proportional to the number of isolates of each SIRU profile. Single-locus variants are linked by lines; CCs are highlighted in gray.

search with different *S. aureus* genomes which showed that the published SIRU05 reverse primer (SIRU05_R) (36) has no similarity with strains MW2, MSSA476, EMRSA-15 (ST22), and RF122 (ST151). Therefore, a new SIRU05R2 reverse primer was designed considering these particular strains (see Materials and Methods), and a new PCR was performed on all isolates for which this locus was not amplified with the previously published SIRU05R primer.

The sequential use of the SIRU05R2 reverse primer increased the typeability from 67% to 89.4%. Moreover, the typeability of the SIRU05 locus alone rose to 95%. Among the 11 nontypeable isolates, 9 isolates showed no amplification for a single locus, 1 isolate showed no amplification for two loci (SIRU05 and SIRU15), and another isolate showed no amplification for three loci (SIRUs 1, 15, and 16).

For further evaluation of the method, an arbitrarily chosen neutral number of repeats (99) was attributed to all nonamplified loci. However, this artifact creates a limitation because two isolates showing no amplification at the same locus are considered identical in this allele.

SIRU locus discriminatory power. Considering the discriminatory power of each locus, SIRU07 showed four different allele numbers, SIRU16 showed five, SIRU01 and SIRU13 showed six, and SIRU15 showed eight. Loci 5 and 21 showed the highest variability among the seven loci, with 14 and 13 different allele numbers, respectively. The SIRU21 locus, the only one located in a coding region, the *spa* gene, had a direct correlation with the *spa* type. However, an identical number of repeat units may contain sequence variations which are not detected by SIRU typing.

SIRU typing clonal assignment. The 104 strains were distributed into 81 SIRU profiles (differing in at least one of the seven loci) that could be clustered into 12 groups and 29 singletons (Table 1). Figure 1 shows the assignment of groups when the eBURST v3 algorithm was applied to the collection. The largest SIRU group, G2 (n = 20), included the majority (19 of 23) of the strains belonging to CC5 (ST5, ST85, and ST100) and the related strain CC228-ST228. SIRU group G9 included the nine strains belonging to CC45 (ST45 and ST508). Similarly, G7 included the four CC22 strains (ST22 and ST79), and G10 included the two CC80-ST80 strains. SIRU group G8 included only three (ST30 and ST36) of the seven CC30 strains. The remaining four strains (ST30 and ST34) were classified as singletons. CC8 strains were divided into four SIRU groups (G3, G4, G5, and G6) and four singletons. Curiously, ST247 and ST250 strains were found in G5 only, while ST239 strains were distributed over G3, G4, and G6 but not in G5. SIRU group G1 included four of the seven CC1 strains.

Unexpectedly, two isolates which were totally different by all other typing methods were grouped in the same SIRU group, G12. In the same way, one CC8 strain appeared to be a single-locus variant of a CC45 strain and was therefore included in

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TABLE 2. Number of types identified and SID for each typing method for the 104 *S. aureus* isolates

Typing method	No. of types identified	SID (%)	95% Confidence interval (%)
PFGE subtyping	97	99.85	99.69–100.00
SIRU profile typing	81	99.23	98.66–99.81
spa typing	61	97.61	96.36–98.86
PFGE typing	33	95.41	94.11–96.70
SIRU group typing	41	93.24	90.62–95.87
MLST			
ST	42	91.77	88.52–95.01
CC	25	84.37	79.68–89.06

the same G9 SIRU group, and a single CC5 strain was clustered with a CC509 strain in G11.

Three of the 12 SIRU groups included a single PFGE type, as follows: G7, PFGE J; G8, PFGE K; and G10, PFGE L. Eleven of the 13 singletons defined by PFGE were also defined as singletons by SIRU typing.

Concerning *spa* typing, all SIRU groups contained related *spa* types, except for (i) one G4 strain (TAW166, t036), (ii) one G9 strain (HU109, t984), (iii) two G11 strains (CHL5, t535; and JP82, t375), and (iv) the two G12 strains. Moreover, each *spa* type was associated with a single SIRU group, with the exception of t037, found in SIRU groups G4 and G6 belonging to the same CC, and t375, found in a G11 isolate and in singleton S20 (Table 1). Among the 11 different SCC*mec* types that characterized the MRSA collection ($n = 78$), with a few exceptions (Table 1), each SIRU profile was associated with a single SCC*mec* type.

Comparison of the discriminatory power of SIRU typing with that of other typing methods. The SID values obtained for the different typing methods are presented in Table 2. Considering the 104 isolates, the SIRU method showed a very high discriminatory power at the profile level (SID = 99.23%), similar to that observed for PFGE at the subtype level (SID = 99.85%) and higher than that observed for *spa* typing, considering both the length and sequence variation (SID = 97.61%). Considering discrimination of the SIRU method at the group level (SID = 93.24%), it was higher than that of MLST (SID = 91.77%) but lower than that of PFGE at the type level (SID = 95.41%). However, since the confidence intervals of the methods overlap (Table 2), we cannot exclude the hypothesis that they have similar discriminatory powers at a 95% confidence level.

Clustering concordance and directional agreement between SIRU typing and other typing methods. The clustering concordance between SIRU typing and the remaining methods (PFGE, *spa* typing, MLST, and SCC*mec* typing) could be traced based on the calculation of AR coefficients for the whole collection (Table 3). The AR values obtained for the SIRU typing method indicated that the highest level of congruence was at the group level for the ST ($AR_{\text{SIRU group-ST}} = 0.5736$), followed by the CC ($AR_{\text{SIRU group-CC}} = 0.4963$). At the profile level, the highest congruence was with *spa* type ($AR_{\text{SIRU profile-}spa\text{ type}} = 0.4313$). Congruence between PFGE (type and subtype levels) and SIRU typing was shown to be particularly low ($AR_{\text{SIRU profile-PFGE type}} = 0.1067$; $AR_{\text{SIRU profile-PFGE subtype}} = -0.0025$).

The W coefficient was calculated to determine the capacity of SIRU typing to predict the classification achieved by other methods (Table 3). The results obtained showed that in this collection, two strains with the same SIRU profile have a 100% probability of belonging to the same CC, a 90% probability of sharing the same *spa* type, and an 83% probability of being classified in the same ST. The capacity of SIRU typing at the profile level to predict the PFGE type was low ($W_{\text{SIRU profile-PFGE type}} = 0.4146$), and there was no correlation with the PFGE subtype ($W_{\text{SIRU profile-PFGE subtype}} = 0$). On the other hand, the SIRU group showed a high correlation with the CC ($W_{\text{SIRU group-CC}} = 0.9006$).

DISCUSSION

In the present study, we evaluated the performance of the SIRU typing method applied to a diverse collection of *S. aureus* isolates previously characterized by well-established typing methods. The SIRU method's typeability, i.e., the method's ability to assign a type to all isolates tested, was 89.4% when new primers designed for SIRU05 and SIRU16 locus amplification were used. The consistent nonamplification of SIRU05 from all strains belonging to ST1, CC22, CC45, and CC80 observed with our collection when we used the previously published SIRU05 primers is in agreement not only with previous studies focusing on SIRU typing of EMRSA-15 (CC22) isolates (29, 35–37) but also with the work of Ikawaty et al., who reported a reduced typeability for this specific locus on CC1, CC5, CC8, CC97, and CC228 isolates as well (39).

The ability of SIRU typing to assign a different type to two unrelated strains randomly sampled from the collection, i.e., its discriminatory power, was found to be very high (>99%) and

TABLE 3. AR and W values for the entire collection ($n = 104$)

Typing method	AR coefficient		W coefficient						
	SIRU group	SIRU profile	SIRU group	SIRU profile	PFGE type	PFGE subtype	*spa* type	MLST—ST	MLST—CC
SIRU group typing		0.1914		0.1113	0.2983	0.0138	0.2928	0.6713	0.9006
SIRU profile typing	0.1914		1.0000		0.4146	0.0000	0.9024	0.8293	1.0000
PFGE typing	0.3180	0.1067	0.4390	0.0691		0.0325	0.1870	0.4959	0.7724
PFGE subtyping	0.0242	−0.0025	0.6250	0.0000	1.0000		0.3750	1.0000	1.0000
spa typing	0.4119	0.4313	0.8281	0.2891	0.3594	0.0234		0.8047	0.9453
MLST									
ST	0.5736	0.1289	0.5510	0.0771	0.2766	0.0181	0.2336		1.0000
CC	0.4963	0.0800	0.3895	0.0490	0.2270	0.0096	0.1446	0.5269	

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was due mainly to the individual high levels of variability of SIRU05 and SIRU21 loci. High variability in SIRU21 is not surprising, since it is located in the known highly variable polymorphic region of the *spa* gene. The discriminatory power of SIRU typing at the profile level (SID = 99.23%) is similar to that of PFGE subtyping (SID = 99.85%) and *spa* typing (SID = 97.61%), considering the overlapping of the confidence intervals at 95%. Our results are concordant with a recent study, besides the fact that only six of the seven SIRU loci were taken into account (39). The observed high discriminatory power makes SIRU typing suitable for outbreak investigations, as also shown in studies by Hardy et al. where strains belonging to an outbreak or consecutively isolated from the same ward had the same or highly related SIRU profiles (35, 37). Moreover, in a study involving seven different outbreaks, variations in the number of SIRU repeats in strains belonging to the same outbreak were found to be rare (40).

The concordance between SIRU typing and well-established typing methods was measured by the calculation of the AR coefficient. We demonstrated that at the group level, SIRU typing showed the highest congruence with MLST (ST and CC), whereas at the profile level it showed the highest congruence with *spa* typing. The correspondence with PFGE was low, in contrast to the study of Ikawaty et al. on 50 *S. aureus* isolates, where the AR coefficient between the MLVA method and PFGE (AR = 0.599) was higher than that for *spa* typing (AR = 0.435) (39).

The major CCs defined by MLST (CC5, CC8, CC22, CC30, and CC45) were maintained when the eBURST v3 algorithm was applied to our SIRU profile data. The exception was CC8, known to include a high degree of variability in STs, which was divided into four well-distinguished groups. Moreover, a single CC8 strain was surprisingly clustered with CC45 isolates (G9). Considering that MLST is based on the variation in housekeeping genes that have a slow evolutionary clock and that the SIRU method looks into variable repeat regions that could evolve more rapidly by an introduction or deletion of a single repeat, clonal types might be affected by genomic rearrangements to different extents in the two methods. The SIRU loci in noncoding regions are less likely to be subject to natural selection that affects some VNTRs located on coding regions or promoters (43). Interestingly, among the seven SIRU loci, different evolutionary clocks could be observed, since SIRU01, SIRU07, SIRU13, SIRU15, and SIRU16 appeared to be generally monomorphic between strains from the same group and therefore more conserved during evolution. SIRU05 and SIRU21 add high levels of variability to the method and could be especially informative for recent levels of evolutionary divergence. Therefore, the SIRU typing method includes different scales of evolutionary divergence within the same system, making it suitable for studies with different purposes.

The predictive power between SIRU typing and other methods, translated by the W index, was maximum for CC and very high for *spa* typing and MLST in our collection. In opposition, the prediction of the PFGE results was low, as SIRU typing was able to distinguish among isolates with the same PFGE subtype and vice versa. Noller et al. (49) showed that during an investigation of an *Escherichia coli* O157:H7 outbreak, MLVA appeared to have a sensitivity equal to that of PFGE and a specificity that was even superior to that of PFGE. On another hand, SIRU typing showed the highest congruence with MLST results, i.e., ST and CC results. It is noteworthy that the predictive power of a method seems to be linked to the variability in the collection studied, as in a recent study involving two collections of *S. aureus* isolates, where for the first collection the MLVA method predicted the *spa* typing results but the reverse was not observed, while for the second collection both methods proved to be mutually predictive (39).

Our results show that SIRU typing analysis adds to the knowledge of the variability of the *S. aureus* genome and contributes to the understanding of genetic relationships among MRSA clones, as seen by Malachowa et al. (44).

In terms of convenience, the SIRU method was shown to be easy to perform, since it is based on single-locus PCRs, which could minimize the drawback of band size determination and even reduce the total time required for practical procedures if allied to the use of automated systems. Automated MLVA approaches were already proposed for *S. aureus* and *Staphylococcus epidermidis* genotyping through different PCR schemes (27, 28). In terms of cost, SIRU typing (11.2€/strain) is comparable to PFGE (13.2€/strain), but it is faster and technically easier to perform and much cheaper than *spa* typing (15.5€/strain) or MLST (80€/strain). Additionally, SIRU typing could be combined with *spa* typing, since the *spa* type may be determined by sequencing of the SIRU21 amplicons of nontypeable strains with conventional primers (1, 56).

In summary, as a PCR-based method, the SIRU typing method is relatively fast, accessible, and not expensive, which combined with its high discriminatory power makes it useful and reliable for short-term epidemiological investigations of *S. aureus*. In addition, its congruence with MLST results (at the CC and ST levels) makes it potentially valuable for evolutionary studies. In order for SIRU typing to be considered a useful tool in terms of epidemiological surveillance networks and evolutionary purposes, a public database similar to the databases available for other bacterial MLVA schemes (34) may be created for SIRU typing, allowing harmonization of *S. aureus* MLVA schemes and the interlaboratory exchange of data.

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