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Assignment of *Staphylococcus* Isolates to Groups by *spa* Typing, SmaI Macrorestriction Analysis, and Multilocus Sequence Typing

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The implementation of the new clustering algorithm *B*ased *U*pon *R*epeat *P*attern (BURP) into the Ridom StaphType software tool enables clustering based on *spa* typing data for *Staphylococcus aureus*. We compared clustering results obtained by *spa* typing/BURP to those obtained by currently well-established methods, i.e., SmaI macrorestriction analysis and multilocus sequence typing/eBURST. A total of 99 clinical *S. aureus* strains, including MRSA and representing major clonal lineages associated with important kinds of infections which have been prevalent in Germany and Central Europe during the last 10 years, were used for comparison. SmaI macrorestriction analysis revealed the highest discriminatory power, and clustering results for all three methods resulted in concordance values ranging from 96.8% between the two sequence-based methods to 93.4% between *spa* typing/BURP and SmaI macrorestriction/cluster analysis. The results of this study indicate that *spa* typing, together with BURP clustering, is a useful tool in *S. aureus* epidemiology, especially because of ease of use and the advantages of unambiguous sequence analysis as well as reproducibility and exchange of typing data.

Staphylococcus aureus is one of the most frequent nosocomial pathogens. The emergence and spread of epidemic strains of methicillin-resistant *S. aureus* in hospitals (hMRSA) and, independent from the nosocomial setting, in the community (cMRSA) require special attention of infection control. Typing is an important prerequisite for targeted control measures. For about 30 years, phage typing has been widely used for strain typing. More recently, SmaI macrorestriction analysis (pulsed-field gel electrophoresis [PFGE]) was introduced as a typing method with high discriminatory power. PFGE is still regarded the "gold standard" of molecular typing of MRSA, despite insufficient comparability of results obtained from different laboratories (21). During the past 5 years, DNA sequence-based typing has become more popular due to progress in large-scale sequencing methodology, ease of data transfer, and excellent comparability of results (2). This first became evident by the application of multilocus sequence typing (MLST) to MRSA (4, 5). At present, however, MLST is not suitable for routine infection control due to high cost, labor intensity, and lack of broad access to high-throughput DNA sequencing.

Several *S. aureus* typing schemes targeting polymorphic DNA repeat regions in genes for microbial surface components recognizing adhesive matrix molecules have been described previously (7, 9, 16, 27, 30). They also include typing methods based on the length polymorphism in *spa* amplimers (9) or, more recently, on polymorphisms in multiple fragments amplified in a multiplex PCR approach for variable-number tandem repeats (7, 27). Among sequence-based approches, *spa* typing was the most promising (8, 12, 13, 15, 31). The X region of the protein A gene (*spa*) consists of direct repeats exhibiting an extensive polymorphism based on point mutations, deletions, duplications, and insertions. Different repeats can be assigned an alpha-numerical code, and the order of specific repeats defines the *spa* type. Two systems of nomenclature are in use for *spa* type determination (13, 15). Ridom StaphType (13) provides a software tool enabling straightforward sequence analysis and designation of *spa* types via synchronization to a central server.

Previous studies have shown that there is a fairly good correlation between clonal groupings of MRSA isolates obtained by *spa* typing and other typing techniques (15, 22, 29, 36). The broader application of *spa* typing revealed a considerable degree of *spa* gene repeat polymorphism within particular clonal groups and clonal lineages of MRSA isolates, as defined by MLST and eBURST, indicating a higher discriminatory power for this method. However, in daily infection control, an unambiguous and quick attribution of newly arising *spa* types to known clonal complexes and clonal lineages is essential because of their differential dynamics of emergence and spread (33). This is exemplified by the occurrence of cMRSA isolates, most often containing the *lukS-lukF* determinant coding for Panton-Valentine leukocidin. They may emerge as (i) clonal lineages not previously reported (40), (ii) derivatives of clonal lineages which have already been known as nosocomial pathogens in the "pre-MRSA era" (23, 26), and (iii) clones belonging to the same clonal lineages as nosocomial MRSA strains and containing both the *mecA* gene and the *lukS-lukF* determinant (17).

In looking at sequence databases for *spa* types, specific repeats and repeat successions seem to be associated with particular MLST sequence types (http://www.spaserver.ridom.de). The recent implementation of the BURP (*B*ased *U*pon *R*epeat *P*attern) algorithm into the Ridom StaphType software (13, 28) makes allowance for this and provides a tool for classifying related *spa* sequence types into different BURP groups.

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TABLE 1. Strains used in this study, ordered numerically

Strain	Yr (country) of isolation[a]	Clinical origin(s)[b]	Virulence-associated determinant(s)[c]	*mecA* (MRSA)[d]	*spa* type
8325				−	t211
00-01004	2000	SSS (h)	*eta*, *etb*	−	t159
00-01437	2000 (Bulgaria)	SSS (h)	*eta*	−	t159
00-01488	2000	Pyodermia (c)	*eta*, *etb*	−	t269
00-01876	2000	SSS (h)	*eta*, *etb*	−	t284
02-01567	2002	Septicemia (h)		+	t045
02-02404	2002	Furuncle (c)	*lukS-lukF*	+	t044
02-02424	2002	Colonization		+	t002
02-02512	2002	VAP (h)		+	t001
02-02712	2002	VAP		+	t026
02-02750	2002	Furuncle (c)	*lukS-lukF*	−	t284
02-02756	2002	Wound infection (h)		+	t038
02-02811	2002	Wound infection (h)		+	t038
02-02878	2002	Furuncle (c)	*lukS-lukF*	−	t159
02-03179	2002	Septicemia (h)		+	t001
02-03534	2002	Wound infection (h)		+	t001
02-03925	2002	Furuncle (c)	*lukS-lukF*	+	t044
03-00220	2003	Urinary tract infection (h)		+	t005
03-00397	2003	Nasal colonization (h)		+	t032
03-01228	2003	Nasal colonization (h)		+	t022
03-01265	2003	Nasal swab	*tst*	+	t021
03-01478	2003	Surgical wound infection (h)		+	t032
03-01486	2003	TSS	*tst*	−	t271
03-01621	2003	TSS	*tst*	−	t021
03-01679	2003	TSS	*tst*	−	t012
03-01816	2003	Furuncle (c)	*lukS-lukF*	+	t019
03-01900	2003	Wound infection (h)		+	t018
03-02106	2003 (Israel)	(h)		+	t002
03-02121	2003 (Israel)	(h)		+	t002
03-02280	2003	Wound infection (h)		+	t008
03-02286	2003	Wound infection (h)		+	t008
03-02309	2003	Pyodermia (h)		+	t008
03-02444	2003	VAP (h)		+	t032
03-02494	2003	Cystic fibrosis	*tst*	+	t268
03-02575	2003	Surgical wound infection (veth)		+	t036
03-02773	2003	Furuncle (c)	*lukS-lukF*	+	t175
04-00608	2004 (United Kingdom)	Skin infection (c)	*lukS-lukF*	+	t310
04-01872	2004	(c)	*lukS-lukF*	+	t019
04-02080	2004	Wound infection (h)		+	t037
04-02936	2004	Abscess (c)	*lukS-lukF*	+	t002
04-02981	2004	VAP (h)		+	t003
05-00043	2005	VAP (h)		+	t003
05-01042	2005	Nasal colonization (h)		+	t030
05-01089	2005	Furunculosis (c)	*lukS-lukF*	+	t310
05-01197-1	2005	Abscess (c)	*lukS-lukF*	+	t008
05-01825	2005	Abscess, skin (c)	*lukS-lukF*	+	t008
05-01851	2005	Vaginal swab		+	t004
05-01977	2005	Wound infection (h)		+	t001
05-02010	2005	Perineal swab (h)		+	t003
05-02040	2005	Furunculosis (c)	*lukS-lukF*	+	t008
05-02065	2005	Furuncle (c)	*lukS-lukF*	−	t1151
05-02086-2	2005	Furuncle (c)	*lukS-lukF*	−	t017
05-02091	2005	Abscess (c)	*lukS-lukF*	−	t318
05-02127	2005	Wound infection (h)		+	t001
05-02139	2005	Wound infection		+	t004
05-02212	2005	Urinary tract infection (h)		+	t003
05-02318	2005	Furuncle (c)	*lukS-lukF*	−	t435
81-01408	1981	Mastitis puerperalis (h)	*lukS-lukF*	−	t021
87-01054	1987	Mastitis puerperalis (h)	*lukS-lukF*	−	t021
93-00134	1993	Wound infection (h)		+	t051
93-00635	1993	Shunt infection, dialysis (h)		+	t037
93-00994	1993	VAP (h)		+	t139
93-01000	1993	Septicemia (h)		+	t009
93-01150	1993	Wound infection (h)		+	t004
94-01450	1994	Pneumonia (h)		+	t051
95-00543	1995	Tropical pyomyositits (c)	*tst*	+	t021

Continued on following page

TABLE 1—*Continued*

Strain	Yr (country) of isolation[a]	Clinical origin(s)[b]	Virulence-associated determinant(s)[c]	*mecA* (MRSA)[d]	*spa* type
95-544	1995 (Uganda)	Tropical pyomyositis (c)	*lukS-lukF*	−	t159
96-00842	1996	Septicemia (h)		+	t004
96-01678	1998	Bacteremia (h)		+	t032
96-32010	1996 (United Kingdom)	HARMONY (h)	*tst*	+	t018
97-00825	1997	Furuncle (c)	*lukS-lukF*	+	t044
97-01451	1997 (Uganda)	Tropical pyomyositis (c)	*lukS-lukF*	−	t308
97-01966	1997	Surgical wound infection (h)		+	t009
97-02594-1	1997	VAP (h)		+	t001
97S101	1997 (Belgium)	HARMONY (h)		+	t045
98-00131	1998	VAP (h)		+	t001
98-00406	1998	Septicemia (h)		+	t051
98-01155-2	1998	Wound infection (h)		+	t001
98-01442	1998	Abscess (h)		+	t009
98-01618	1998	SSS (c)	*eta*	−	t159
98-01907	1998	VAP (h)	*tst*	+	t018
98-01976	1998	Osteomyelitis (h)	*tst*	+	t018
98-02088	1998	Nasal colonization		+	t004
99-00653	1998	Wound infection (h)		+	t001
99-159	1999 (United Kingdom)	HARMONY (h)		+	t018
C2SAU0010	2002	Wound infection		−	t216
C2SAU0032	2002	Nasal colonization	*tst*	−	t276
C2SAU0077	2002	Nasal colonization		−	t275
M1SAU0039	2005	Nasal colonization	*tst*	−	t138
M3SAU0012	2002	Nasal colonization	*tst*	−	t012
MSAU192	2002	Nasal colonization		−	t274
NCTC11939	1985 (United Kingdom)	Nosocomial environment		+	t037
Pl25	1999 (Poland)	HARMONY (h)		+	t001
PS80	1955	Mastitis puerperalis (h)	*lukS-lukF*	−	t021
PS95	1972 (United States)	Reference strain for phage 95		−	t065
sau274	2004	VAP (h)		−	t586
sau285	2004	Nasal colonization (h)		−	t242
Slovenia 14	1999 (Slovenia)	HARMONY (h)		+	t001
Slovenia 30	1999 (Slovenia)	HARMONY (h)		+	t178

[a] Isolated in Germany, unless otherwise noted.
[b] VAP, ventilator-associated pneumonia; TSS, toxic shock syndrome; SSS staphylococcal scaled skin syndrome; (h), hospital acquired; (c), community acquired; (veth), veterinary hospital acquired.
[c] As determined by PCR.
[d] +, presence; −, absence.

Here, we report about the application of *spa* typing and subsequent BURP clustering to a collection of *S. aureus* isolates, including all major clonal lineages of hMRSA and cMRSA isolates, as well as methicillin-susceptible *S. aureus* (MSSA) isolates of the same clonal lineages representing probable ancestors. Furthermore, MSSA isolates with particular virulence genes associated with important kinds of disease, such as *tst* (toxic shock syndrome), *eta* and *etb* (exfoliative dermatitis), and *lukS-lukF* (furunculosis and necrotizing pneumonia), were included. All isolates were collected at different times and from different geographical areas, mainly in Central Europe. The resulting groups were compared to those obtained with SmaI macrorestriction and MLST/eBURST analyses.

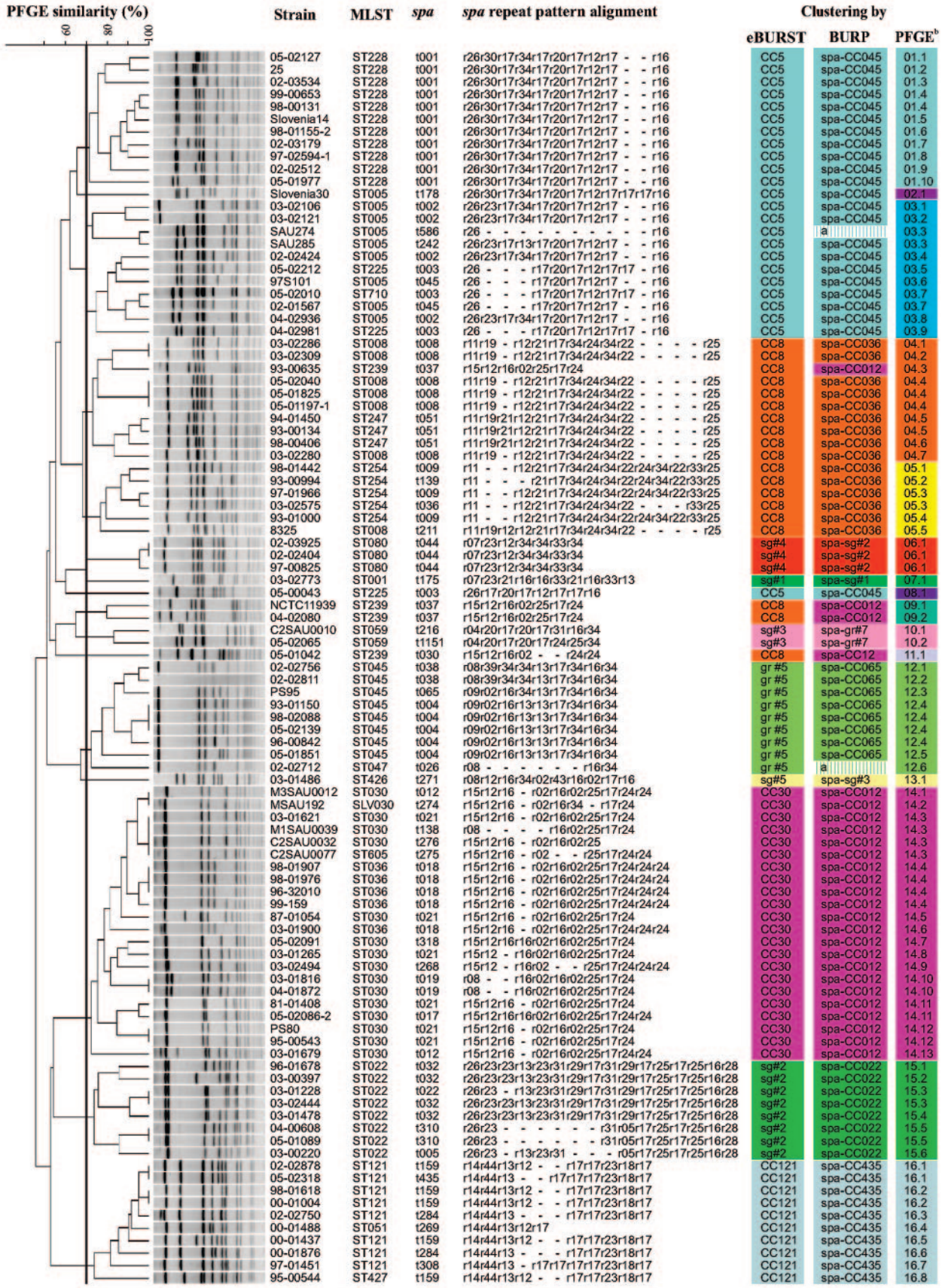
MATERIALS AND METHODS

Bacterial strains. A total of 99 *S. aureus* isolates, including methicillin-sensitive as well as -resistant ones, were used in this study. Strains were selected from the strain collection of the German reference center for staphylococci situated at our laboratory and represent the majority of clonal lineages prevalent in Germany and Central Europe during the last 10 years, including recently emerging cMRSA isolates. The reference strains previously used in the "HARMONY" study on harmonization of PFGE protocols for MRSA strain typing (21) were included. Isolates were collected at different time points over a period of approximately 10 years. More-detailed information about strain characteristics, also including demonstrated virulence determinants for each isolate, can be found in Table 1.

SmaI macrorestriction and cluster analyses. SmaI macrorestriction analysis was conducted according to the HARMONY protocol (21). Resulting gel images were analyzed using the guidelines proposed by Tenover et al. (37). Accordingly, strains were supposed to be identical or very closely related if they differed by at most three bands. Additionally, cluster analysis was performed with the BioNumerics software package (Applied Maths, Sint-Martens-Latem, Belgium), using the Dice coefficient, and visualized as a dendrogram by the unweighted-pair group method, using average linkages with 1% tolerance and 1% optimization settings. A similarity cutoff of 70% was used to define a cluster.

DNA extraction. Genomic DNA for subsequent PCRs was isolated from a 2-ml overnight culture with the DNeasy tissue kit (QIAGEN, Hilden, Germany), using lysostaphin (100 mg/liter; Sigma, Taufkirchen, Germany) to achieve bacterial lysis.

***spa* typing and BURP.** The polymorphic X region of the protein A gene (*spa*) was amplified using the primers spa-1113f (5′ TAA AGA CGA TCC TTC GGT GAG C 3′) and spa-1514r (5′ CAG CAG TAG TGC CGT TTG CTT 3′). All sequencing reactions were carried out using the ABI PRISM BigDye Terminator cycle sequencing ready reaction kit (Applied Biosystems, Foster City, Calif.). *spa* types as well as BURP *spa* clonal complexes (*spa*-CCs) were assigned using the Ridom StaphType software version 1.3 (Ridom GmbH, Würzburg, Germany) as described by Harmsen et al. (13). Applying the newly implemented algorithm BURP, *spa* types were clustered into different groups, with the calculated cost between members of a group less than or equal to 8. *spa* types shorter than five repeats were excluded from analysis because no reliable deduction about ancestries can be made from these types. The new algorithm takes repeat duplication/



Strain	MLST	*spa*	*spa* repeat pattern alignment	Clustering by eBURST	BURP	PFGE[b]
05-02127	ST228	t001	r26r30r17r34r17r20r17r12r17 - - r16	CC5	spa-CC045	01.1
25	ST228	t001	r26r30r17r34r17r20r17r12r17 - - r16	CC5	spa-CC045	01.2
02-03534	ST228	t001	r26r30r17r34r17r20r17r12r17 - - r16	CC5	spa-CC045	01.3
99-00653	ST228	t001	r26r30r17r34r17r20r17r12r17 - - r16	CC5	spa-CC045	01.4
98-00131	ST228	t001	r26r30r17r34r17r20r17r12r17 - - r16	CC5	spa-CC045	01.4
Slovenia14	ST228	t001	r26r30r17r34r17r20r17r12r17 - - r16	CC5	spa-CC045	01.5
98-01155-2	ST228	t001	r26r30r17r34r17r20r17r12r17 - - r16	CC5	spa-CC045	01.6
02-03179	ST228	t001	r26r30r17r34r17r20r17r12r17 - - r16	CC5	spa-CC045	01.7
97-02594-1	ST228	t001	r26r30r17r34r17r20r17r12r17 - - r16	CC5	spa-CC045	01.8
02-02512	ST228	t001	r26r30r17r34r17r20r17r12r17 - - r16	CC5	spa-CC045	01.9
05-01977	ST228	t001	r26r30r17r34r17r20r17r12r17 - - r16	CC5	spa-CC045	01.10
Slovenia30	ST005	t178	r26r30r17r34r17r20r17r12r17r17r17r16	CC5	spa-CC045	02.1
03-02106	ST005	t002	r26r23r17r34r17r20r17r12r17 - - r16	CC5	spa-CC045	03.1
03-02121	ST005	t002	r26r23r17r34r17r20r17r12r17 - - r16	CC5	spa-CC045	03.2
SAU274	ST005	t586	r26 - - - - - - - - - - r16	CC5	a	03.3
SAU285	ST005	t242	r26r23r17r13r17r20r17r12r17 - - r16	CC5	spa-CC045	03.3
02-02424	ST005	t002	r26r23r17r34r17r20r17r12r17 - - r16	CC5	spa-CC045	03.4
05-02212	ST225	t003	r26 - - - r17r20r17r12r17r17 - r16	CC5	spa-CC045	03.5
97S101	ST005	t045	r26 - - - r17r20r17r12r17 - - r16	CC5	spa-CC045	03.6
05-02010	ST710	t003	r26 - - - r17r20r17r12r17r17 - r16	CC5	spa-CC045	03.7
02-01567	ST005	t045	r26 - - - r17r20r17r12r17 - - r16	CC5	spa-CC045	03.7
04-02936	ST005	t002	r26r23r17r34r17r20r17r12r17 - - r16	CC5	spa-CC045	03.8
04-02981	ST225	t003	r26 - - - r17r20r17r12r17r17 - r16	CC5	spa-CC045	03.9
03-02286	ST008	t008	r11r19 - r12r21r17r34r24r34r22 - - - - - r25	CC8	spa-CC036	04.1
03-02309	ST008	t008	r11r19 - r12r21r17r34r24r34r22 - - - - - r25	CC8	spa-CC036	04.2
93-00635	ST239	t037	r15r12r16r02r25r17r24	CC8	spa-CC012	04.3
05-02040	ST008	t008	r11r19 - r12r21r17r34r24r34r22 - - - - - r25	CC8	spa-CC036	04.4
05-01825	ST008	t008	r11r19 - r12r21r17r34r24r34r22 - - - - - r25	CC8	spa-CC036	04.4
05-01197-1	ST008	t008	r11r19 - r12r21r17r34r24r34r22 - - - - - r25	CC8	spa-CC036	04.4
94-01450	ST247	t051	r11r19r21r12r21r17r34r24r34r22 - - - - - r25	CC8	spa-CC036	04.5
93-00134	ST247	t051	r11r19r21r12r21r17r34r24r34r22 - - - - - r25	CC8	spa-CC036	04.5
98-00406	ST247	t051	r11r19r21r12r21r17r34r24r34r22 - - - - - r25	CC8	spa-CC036	04.6
03-02280	ST008	t008	r11r19 - r12r21r17r34r24r34r22 - - - - - r25	CC8	spa-CC036	04.7
98-01442	ST254	t009	r11 - - r12r21r17r34r24r34r22r24r34r22r33r25	CC8	spa-CC036	05.1
93-00994	ST254	t139	r11 - - - r21r17r34r24r34r22r24r34r22r33r25	CC8	spa-CC036	05.2
97-01966	ST254	t009	r11 - - r12r21r17r34r24r34r22r24r34r22r33r25	CC8	spa-CC036	05.3
03-02575	ST254	t036	r11 - - r12r21r17r34r24r34r22 - - - r33r25	CC8	spa-CC036	05.3
93-01000	ST254	t009	r11 - - r12r21r17r34r24r34r22r24r34r22r33r25	CC8	spa-CC036	05.4
8325	ST008	t211	r11r19r12r12r21r17r34r24r34r22 - - - - - r25	CC8	spa-CC036	05.5
02-03925	ST080	t044	r07r23r12r34r34r33r34	sg#4	spa-sg#2	06.1
02-02404	ST080	t044	r07r23r12r34r34r33r34	sg#4	spa-sg#2	06.1
97-00825	ST080	t044	r07r23r12r34r34r33r34	sg#4	spa-sg#2	06.1
03-02773	ST001	t175	r07r23r21r16r16r33r21r16r33r13	sg#1	spa-sg#1	07.1
05-00043	ST225	t003	r26r17r20r17r12r17r17r16	CC5	spa-CC045	08.1
NCTC11939	ST239	t037	r15r12r16r02r25r17r24	CC8	spa-CC012	09.1
04-02080	ST239	t037	r15r12r16r02r25r17r24	CC8	spa-CC012	09.2
C2SAU0010	ST059	t216	r04r20r17r20r17r31r16r34	sg#3	spa-gr#7	10.1
05-02065	ST059	t1151	r04r20r17r20r17r24r25r34	sg#3	spa-gr#7	10.2
05-01042	ST239	t030	r15r12r16r02 - - r24r24	CC8	spa-CC12	11.1
02-02756	ST045	t038	r08r39r34r34r13r17r34r16r34	gr #5	spa-CC065	12.1
02-02811	ST045	t038	r08r39r34r34r13r17r34r16r34	gr #5	spa-CC065	12.2
PS95	ST045	t065	r09r02r16r34r13r17r34r16r34	gr #5	spa-CC065	12.3
93-01150	ST045	t004	r09r02r16r13r13r17r34r16r34	gr #5	spa-CC065	12.4
98-02088	ST045	t004	r09r02r16r13r13r17r34r16r34	gr #5	spa-CC065	12.4
05-02139	ST045	t004	r09r02r16r13r13r17r34r16r34	gr #5	spa-CC065	12.4
96-00842	ST045	t004	r09r02r16r13r13r17r34r16r34	gr #5	spa-CC065	12.4
05-01851	ST045	t004	r09r02r16r13r13r17r34r16r34	gr #5	spa-CC065	12.5
02-02712	ST047	t026	r08 - - - - - - r16r34	gr #5	a	12.6
03-01486	ST426	t271	r08r12r16r34r02r43r16r02r17r16	sg#5	spa-sg#3	13.1
M3SAU0012	ST030	t012	r15r12r16 - r02r16r02r25r17r24r24	CC30	spa-CC012	14.1
MSAU192	SLV030	t274	r15r12r16 - r02r16r34 - r17r24	CC30	spa-CC012	14.2
03-01621	ST030	t021	r15r12r16 - r02r16r02r25r17r24	CC30	spa-CC012	14.3
M1SAU0039	ST030	t138	r08 - - - - r16r02r25r17r24	CC30	spa-CC012	14.3
C2SAU0032	ST030	t276	r15r12r16 - r02r16r02r25	CC30	spa-CC012	14.3
C2SAU0077	ST605	t275	r15r12r16 - r02 - - r25r17r24r24	CC30	spa-CC012	14.3
98-01907	ST036	t018	r15r12r16 - r02r16r02r25r17r24r24r24	CC30	spa-CC012	14.4
98-01976	ST036	t018	r15r12r16 - r02r16r02r25r17r24r24r24	CC30	spa-CC012	14.4
96-32010	ST036	t018	r15r12r16 - r02r16r02r25r17r24r24r24	CC30	spa-CC012	14.4
99-159	ST036	t018	r15r12r16 - r02r16r02r25r17r24r24r24	CC30	spa-CC012	14.4
87-01054	ST030	t021	r15r12r16 - r02r16r02r25r17r24	CC30	spa-CC012	14.5
03-01900	ST036	t018	r15r12r16 - r02r16r02r25r17r24r24r24	CC30	spa-CC012	14.6
05-02091	ST030	t318	r15r12r16r16r02r16r02r25r17r24	CC30	spa-CC012	14.7
03-01265	ST030	t021	r15r12 - r16r02r16r02r25r17r24	CC30	spa-CC012	14.8
03-02494	ST030	t268	r15r12 - r16r02 - - r25r17r24r24r24	CC30	spa-CC012	14.9
03-01816	ST030	t019	r08 - - r16r02r16r02r25r17r24	CC30	spa-CC012	14.10
04-01872	ST030	t019	r08 - - r16r02r16r02r25r17r24	CC30	spa-CC012	14.10
81-01408	ST030	t021	r15r12r16 - r02r16r02r25r17r24	CC30	spa-CC012	14.11
05-02086-2	ST030	t017	r15r12r16r16r02r16r02r25r17r24r24	CC30	spa-CC012	14.11
PS80	ST030	t021	r15r12r16 - r02r16r02r25r17r24	CC30	spa-CC012	14.12
95-00543	ST030	t021	r15r12r16 - r02r16r02r25r17r24	CC30	spa-CC012	14.12
03-01679	ST030	t012	r15r12r16 - r02r16r02r25r17r24r24	CC30	spa-CC012	14.13
96-01678	ST022	t032	r26r23r23r13r23r31r29r17r31r29r17r25r17r25r16r28	sg#2	spa-CC022	15.1
03-00397	ST022	t032	r26r23r23r13r23r31r29r17r31r29r17r25r17r25r16r28	sg#2	spa-CC022	15.2
03-01228	ST022	t022	r26r23 - r13r23r31r29r17r31r29r17r25r17r25r16r28	sg#2	spa-CC022	15.3
03-02444	ST022	t032	r26r23r23r13r23r31r29r17r31r29r17r25r17r25r16r28	sg#2	spa-CC022	15.3
03-01478	ST022	t032	r26r23r23r13r23r31r29r17r31r29r17r25r17r25r16r28	sg#2	spa-CC022	15.4
04-00608	ST022	t310	r26r23 - - - - - - - r31r05r17r25r17r25r16r28	sg#2	spa-CC022	15.5
05-01089	ST022	t310	r26r23 - - - - - - - r31r05r17r25r17r25r16r28	sg#2	spa-CC022	15.5
03-00220	ST022	t005	r26r23 - r13r23r31 - - - r05r17r25r17r25r16r28	sg#2	spa-CC022	15.6
02-02878	ST121	t159	r14r44r13r12 - - r17r17r23r18r17	CC121	spa-CC435	16.1
05-02318	ST121	t435	r14r44r13 - - r17r17r17r23r18r17	CC121	spa-CC435	16.1
98-01618	ST121	t159	r14r44r13r12 - - r17r17r23r18r17	CC121	spa-CC435	16.2
00-01004	ST121	t159	r14r44r13r12 - - r17r17r23r18r17	CC121	spa-CC435	16.2
02-02750	ST121	t284	r14r44r13 - - - r17r17r23r18r17	CC121	spa-CC435	16.3
00-01488	ST051	t269	r14r44r13r12r17	CC121	spa-CC435	16.4
00-01437	ST121	t159	r14r44r13r12 - - r17r17r23r18r17	CC121	spa-CC435	16.5
00-01876	ST121	t284	r14r44r13 - - - r17r17r23r18r17	CC121	spa-CC435	16.6
97-01451	ST121	t308	r14r44r13 - r17r17r17r17r23r18r17	CC121	spa-CC435	16.7
95-00544	ST427	t159	r14r44r13r12 - - r17r17r23r18r17	CC121	spa-CC435	16.8

deletion in addition to point mutation events into account when calculating the relatedness of different *spa* types. Due to speed constraints, a heuristic version (secondary duplication events within primary duplications are not detected) of the EDSI alignment (excisions, duplications, substitutions, and insertions), as described by Sammeth et al. (28), was used.

MLST and eBURST. MLST was conducted as previously described (4). Allele types and resulting sequence types were assigned at the *S. aureus* MLST database via the Internet (http://www.mlst.net). Sequence types were clustered into groups using eBURST, employing the relaxed group definition with five of seven loci (i.e., members of a group differ at a single locus or two loci [6]).

Discriminatory power. An index of discrimination (DI) for each typing method was calculated, defined as the average probability that the typing system will assign a different type to two unrelated strains randomly sampled in the microbial population of a given taxon (14). The DI depends on the number of strain types and on the homogeneity of frequency distribution of strains into types. Confidence intervals (CIs) for discriminatory indices were calculated as previously described (11).

Calculation of typing system concordance. The agreement between two strain typing tests was calculated as described by Robinson et al. (25). Ideally, the DI and the typing system concordance should be calculated using a test population that includes epidemiologically unrelated strains. This is most likely not true in our study, and therefore, the absolute figures should be treated with caution. Nevertheless, the relative ordering of the typing schemes according to the DIs and the typing system concordance is meaningful. Calculation of both methods is implemented in the Ridom StaphType software version 1.3.

RESULTS

SmaI macrorestriction and cluster analyses. All 99 isolates were typeable by SmaI macrorestriction and produced 74 different macrorestriction patterns according to the criteria defined by Tenover et al. (37). Employing a cutoff similarity value of 70% in subsequent cluster analysis, we assigned the isolates to 16 different groups, with 5 groups containing only a single isolate (Fig. 1).

***spa* typing and BURP.** All isolates were assigned to 44 different *spa* types, varying in length between 2 (t586) and 16 (t032) repeats (Fig. 1 and Table 1). Using the algorithm BURP, newly implemented in the Ridom StaphType software, *spa* types were clustered into 10 different groups, with 7 groups comprising more than one *spa* type and three so-called "singletons." Thereby, *spa* types were grouped together if the calculated cost between members of a group was less than or equal to 8. Since clustering parameters excluded *spa* types shorter than five repeats, two types (t026 and t586) were excluded from BURP grouping. Nevertheless, t026 and t586 could be classified into *spa* CC045 and *spa* CC065, respectively, after visual inspection of the corresponding repeat patterns. Using these parameters, the majority of isolates were grouped together as expected regarding their evolutionary origin, as reflected by MLST analysis, although in most cases a variety of *spa* types corresponded to a single MLST. (ST5, six different *spa* types; ST254, three different *spa* types; ST45, three different *spa* types; ST30, eight different *spa* types; ST22, four different *spa* types; ST121, four different *spa* types).

TABLE 2. Discriminatory power of different typing methods

Method	No. of strains included	No. of different types/groups	DI	95% CI
spa typing	99	44	0.969	0.957–0.981
spa typing/BURP	97[a]	10	0.831	0.797–0.865
MLST	99	22	0.931	0.914–0.947
MLST/eBURST	99	10	0.837	0.808–0.866
PFGE[b]	99	74	0.993	0.988–0.997
PFGE/cluster analysis	99	16	0.894	0.868–0.919

[a] Two *spa* types excluded due to insufficient length.
[b] As defined by Tenover et al. (37).

MLST and eBURST. Twenty-two different sequence types were identified. Using the relaxed group definition in eBURST, 10 different groups were defined, with five groups including more than one sequence type and five singletons. Groups corresponded to the most abundant clonal complexes present in Middle Europe during the last 10 years, i.e., CC-5, CC-8, CC-22, CC-30, CC-45, and CC-121, and included ST80 and ST1 representing the predominant cMRSA isolates in Central Europe and North America, respectively (Fig. 1).

Discriminatory power and concordance between methods. The ability of each method to discriminate different strain types was assessed by calculation of the DI; DIs and corresponding CIs are summarized in Table 2.

Using MLST/eBURST data as a reference method, we evaluated the concordance between typing methods applied in this study for the given strain collection (Fig. 1). Regarding the isolates of the clonal complexes CC-121, CC-22, and CC-45 and sequence types ST426, ST59, ST81, and ST1, typing results were identical for all three methods. Each of these clonal complexes and sequence types corresponded to a single group after *spa* typing/BURP and SmaI macrorestriction analyses, respectively (Fig. 1). Similar results were obtained for isolates of the clonal complex CC-30, which clustered in a single group after *spa* typing/BURP (*spa* CC012) as well as after macrorestriction analysis (PFGE14). However, *spa* typing revealed very similar *spa* types (t037 and t030) for ST239 (CC-8) isolates compared to *spa* types of *spa* CC012. As a consequence, those *spa* types were grouped together in *spa* CC012 by BURP. This phenomenon was described previously and could be attributed to a chromosomal replacement in the evolution of ST239 MRSA isolates descending from ST8 by integration of a large genetic element from ST30, also encompassing the *spa* locus (24). *spa* types for the remaining isolates of clonal complex CC-8 (showing sequence types ST8, ST247, and ST254) were clustered into a single group (*spa* CC036) by BURP. Using SmaI macrorestriction analysis, all 19 isolates of CC-8 were classified into a total of four different groups (PFGE04,

FIG. 1. Typing results obtained by SmaI macrorestriction analysis, MLST, and *spa* typing. SmaI macrorestriction patterns analyzed using the Dice coefficient and visualized by the unweighted-pair group method, using average linkages with 1% tolerance and 1% optimization settings. The similarity cutoff of 70% is indicated by a vertical line. Results of clustering analyses are compared on the right. Resulting groups are color coded. Identical colors represent corresponding groups obtained from the different clustering methods. [a], excluded from BURP clustering due to insufficient length. [b], PFGE groups determined by cluster analysis are numbered from 1 to 16. Suffixes indicate different PFGE patterns as defined by Tenover et al. (37), e.g., 12.1 means PFGE group 12, pattern 1.

TABLE 3. Concordance between the three typing methods for *S. aureus* applied in this study

Typing method	Concordance between methods (%)	
	spa typing/BURP	PFGE/cluster analysis
MLST/eBURST	96.8	94.5
spa typing/BURP		93.4

PFGE05, PFGE09, and PFGE11) containing 1 to 10 isolates. Although those PFGE groups contained predominantly isolates of one or two sequence types (PFGE04, ST8 and ST247; PFGE05, ST254; PFGE09 and PFGE11, ST239), cluster analysis was not able to group respective sequence types into separate groups unambiguously. Similar results were obtained for isolates of the clonal complex CC-5 with sequence types ST5, ST225, and ST228. While eBURST and BURP clustered all isolates into one group, SmaI macrorestriction analysis was able to group all ST228 isolates into a separate cluster; however, isolates of ST5 and ST225 were not separated by this method and macrorestriction patterns of isolates belonging to those sequence types were quite diverse. These results indicate a high concordance between results of BURP and eBURST for the data set used in this study, while concordance between both sequence-based methods and SmaI macrorestriction analysis is comparatively lower. This is also reflected by concordance values between the three typing methods for the given data set which are summarized in Table 3.

DISCUSSION

A variety of genotyping techniques are available for classifying *S. aureus* strains for epidemiological investigation, including "band-based" as well as "sequence-based" methods. Thereby, sequence-based typing methods, such as *spa* typing and MLST, have some obvious advantages, such as ease of use, reproducibility, transportability, and comparability of results, compared to band-based methods, such as SmaI macrorestriction analysis (2). SmaI macrorestriction analysis, the current gold standard in *S. aureus* strain typing, is accepted for outbreak investigations, but some authors question its use for phylogenetic analyses (34).

In contrast to MLST (combined with eBURST grouping), which is widely used for evolutionary investigation in *S. aureus*, *spa* typing proved to be a tool for routine investigation. Moreover, *spa* typing was more discriminatory than MLST in previous studies. This was confirmed in the present study, where most MLST types encompassed several *spa* types. However, until now, no algorithm was available to group related *spa* types together for epidemiological investigations. The implementation of BURP into the Ridom StaphType software allows clustering of different *spa* types based on a new algorithm for the alignment of repeat sequences (28). Thus, the aim of the present study was to compare clustering results obtained by *spa* typing/BURP analysis to those obtained by well-established methods (SmaI macrorestriction analysis and MLST/eBURST).

Our study demonstrated a wide congruence of clustering results obtained by *spa* typing/BURP, SmaI macrorestriction analysis, and MLST/eBURST. Similar results were previously reported for clonal complexes containing major epidemic nosocomial MRSA isolates, such as CC5, CC8, and CC45 (15, 32), as well as for MSSA isolates of CC5, CC30, and CC121 (1, 15). Additionally, we found a good congruence for epidemic nosocomial MRSA isolates of ST22 (in CC22) and ST228 (in CC5), as well as for community-acquired MRSA isolates of ST80. We also confirmed the divergent *spa* types t037 and t030 in MRSA isolates of ST239 clustering together with *spa* types found in CC30 (15, 18). This has been explained previously by recombinative replacement of a large stretch of chromosomal DNA in MRSA isolates of CC8 by a stretch originating from CC30 and including the *spa* gene (24).

The different *spa* CCs are characterized by one or two repeats specific for a particular complex, such as r15 for *spa* CC012, r14 and r44 for *spa* CC435, r28 and r29 for *spa* CC022, r11 and r19 for *spa* CC036, r20 and r30 for *spa* CC045, r07 for t044, and r02, r08, and r09 for *spa* CC065, as well as by the repeat succession. The types within a *spa* CC in most instances differ by deletion, duplication, or insertion of repeats, but there are also point mutations leading to new repeats. Although we find the same or closely related *spa* sequence types in isolates of the same clonal lineage (as defined by MLST) collected at very different times and from different geographical locations, we should be careful with conclusions on descent and direct epidemiological relations of isolates within a *spa* CC based only on *spa* sequence types. In the following, these aspects will be discussed in more detail.

The majority of isolates belonging to *spa* CC012 (which corresponds to MLST CC30) have repeat r15 as the first repeat unit in common, which is followed by repeats r12, r16, and r02 in a rather conserved order. Besides *spa* type t019, most of the other types included in *spa* CC012 differ by various numbers of the final repeat r24. *spa* type t021 is already represented by MSSA isolates containing the *lukS-lukF* determinant coding for Panton-Valentine leukocidin from the 1960s, such as PS80, and by MSSA isolates from the 1980s. These isolates represent the so-called 80, 81 complex, a major nosocomial pathogen of the 1960s and 1990s (23). This *spa* type is, however, also seen in more-recent MSSA and MRSA isolates which contain *tst* but not *lukS-lukF*. An interpretation in the sense of a direct common ancestral origin of both pathotypes of this clonal lineage would be rather speculative, since *spa* type t021 could have been derived from other *spa* types by loss of one or more final repeats of r24 (e.g., from type t012 or t018). MSSA isolates of MLST CC30 exhibiting *spa* types t012, t018, and t021 from the United States and from Poland have also been described previously (15, 16, 18).

Both cMRSA isolates of MLST ST30 in our collection exhibit *spa* type t019; this type was also described for cMRSA from Belgium (3), Poland (18), and Japan (35). In this case, a wide geographic dissemination of a particular clone cannot be excluded, as t019 differs from other *spa* types in *spa* CC012 by two point mutations in the first repeat (r08 instead of r15 [for details, see http://www.spaserver.ridom.de]).

MSSA isolates of MLST ST121 are grouped in *spa* CC435 and share repeats r14, r44, and r13 as the first ones; repeats r14 and r44 have not been found in any other isolates of the collection. As already seen in isolates of CC30, there is no association of virulence-associated genes (*eta* and *etb* versus *lukS-lukF*) with particular *spa* types.

spa types of MLST ST22 MRSA isolates cluster in *spa* CC022. Among them, isolate 96-1678 exhibits the prototype SmaI macrorestriction pattern of ST22; the other isolates of this lineage have been selected for different fragment patterns. Four of them exhibit t032, one exhibits t022 (one deletion of r23), and one exhibits t005 (one point mutation in r29 leading to r05). *spa* type t310 was found for both cMRSA isolates of ST22 originating from Scotland and Germany, which suggests a more direct relation.

CC8 contains four major clonal lineages of epidemic hMRSA: ST8, ST239, ST247, and ST254 revealing *spa* types t008, t037/t030, t051, and t009, respectively. Besides t037/t030 (see above), they are grouped into *spa* CC08. The MRSA isolate of lineage ST254 isolated from a horse (isolate 03-2575) exhibits t036 (deletion of three repeats from t009). The finding of the same *spa* types for isolates from each clonal lineage collected in different years and from rather dispersed geographical areas suggests that *spa* types in *spa* CC08 are quite stable. *spa* types t008 and t037 have also been described for MRSA isolates of lineages ST8 and ST239 from the United States (15). There are, however, additional types (t388) among MRSA isolates of ST239 in Poland; Polish MRSA isolates of ST247 exhibit t052, differing from t051, which was found in Central European isolates of this lineage, by deletion of one repeat (18). cMRSA isolates belonging to ST8 cannot be discriminated from hMRSA isolates of this lineage by *spa* sequence typing; they also exhibit t008.

For CC5, isolates belonging to clonal lineages ST5, ST225, and ST228 have been investigated. Clustering of their *spa* types groups them in *spa* CC045. *spa* type t001 was found for isolates of ST228 collected from different locations in Germany, Poland, and Slovenia over 6 years. Besides t002, which had already been reported for MRSA isolates of lineage ST5 from Central Europe (13), two other *spa* types have been detected (t045 and t178). Type t002 was also found in MRSA isolates of ST5 from the United States (15, 16) and in the majority of MRSA isolates of these lineages from Japan and southern Korea. In this study, seven "subtypes" due to deletion, insertion, and point mutations were reported (32). The majority of MRSA isolates of ST5 reported from Poland exhibited t053, which differs from t002 by three point mutations within the final repeat (18). *spa* type t003 was found in three isolates of ST225 from different locations in Germany.

MRSA isolates of CC45 had first been reported in 1993 from Berlin hospitals (39) and afterwards from other European countries (26); they are obviously also disseminated in Northern America. *spa* types in ST45 are rather heterogeneous and are grouped in *spa* CC065; type t004 was found in five of eight isolates which have been collected from 1993 until now. Type t004 seems to be characteristic for MRSA isolates of ST45 originating from Central Europe, whereas t015 was reported for isolates from the United States (15) and Poland (18); it is substantially different from t004.

Among the cMRSA isolates investigated, clonal lineages ST80, which includes the most widely disseminated cMRSA isolates in Europe (38), and ST1, which is sporadic in central Germany but frequent in northern states of the United States (20), exhibit *spa* types t044 and t175, respectively, by which they can easily be recognized, as these types have not been reported for any other MSSA or MRSA isolates so far (http://www.spaserver.ridom.de). Type t044 was also reported for cMRSA isolates of ST80 from Belgium (3).

In conclusion, we could demonstrate a high degree of concordance between the three different typing and clustering methods applied in this study. Although SmaI macrorestriction analysis proved to be superior in discriminatory power, *spa* typing/BURP was shown to be a feasible tool for elucidating epidemiological questions, providing results comparable to those obtained with MLST/eBURST. However, the user must be aware of certain particularities (e.g., t037/t030 grouping; see above). A specialized software tool such as Ridom StaphType enables the implementation of *spa* type-specific alerts, thus preventing "misclassification." Recently, such a software tool was used to establish a DNA sequence-based early warning system for outbreak investigations in hospitals (19). Additionally, it ensures a common typing nomenclature and thus greatly facilitates the exchange of typing data for *S. aureus* (2) as well as the setup of supranational typing networks (10).

REFERENCES

1. **Aires de Sousa, M., T. Conceicao, C. Simas, and H. de Lencastre.** 2005. Comparison of genetic backgrounds of methicillin-resistant and -susceptible *Staphylococcus aureus* isolates from Portuguese hospitals and the community. J. Clin. Microbiol. **43:**5150–5157.
2. **Aires de Sousa, M., K. Boye, H. de Lencastre, A. Deplano, M. C. Enright, J. Etienne, A. Friedrich, D. Harmsen, A. Holmes, X. W. Huijsdens, A. M. Kearns, A. Mellmann, H. Meugnier, J. K. Rasheed, E. Spalburg, B. Strommenger, M. J. Struelens, F. C. Tenover, J. Thomas, U. Vogel, H. Westh, J. Xu, and W. Witte.** 2006. High interlaboratory reproducibility of DNA sequence-based typing of bacteria in a multicenter study. J. Clin. Microbiol. **44:**619–621.
3. **Denis, O., A. Deplano, H. De Beenhouwer, M. Hallin, G. Huysmans, M. G. Garrino, Y. Glupczynski, X. Malaviolle, A. Vergison, and M. J. Struelens.** 2005. Polyclonal emergence and importation of community-acquired methicillin-resistant *Staphylococcus aureus* strains harbouring Panton-Valentine leucocidin genes in Belgium. J. Antimicrob. Chemother. **56:**1103–1106.
4. **Enright, M. C., N. P. Day, C. E. Davies, S. J. Peacock, and B. G. Spratt.** 2000. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. J. Clin. Microbiol. **38:**1008–1015.
5. **Enright, M. C., D. A. Robinson, G. Randle, E. J. Feil, H. Grundmann, and B. G. Spratt.** 2002. The evolutionary history of methicillin-resistant *Staphylococcus aureus* (MRSA). Proc. Natl. Acad. Sci. USA **99:**7687–7692.
6. **Feil, E. J., B. C. Li, D. M. Aanensen, W. P. Hanage, and B. G. Spratt.** 2004. eBURST: inferring patterns of evolutionary descent among clusters of related bacterial genotypes from multilocus sequence typing data. J. Bacteriol. **186:**1518–1530.
7. **Francois, P., A. Huyghe, Y. Charbonnier, M. Bento, S. Herzig, I. Topolski, B. Fleury, D. Lew, P. Vaudaux, S. Harbarth, W. van Leeuwen, A. van Belkum, D. S. Blanc, D. Pittet, and J. Schrenzel.** 2005. Use of an automated multiple-locus, variable-number tandem repeat-based method for rapid and high-throughput genotyping of *Staphylococcus aureus* isolates. J. Clin. Microbiol. **43:**3346–3355.
8. **Frenay, H. M., A. E. Bunschoten, L. M. Schouls, W. J. van Leeuwen, C. M. Vandenbroucke-Grauls, J. Verhoef, and F. R. Mooi.** 1996. Molecular typing of methicillin-resistant *Staphylococcus aureus* on the basis of protein A gene polymorphism. Eur. J. Clin. Microbiol. Infect. Dis. **15:**60–64.
9. **Frenay, H. M., J. P. Theelen, L. M. Schouls, C. M. Vandenbroucke-Grauls, J. Verhoef, W. J. van Leeuwen, and F. R. Mooi.** 1994. Discrimination of epidemic and nonepidemic methicillin-resistant *Staphylococcus aureus* strains on the basis of protein A gene polymorphism. J. Clin. Microbiol. **32:**846–847.
10. **Friedrich, A., W. Witte, D. Harmsen, H. de Lencastre, W. Hryniewicz, J. Scheres, H. Westh, and on behalf of the SeqNet.org participants.** 2006. SeqNet.org: a European laboratory network for sequence-based typing of microbial pathogens. Euro Surveill. **11**. [Online.] http://www.eurosurveillance.org/ew/2006/060112.asp#4.
11. **Grundmann, H., S. Hori, and G. Tanner.** 2001. Determining confidence intervals when measuring genetic diversity and the discriminatory abilities of typing methods for microorganisms. J. Clin. Microbiol. **39:**4190–4192.
12. **Harmsen, D., H. Claus, and U. Vogel.** 2005. DNA sequence-based tandem repeat analysis of the *clfB* gene is less discriminatory than spa typing for methicillin-resistant *Staphylococcus aureus*. Int. J. Med. Microbiol. **294:**525–528.
13. **Harmsen, D., H. Claus, W. Witte, J. Rothgänger, H. Claus, D. Turnwald, and**

U. Vogel. 2003. Typing of methicillin-resistant *Staphylococcus aureus* in a university hospital setting by using novel software for *spa* repeat determination and database management. J. Clin. Microbiol. **41:**5442–5448.
14. **Hunter, P. R., and M. A. Gaston.** 1988. Numerical index of the discriminatory ability of typing systems: an application of Simpson's index of diversity. J. Clin. Microbiol. **26:**2465–2466.
15. **Koreen, L., S. V. Ramaswamy, E. A. Graviss, S. Naidich, J. M. Musser, and B. N. Kreiswirth.** 2004. *spa* typing method for discriminating among *Staphylococcus aureus* isolates: implications for use of a single marker to detect genetic micro- and macrovariation. J. Clin. Microbiol. **42:**792–799.
16. **Koreen, L., S. V. Ramaswamy, S. Naidich, I. V. Koreen, G. R. Graff, E. A. Graviss, and B. N. Kreiswirth.** 2005. Comparative sequencing of the serine-aspartate repeat-encoding region of the clumping factor B gene (*clfB*) for resolution within clonal groups of *Staphylococcus aureus*. J. Clin. Microbiol. **43:**3985–3994.
17. **Linde, H., F. Wagenlehner, B. Strommenger, I. Drubel, J. Tanzer, U. Reischl, U. Raab, C. Holler, K. G. Naber, W. Witte, F. Hanses, B. Salzberger, and N. Lehn.** 2005. Healthcare-associated outbreaks and community-acquired infections due to MRSA carrying the Panton-Valentine leucocidin gene in southeastern Germany. Eur. J. Clin. Microbiol. Infect. Dis. **24:**419–422.
18. **Malachowa, N., A. Sabat, M. Gniadkowski, J. Krzyszton-Russjan, J. Empel, J. Miedzobrodzki, K. Kosowska-Shick, P. C. Appelbaum, and W. Hryniewicz.** 2005. Comparison of multiple-locus variable-number tandem-repeat analysis with pulsed-field gel electrophoresis, *spa* typing, and multilocus sequence typing for clonal characterization of *Staphylococcus aureus* isolates. J. Clin. Microbiol. **43:**3095–3100.
19. **Mellmann, A., A. W. Friedrich, N. Rosenkotter, J. Rothganger, H. Karch, R. Reintjes, and D. Harmsen.** 2006. Automated DNA sequence-based early warning system for the detection of methicillin-resistant *Staphylococcus aureus* outbreaks. PLOS Med. **3:**e33.
20. **Mongkolrattanothai, K., S. Boyle, M. D. Kahana, and R. S. Daum.** 2003. Severe *Staphylococcus aureus* infections caused by clonally related community-acquired methicillin-susceptible and methicillin-resistant isolates. Clin. Infect. Dis. **37:**1050–1058.
21. **Murchan, S., M. E. Kaufmann, A. Deplano, R. de Ryck, M. Struelens, C. E. Zinn, V. Fussing, S. Salmenlinna, J. Vuopio-Varkila, N. El Solh, C. Cuny, W. Witte, P. T. Tassios, N. Legakis, W. van Leeuwen, A. van Belkum, A. Vindel, I. Laconcha, J. Garaizar, S. Haeggman, B. Olsson-Liljequist, U. Ransjo, G. Coombes, and B. Cookson.** 2003. Harmonization of pulsed-field gel electrophoresis protocols for epidemiological typing of strains of methicillin-resistant *Staphylococcus aureus*: a single approach developed by consensus in 10 European laboratories and its application for tracing the spread of related strains. J. Clin. Microbiol. **41:**1574–1585.
22. **Oliveira, D. C., A. Tomasz, and H. de Lencastre.** 2001. The evolution of pandemic clones of methicillin-resistant *Staphylococcus aureus*: identification of two ancestral genetic backgrounds and the associated *mec* elements. Microb. Drug Resist. **7:**349–361.
23. **Parker, M. T., E. H. Asheshov, J. H. Hewitt, L. S. Nakhla, and B. M. Brock.** 1974. Endemic staphylococcal infections in hospitals. Ann. N. Y. Acad. Sci. **236:**466–484.
24. **Robinson, D. A., and M. C. Enright.** 2004. Evolution of *Staphylococcus aureus* by large chromosomal replacements. J. Bacteriol. **186:**1060–1064.
25. **Robinson, D. A., S. K. Hollingshead, J. M. Musser, A. J. Parkinson, D. E. Briles, and M. J. Crain.** 1998. The IS1167 insertion sequence is a phylogenetically informative marker among isolates of serotype 6B *Streptococcus pneumoniae*. J. Mol. Evol. **47:**222–229.
26. **Robinson, D. A., A. M. Kearns, A. Holmes, D. Morrison, H. Grundmann, G. Edwards, F. G. O'Brien, F. C. Tenover, L. K. McDougal, A. B. Monk, and M. C. Enright.** 2005. Re-emergence of early pandemic *Staphylococcus aureus* as a community-acquired meticillin-resistant clone. Lancet **365:**1256–1258.
27. **Sabat, A., J. Krzyszton-Russjan, W. Strzalka, R. Filipek, K. Kosowska, W. Hryniewicz, J. Travis, and J. Potempa.** 2003. New method for typing *Staphylococcus aureus* strains: multiple-locus variable-number tandem repeat analysis of polymorphism and genetic relationships of clinical isolates. J. Clin. Microbiol. **41:**1801–1804.
28. **Sammeth, M., W. Weniger, D. Harmsen, and J. Stoye.** 2006. Alignment of tandem repeats with excision, duplication, substitution and indels (EDSI), p. 276–290. *In* R. Casadio and G. Myers (ed.), Proceedings of the 5th Workshop on Algorithms in Bioinformatics (WABI 2005). Springer Verlag, Berlin, Heidelberg, Germany.
29. **Shopsin, B., M. Gomez, S. O. Montgomery, D. H. Smith, M. Waddington, D. E. Dodge, D. A. Bost, M. Riehman, S. Naidich, and B. N. Kreiswirth.** 1999. Evaluation of protein A gene polymorphic region DNA sequencing for typing of *Staphylococcus aureus* strains. J. Clin. Microbiol. **37:**3556–3563.
30. **Shopsin, B., M. Gomez, M. Waddington, M. Riehman, and B. N. Kreiswirth.** 2000. Use of coagulase gene (*coa*) repeat region nucleotide sequences for typing of methicillin-resistant *Staphylococcus aureus* strains. J. Clin. Microbiol. **38:**3453–3456.
31. **Shopsin, B., and B. N. Kreiswirth.** 2001. Molecular epidemiology of methicillin-resistant *Staphylococcus aureus*. Emerg. Infect. Dis. **7:**323–326.
32. **Soo, K. K., K. R. Peck, O. W. Sup, N. Y. Lee, K. Hiramatsu, and J. H. Song.** 2005. Genetic differentiation of methicillin-resistant *Staphylococcus aureus* strains from Korea and Japan. Microb. Drug Resist. **11:**279–286.
33. **Strommenger, B., C. Cuny, G. Werner, and W. Witte.** 2004. Obvious lack of association between dynamics of epidemic methicillin-resistant *Staphylococcus aureus* in central Europe and *agr* specificity groups. Eur. J. Clin. Microbiol. Infect. Dis. **23:**15–19.
34. **Swofford, D. L., and G. J. Olsen.** 1990. Phylogenetic reconstruction, p. 411–501. *In* D. M. Hillis and C. Moritz (ed.), Molecular systematics. Sinauer Associates, Sunderland, Mass.
35. **Takizawa, Y., I. Taneike, S. Nakagawa, T. Oishi, Y. Nitahara, N. Iwakura, K. Ozaki, M. Takano, T. Nakayama, and T. Yamamoto.** 2005. A Panton-Valentine leucocidin (PVL)-positive community-acquired methicillin-resistant *Staphylococcus aureus* (MRSA) strain, another such strain carrying a multiple-drug resistance plasmid, and other more-typical PVL-negative MRSA strains found in Japan. J. Clin. Microbiol. **43:**3356–3363.
36. **Tang, Y. W., M. G. Waddington, D. H. Smith, J. M. Manahan, P. C. Kohner, L. M. Highsmith, H. Li, F. R. Cockerill III, R. L. Thompson, S. O. Montgomery, and D. H. Persing.** 2000. Comparison of protein A gene sequencing with pulsed-field gel electrophoresis and epidemiologic data for molecular typing of methicillin-resistant *Staphylococcus aureus*. J. Clin. Microbiol. **38:**1347–1351.
37. **Tenover, F. C., R. D. Arbeit, R. V. Goering, P. A. Mickelsen, B. E. Murray, D. H. Persing, and B. Swaminathan.** 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J. Clin. Microbiol. **33:**2233–2239.
38. **Vandenesch, F., T. Naimi, M. C. Enright, G. Lina, G. R. Nimmo, H. Heffernan, N. Liassine, M. Bes, T. Greenland, M. E. Reverdy, and J. Etienne.** 2003. Community-acquired methicillin-resistant *Staphylococcus aureus* carrying Panton-Valentine leukocidin genes: worldwide emergence. Emerg. Infect. Dis. **9:**978–984.
39. **Witte, W., C. Cuny, E. Halle, J. Wagner, and H. Mauch.** 1993. Methicillin resistance in an epidemic *Staphylococcus aureus* strain with genomic fingerprints corresponding to those of a sensitive strain in the community. Med. Microbiol. Lett. **3:**388–395.
40. **Witte, W., C. Cuny, B. Strommenger, C. Braulke, and D. Heuck.** 2004. Emergence of a new community acquired MRSA strain in Germany. Euro Surveill. **9:**1–2. [Online.]