JOURNAL OF CLINICAL MICROBIOLOGY, July 2006, p. 2442–2448 Vol. 44, No. 7
0095-1137/06/$08.00+0 doi:10.1128/JCM.00113-06

Classifying *spa* Types in Complexes Improves Interpretation of Typing Results for Methicillin-Resistant *Staphylococcus aureus*

Werner Ruppitsch,* Alexander Indra, Anna Stöger, Barbara Mayer, Silke Stadlbauer, Günther Wewalka, and Franz Allerberger

Austrian Agency for Health and Food Safety, Institute of Medical Microbiology and Hygiene, Spargelfeldstrasse 191, 1226 Vienna, Austria

Received 18 January 2006/Returned for modification 23 February 2006/Accepted 6 May 2006

A total of 382 isolates of methicillin-resistant *Staphylococcus aureus* originating from three Austrian regions and one adjacent Italian region (Vienna, Lower Austria, North Tyrol, and South Tyrol) were typed by DNA sequence analysis of the variable repeat region of the protein A gene (*spa* typing). The strain collection consisted of arbitrarily chosen isolates originating from clinical specimens taken in the years 2003 to 2005 at 17 hospitals. The most common *spa* types found were t001 (28.8% of all isolates), t190 (27.0%), t008 (14.1%), and t041 (11.3%). The 42 remaining *spa* types accounted for ≤2.4% each. The dominating *spa* types varied between the different regions. As short sequence DNA repeat units are unstable entities, the 46 *spa* types were classified into seven *spa* complexes with respect to short sequence repeat unit composition and organization. Such classification into complexes can provide additional information for the hospital epidemiologist, empowering one to differentiate the introduction of a new strain from mere variation of endemic *spa* types.

Staphylococcus aureus is one of the most significant health care-associated pathogens and is responsible for a wide range of hospital infections (7, 27, 44). Since the first identification of methicillin-resistant *Staphylococcus aureus* (MRSA) in the early 1960s (18), the incidence of *S. aureus* bacteremia has been rising and has multiplied in some European countries in the past 25 years (11, 25). This increase coincides with an increased rate of community-acquired MRSA infections (5, 17, 26, 36). The emergence of community-associated MRSA strains has further increased public health concerns (26). Therefore, *S. aureus* typing has become an important tool in the study of strain origin, surveillance of health care-associated infections, and epidemiological outbreak investigation. A large number of molecular methods have been developed for typing of MRSA strains. The most widely used molecular typing method for the study of MRSA epidemiology is pulsed-field gel electrophoresis (PFGE) (1, 29). However, the use of PFGE remains limited by problematic interlaboratory comparison, problems of interpretation, low throughput, and high costs (6, 42). PFGE is a suitable method for the determination of clonal relationships but not for long-term epidemiological investigations (2, 3). Typing techniques based on DNA sequencing have an obvious advantage in speed, unambiguous data interpretation, simplicity of database creation, and standardization among diverse laboratories (24). Multilocus sequence typing (MLST) is a highly discriminatory method for strain typing and characterizes bacterial isolates on the basis of the sequence fragments of seven housekeeping genes. An isolate is defined by the allelic profile, or sequence type (9). Similar sequence types are grouped into clonal complexes. The low mutation rate of the seven housekeeping genes makes MLST more suitable for long-term and global epidemiological studies (10, 24). However, MLST of *S. aureus* is an expensive and laborious method that requires the sequencing of approximately 3,500 nucleotides and does not have the resolving power of PFGE. Recently, DNA sequencing of short sequence repeats (SSR) of the polymorphic X region of the protein A gene (*spa*) was proposed as an accurate method for typing *S. aureus* (13, 14, 34, 37). Although *spa* typing does not have the fine resolution of PFGE, it has advantages in terms of speed, interpretation, and interlaboratory comparison (34, 44). The polymorphic X region consists of a variable number of 21-bp to 27-bp repeats and is located upstream of the region encoding the cell wall attachment sequence (38). The determination of *spa* types was simplified by developing appropriate software (16, 34), and to date 111 diverse repeat units and more than 1,400 *spa* types have been described (http://spaserver.ridom.de). Although the function of the octapeptide of the polymorphic X region is not known, SSR variations are clearly related to bacterial pathogenesis and virulence (22, 32, 41, 43).

Despite the variability of the DNA sequence of the variable X region, the amino acid sequences of the repeats remain relatively constant. The peptides of diverse *spa* types show similar amino acid hydropathicities and similar secondary structures (20). Thus, mutations mainly affect the wobble base and all repeats are in frame, so that deletions or additions of repeat units will not alter the reading frame.

In most cases, rearrangements of repetitive sequences are caused by replication slippage or the repair of double-strand breaks during DNA replication (4, 12, 43). Conflicting results on the variability of the X region of the *spa* gene have been reported (13, 19, 34, 37, 39). Without selective pressure (i.e., under laboratory conditions), the frequency of genetic variation of SSR is low (13, 31, 37, 39). However, under selective pressure (i.e., in vivo), up to 10% of the analyzed MRSA

* Corresponding author. Mailing address: Austrian Agency for Health and Food Safety, Institute of Medical Microbiology and Hygiene, Spargelfeldstrasse 191, A-1226 Vienna, Austria. Phone: 0043 50 555 32204. Fax: 0043 50 555 32219. E-mail: werner.ruppitsch@ages.at.

TABLE 1. *spa* complex I

spa type[a]	SSR profile																No. of isolates[b]				
																	ST	NT	LA	VIE	Total
t661	26	30	17	34	17	20	17	34	17	20	17	12	17	12	17	16				1	1
t838	26	30	17	34	17	20	17	34	17	20	17	12	17		17	16			1	1	2
t896	26	30	17	34	17	20	17	34	17		12	12		12	17	16		4			4
t041	26	30	17	34	17	20	17	34	17	20	17	12			17	16	11	5	13	14	43
t001	26	30	17	34	17	20	17					12			17	16	5	56	38	11	110
t002	26	23	17	34	17	20	17					12			17	16		1	2		3
t1062	26	23	17	34	17	2	17					12			17	16				2	2
t1003	26				17	20	17	34	17	20	17	12			17	16		1			1
t003	26				17	20	17					12	17		17	16				2	2
t811	26	30	17	34	17	20	17								17	16			1	1	2
t551	26	30	17	34	17	20									17	16			1		1
t288	26	30													17	16			2		2
Total																	16	67	58	32	173

[a] t001, t002, t003, and t041 were assigned to CC5.
[b] The frequency of each *spa* type within a region is shown. ST, South Tyrol; NT, North Tyrol; LA, Lower Austria; VIE, Vienna.

strains showed mutations within the polymorphic X region of the *spa* gene (19, 39). It is probably a limitation to use the X region as a sound target for epidemiological investigations (40). The hypervariability of the SSR region and the huge number of *spa* types identified demand further classification into *spa* complexes with respect to SSR unit similarity (10, 30). The *spa* complexes so determined showed a good correlation to the recently determined clonal complexes (10, 21, 30), amplified fragment length polymorphism complexes (25), and DNA microarray data (20). Thus, analysis of *spa* complexes may increase the usefulness of *spa* typing for short-term as well as long-term epidemiological investigations.

For the first time, MRSA isolates collected from three Austrian regions and one adjacent Italian region were analyzed by *spa* typing using this bifunctional approach to view the MRSA population. The diversity of the MRSA isolates was determined by the number of distinct *spa* types. The clonal structure of the MRSA population was determined by the number of *spa* complexes, which were defined as a group of *spa* types with similar SSR profiles. These *spa* complexes were correlated to clonal complexes or to an MLST if the specific sequence type had not been assigned to a clonal complex.

MATERIALS AND METHODS

Bacterial strains. The 382 MRSA isolates *spa* typed originated from the region of South Tyrol, Italy (isolates provided by Sanitary Service Bozen, $n = 56$), and the Austrian regions of North Tyrol (University Hospital Innsbruck, $n = 76$), Lower Austria (Hospital Gmuend, Danube-Clinic Tulln, Hospital St. Poelten, Hospital Korneuburg/Stockerau, Danube-Clinic Gugging, and Waldviertel-Clinic Horn, $n = 80$), and Vienna (Hospital Rudolfstiftung, Hospital Meidling, Lorenz-Boehler Hospital, Hospital Speising, Hospital Lainz, Hospital Kaiser-Franz-Josef, Vienna Medical Center East, Hospital Wilheminen, and Hospital St. Elisabeth, $n = 170$).

***spa* sequencing.** A loopful of material from an overnight culture (grown on blood agar plates) was suspended in 0.5 ml sterile H_2O. The suspension was heated to 95°C for 10 min and was immediately frozen at −20°C for at least 30 min. After thawing, 1.0 μl of the suspension was used for PCR.

The variable X region of the *spa* gene was amplified in a 50-μl reaction volume using RedTaq Ready mix (Sigma, St. Louis, Mo.). The oligonucleotides used for amplification correspond to the 5′ end (1113F, 5′-TGTAAAACGACGGCCAG TTAAAGACGATCCTTCGGTGAGC) and the 3′ end (1514R, 5′-CAGGAA ACAGCTATGACCCAGCAGTAGTGCCGTTTGCTT) (16, 28) containing an M13 primer sequence (31). PCR conditions were 95°C for 5 min; 35 cycles each of 95°C for 15 s, 58°C for 30 s, and 72°C for 45 s; and a final step at 72°C for 10 min. Prior to sequencing, 10 μl of the amplified products was analyzed on 1.5% agarose gels. The remaining 40 μl was purified using a GenElute PCR clean-up kit (Sigma) according to the manufacturer's instructions.

Sequence analysis was performed by cycle sequencing (SequiTerm Excel II cycle sequencing kit; Epicenter, Madison, Wis.) with fluorescent-labeled primers

TABLE 2. *spa* complex II

spa type[a]	SSR profile											No. of isolates[b]				
												ST	NT	LA	VIE	Total
t051	11	19	21	12	21	17	34	24	34	22	25	1				1
t008	11	19		12	21	17	34	24	34	22	25	33	6	2	13	54
t024	11			12	21	17	34	24	34	22	25			1		1
t746	11	19		12	21	17				22	25	1				1
t190	11					17	34	24	34	22	25			13	90	103
t662	11					17			34	22	25				1	1
t810	11					17				22	25			1		1
Total												35	6	17	104	162

[a] t051, a North German MRSA, Archaic/Iberian type (ST247:MRSA:SCC*mec* I), may represent the prototype of *spa* complex II. t051, t008, t024, and t190 were assigned to CC8.
[b] The frequency of each *spa* type within a region is shown. ST, South Tyrol; NT, North Tyrol; LA, Lower Austria; VIE, Vienna.

TABLE 3. *spa* complex III

spa type[a]	SSR profile											No. of isolates[b]				
												ST	NT	LA	VIE	Total
t018	15	12	16	2	16	2	25	17	24	24	24			1		1
t012	15	12	16	2	16	2	25	17		24	24				1	1
t582	1	12	16	2	16	2	25			24	24				1	1
t019	8		16	2	16	2	25	17			24				1	1
t037	15	12	16	2			25	17		24	24		1		1	2
t030	15	12	16	2						24	24		1		4	5
t425	15	12	16	2			25	17	25						1	1
t930	15		16	2						24	24				2	2
t633	8										24				1	1
Total													2	1	12	15

[a] t018 may represent the prototype of *spa* complex III. t012, t018, and t019 were assigned to CC30, whereas t030 and t037 were assigned to CC239.
[b] The frequency of each *spa* type within a region is shown. ST, South Tyrol; NT, North Tyrol; LA, Lower Austria; VIE, Vienna.

M13 univ. (5′-TGTAAAACGACGGCCAGT) and M13 rev. (5′-CAGGAAAC AGCTATGACC) (MWG-Biotech, Ebersberg, Germany), using a LI-COR 4200S automated DNA sequencer (LI-COR Bioscience, Lincoln, Nebr.) according to the manufacturer's instructions.

Sequence data analysis. The standard chromatogram files of the forward and reverse sequences obtained from each sample were assembled and edited and *spa* types were determined using Ridom StaphType software (16).

Classification of *spa* types. *spa* types with similar repeat profiles were grouped into a *spa* complex. Since sequence-based alignment using algorithms is not useful for analysis of repeat regions, visual analysis of repeat organization is a more reliable and easier method for comparing related *spa* types. A *spa* complex was characterized by *spa* types that shared identical SSR units arranged in an identical order. Within a complex, *spa* types were differentiated due to deletions or duplications of SSR units, which is the major cause of genetic rearrangement within an SSR region (4, 41). Exceptions were due to less frequent mutation events such as deletion, duplication, and exchange of triplets or single nucleotide polymorphisms, resulting in a change of single SSR units within a repeat region. The general structure of repeat units of a repeat region may not be changed by single nucleotide polymorphisms.

RESULTS

Typing of the 382 clinical MRSA isolates from the four regions, South Tyrol, North Tyrol, Lower Austria, and Vienna, yielded 46 *spa* types (Tables 1 to 7). The most common *spa* types identified were t001 (28.8% of all isolates), t190 (27.0%), t008 (14.1%), and t041 (11.3%). The 42 remaining *spa* types found accounted for ≤2.4% each. Considering SSR unit similarity of the SSR region, the 46 *spa* types identified were classified into seven *spa* complexes (Tables 1 to 7). *spa* complexes were defined by the following repeat profiles: complex I, 26-(30/23)-(17-34-17-20/2)$_n$-(17-12)$_n$-17-16; complex II, 11-(19-21-12-21)-17-(34-24-34)-22-25; complex III, 15/1/8-(12)-(16-2)$_n$-(25)-(17)-(24/25)-24$_n$; complex IV, 8/9-(16)-(2-16-34-13-17-34)-16-34; complex V, 26-(23)$_n$-13-23-(31-29/5-17)$_n$-25-17-25-16-28; complex VI, which might consist of two subcomplexes, 4-20/21-(12-17)$_n$-20-17-12-12/17-17 and 7-23-12-21-21-(17)-20-17-(12)$_n$-(17); and complex VII, which might also consist of subcomplexes, 7-23-(21)-12/17/16-(34)$_n$-(33)-34/12/13-(12-23)-(2-12-23) (Tables 1 to 7).

One hundred seventy-three isolates, or 45.4%, belonged to *spa* complex I (Table 1). The *spa* types t001, t002, t003, and t041 of complex I were assigned to clonal complex 5 (CC5). The South German MRSA type t001 and the Rhine Hesse MRSA type t002 represent the prototypes within this complex. In North Tyrol and Lower Austria, *spa* types of complex I prevailed, with frequencies of 88.2% and 72.5%, respectively (Table 1). In both regions, t001 was the most prevalent *spa* type, with a frequency of 73.7% in North Tyrol and with a frequency of 47.5% in Lower Austria (Table 1).

One hundred sixty-two isolates, or 42.5%, belonged to *spa* complex II (Table 2). The *spa* types t008, t190, t051, and t024 were assigned to CC8. *spa* types of complex II were most frequent for Vienna and South Tyrol, with frequencies of 60.5% and 62.5%, respectively (Table 2). With a frequency of 52.3%, *spa* type t190 was the predominant *spa* type in Vienna. *spa* type t190 was not found in South Tyrol. In this region, *spa* type t008 was the dominant *spa* type, with a frequency of 58.9%. *spa* type t008 was the exclusive representative of complex II in North Tyrol (Table 2).

Fifteen isolates, or 3.9%, belonged to *spa* complex III (Table 3). The *spa* types t012, t018, and t019 of complex III were

TABLE 4. *spa* complex IV

spa type[a]	SSR profile										No. of isolates[b]				
											ST	NT	LA	VIE	Total
t015	8	16	2	16	34	13	17	34	16	34				2	2
t065	9		2	16	34	13	17	34	16	34				1	1
t550	8						17	34	16	34				1	1
t390	8	16							16	34			1		1
Total													1	4	5

[a] t015, t065, and t390 were assigned to CC45. t004 (ST45:MRSA:SCC*mec* IV) may represent the prototype of *spa* complex IV.
[b] The frequency of each *spa* type within a region is shown. ST, South Tyrol; NT, North Tyrol; LA, Lower Austria; VIE, Vienna.

TABLE 5. *spa* complex V

spa type[a]	SSR profile																No. of isolates[b]				
																	ST	NT	LA	VIE	Total
t032	26	23	23	13	23	31	29	17	31	29	17	25	17	25	16	28				5	5
t379	26	23	23	13	23	31	29	17				25	17	25	16	28				2	2
t005	26	23		13	23	31	5	17				25	17	25	16	28	1				1
t223	26	23		13	23		5	17				25	17	25	16	28				1	1
Total																	1			8	9

[a] The Barnim MRSA type t032 is the prototype of *spa* complex V. t032, t005, and t223 were assigned to CC22.
[b] The frequency of each *spa* type within a region is shown. ST, South Tyrol; NT, North Tyrol; LA, Lower Austria; VIE, Vienna.

assigned to CC30/39, and *spa* types t030 and t037 of this complex were assigned to CC239. The *spa* type t018 and the Vienna MRSA type t037 might represent the prototypes of this complex. Thirteen of 15 isolates of complex III were found in the Vienna region (Vienna and bordering districts of Lower Austria).

The *spa* complex IV consisted of four different *spa* types, derived from five isolates (Table 4). The *spa* types t015, t065, and t390 were assigned to CC45. The Berlin MRSA type t004 (ST45:MRSA:SCC*mec* IV) (where ST45 is sequence type 45 and SCC*mec* IV is staphylococcal chromosome cassette *mec* type IV) might represent the prototype of this complex. All isolates were from the adjacent geographical areas Vienna and Lower Austria.

The *spa* complex V consisted of nine isolates (Table 5). With the exception of *spa* type t005, which was a single isolate from South Tyrol, all other isolates were from Vienna. The *spa* types t005, t032, and t223 were assigned to CC22.

The *spa* complex VI consisted of four isolates that belong to four different *spa* types (Table 6). The *spa* types of this complex might consist of two different subcomplexes due to the first SSR units of the SSR region and show, at least to some extent, similarity to the *spa* types of complex I and complex VII. Similar *spa* types of complex VI were assigned to CC25.

Complex VII was a heterogeneous group (Table 7), and might consist of two or even more subcomplexes. However, because of common repeat units and due to the limited number of similar *spa* types these *spa* types were grouped into a common complex. Nine of 14 isolates (64%) of the six *spa* types within this complex belonged to *spa* type t044. The *spa* types of this complex were assigned to CC1 (t127), CC15 (t084), ST7 (t091), and ST80 (t044 and t416). *spa* types within this complex were found in South Tyrol and the Vienna region.

DISCUSSION

The Antibiotic Resistance, Prevention and Control (ARPAC) project, a European Commission DG Research-funded concerted action, identified *S. aureus* resistant to methicillin/oxacillin as an alert organism of concern in Europe (23). Typing alert organisms by use of molecular techniques is considered a high-priority recommendation for national health authorities.

To determine the diversity of the MRSA population in Austria, isolates from diverse regions and hospitals were typed. Because of the high level of polymorphism within the X region of the protein A gene and the fact that *spa* typing is associated with speed, low costs, and comparability of data, *spa* typing was used in this study for typing of MRSA isolates. Forty-six different *spa* types were found, of which the majority, 82%, belong to four *spa* types only. However, as SSR are generally described as unstable entities that undergo frequent DNA sequence variation (19, 33, 39, 40, 41), these 46 *spa* types were grouped into *spa* complexes with respect to SSR unit composition and organization (34). This system of classification of related types had already been used to group MLSTs into clonal complexes (8). The grouping of related types (*spa*, MLST, or PFGE) is a valuable tool for strain analysis and supports a hospital epidemiologist's work by simplifying the identification of epidemiologically related strains. However, molecular subtyping on its own is not able to elucidate whether distinct subtypes have evolved from one another or whether they represent single introductions from outside. Without additional epidemiological information, it is, for instance, impossible to say whether *spa* type t008 in Vienna is the precursor of the most frequent *spa* type, t190, or whether these *spa* types occurred independently. Moreover, without epidemiological data it is even impossible to postulate a correlation between

TABLE 6. *spa* complex VI

spa type[a]	SSR profile													No. of isolates[b]				
														ST	NT	LA	VIE	Total
t897	4			20	12	17	12	17	20	17	12	17	17		1			1
t349	4			21	12	17			20	17	12	12	17			1		1
t585	7	23	12	21	12				20	17	12	12					1	1
t148	7	23	12	21	12	17			20	17	12	12	17				1	1
Total															1	1	2	4

[a] MLST profiles of these *spa* types were not available. Similar *spa* types were assigned to CC25.
[b] The frequency of each *spa* type within a region is shown. ST, South Tyrol; NT, North Tyrol; LA, Lower Austria; VIE, Vienna.

TABLE 7. *spa* complex VII

spa type[a]	SSR profile												No. of isolates[b]				
													ST	NT	LA	VIE	Total
t084	7	23		12	34	34		12	12	23	2	12	23	1				1
t091	7	23	21	17		34			12	23	2	12	23	1				1
t593	7	23		12	34						2	12	23				1	1
t127	7	23	21	16		34	33	13								1		1
t044	7	23		12	34	34	33	34						2		1	6	9
t416	7	23		12	34												1	1
Total														4		2	8	14

[a] *spa* complex VII probably consists of at least two subcomplexes, which is reflected by the assignment of the *spa* types to diverse clonal complexes and sequence types (CC1, CC15, ST7, and ST80).
[b] The frequency of each *spa* type within a region is shown. ST, South Tyrol; NT, North Tyrol; LA, Lower Austria; VIE, Vienna.

rare *spa* types, for example, t037 in Vienna and in North Tyrol. Nevertheless, these rare *spa* types are those of special interest for epidemiological investigations, because the chance of elucidating chains of infection is increased compared to doing so with widely distributed types. Thus, dominant *spa* types may be too frequent to provide useful hints in investigating the chains of transmission. Additional markers are necessary to differentiate these frequent strains.

The allocation of similar *spa* types into *spa* complexes facilitates the differentiation of new, emerging *spa* types into descendants of the local *spa* complexes and new *spa* types and unrelated types. The grouping of similar *spa* types considers that various *spa* types are encountered among strains with similar overall genotypes, which is an indication that the speed of SSR evolution does not reflect the speed of overall genome evolution (25, 39). The *spa* complexes generated with respect to these features showed a good correlation to recently determined clonal complexes, coagulase groups, and amplified fragment length polymorphism clusters (8, 10, 25, 35). Moreover, recently reported microarray data correlate with SSR composition and organization (20). Thus, this bifunctional application combines a rapidly evolving marker useful for outbreak investigations with the more stable core structure of a complex useful for long-term epidemiology by sequencing a single DNA fragment.

However, one discrepancy in comparison to the MLST complexes could be observed. Although *spa* types of *spa* complex III have a uniform SSR structure, *spa* types of this complex were assigned to the different clonal complexes CC30/39 and CC239. Strains of the clonal complex CC239 are related to strains of the clonal complex CC8 (8). CC8 is equivalent to *spa* complex II. Thus, we might have a situation where similar *spa* types have evolved from different precursors. The opposite, the evolution of different MLSTs from a common *spa* type, cannot be ruled out. Further work has to be done to elucidate this question.

Due to the lack of a sufficient number of *spa* types and isolates, the *spa* complex VI may contain *spa* types that belong to two related but different *spa* complexes. Although the MLST of our isolates of *spa* complex VI is not known, similar *spa* types were assigned to CC25. The SSR structure of *spa* complex VI shows a distant relation to *spa* complex I.

As with complex VI, there is a high probability that the *spa* types of *spa* complex VII belong to different groups. This is supported by the fact that *spa* types of *spa* complex VII have been assigned to different MLSTs (ST1, ST15, ST7, and ST80). Nevertheless, *spa* types of *spa* complex VII have similar SSR structures. Moreover, it was previously shown that isolates of ST1 and ST80 both carry the Panton-Valentine leucocidin gene and SCC*mec* IV (15). Thus, there might be a phylogenetic correlation of these *spa* types within complex VII that is reflected by a related SSR structure, although the MLST is different.

With respect to the classification of *spa* types into *spa* complexes, each region has its distinct *spa* population. Interestingly, the neighboring regions of South Tyrol and North Tyrol and those of Vienna and Lower Austria have quite different *spa* populations. There is a higher similarity between the *spa* populations of the geographically distant regions North Tyrol and Lower Austria on the one hand and Vienna and South Tyrol on the other hand. In our opinion, this finding underlines the importance of local efforts to cope with local MRSA problems. Whereas South Tyrol, North Tyrol, and Lower Austria have quite uniform *spa* populations, consisting of mainly two *spa* complexes each (with a few *spa* types belonging to other complexes), due to a homogenous population, the occurrence of all *spa* complexes in Vienna probably reflects the multinational population structure of this large city.

It is of interest that the prevalent *spa* type within *spa* complex II in South Tyrol is t008, whereas it is t190 in Vienna. This prevalence of different *spa* types, both of one complex, may be the result of adaptation to different ecological conditions (39).

In conclusion, we consider *spa* typing a highly effective and rapid typing tool for *S. aureus* that has significant advantages over other typing techniques, with the potential to benefit largely the surveillance of antimicrobial resistance and infection control. The system presented here of reporting complexes in addition to the mere *spa* type should foster the use of *spa* typing as a tool for long- and short-term epidemiological investigations. Although some *spa* types cannot be grouped unambiguously into a distinct *spa* complex, the grouping of *spa* types into complexes, in addition to the simple reporting of *spa* types, seems to be a promising tool to simplify the use of *spa* typing for epidemiological investigations. We recommend a reclassification of the >1,400 already-determined *spa* types that would consider the relatedness of certain *spa* types, for example, I-001 (I for the *spa* complex and 001 for the *spa* type). The grouping of the large number of *spa* types of the Ridom database and the determination of MLSTs of so far-unclassified isolates will show a more complete and accurate situation as to the numbers and types of *spa* complexes. Even difficult-

to-group types (i.e., *spa* types consisting of one, two, or three SSR units) should be amenable to grouping into a complex with the additional information of the MLST.

ACKNOWLEDGMENT

This work was supported by funding from the Austrian Agency for Health and Food Safety, project no. 20105/05.

REFERENCES

1. **Bannerman, T. L., G. A. Hancock, F. C. Tenover, and J. M. Miller.** 1995. Pulsed-field gel electrophoresis as a replacement for bacteriophage typing of *Staphylococcus aureus*. J. Clin. Microbiol. **33:**551–555.
2. **Blanc, D. S., M. J. Struelens, A. Deplano, R. de Ryck, P. M. Hauser, C. Petignat, and P. Francioli.** 2001. Epidemiological validation of pulsed-field gel electrophoresis patterns for methicillin-resistant *Staphylococcus aureus*. J. Clin. Microbiol. **39:**3442–3445.
3. **Blanc, D. S., P. Francioli, and P. M. Hauser.** 2002. Poor value of pulsed-field gel electrophoresis to investigate long-term scale epidemiology of methicillin-resistant *Staphylococcus aureus*. Infect. Genet. Evol. **2:**145–148.
4. **Bzymek, M., and S. T. Lovett.** 2001. Instability of repetitive DNA sequences: role of replication in multiple mechanisms. Proc. Natl. Acad. Sci. USA **93:**7120–7124.
5. **Chambers, H. F.** 2001. The changing epidemiology of *Staphylococcus aureus*? Emerg. Infect. Dis. **7:**178–182.
6. **Cookson, B. D., P. Aparicio, A. Deplano, M. Struelens, R. Goering, and R. Marples.** 1996. Inter-centre comparison of pulsed-field gel electrophoresis for the typing of methicillin-resistant *Staphylococcus aureus*. J. Med. Microbiol. **44:**179–184.
7. **Di Fiore, J.** 1956. *Staphylococcus aureus* septicemia, meningitis, endocarditis, and septic embolization. N. Y. State J. Med. **56:**1948–1949.
8. **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.
9. **Enright, M. C., N. P. J. 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.
10. **Feil, E. J., J. E. Cooper, H. Grundmann, D. A. Robinson, M. C. Enright, T. Berendt, S. J. Peacock, J. M. Smith, M. Murphy, B. G. Spratt, C. E. Moore, and N. P. J. Day.** 2003. How clonal is *Staphylococcus aureus*? J. Bacteriol. **185:**3307–3316.
11. **Fitzgerald, J. R., D. E. Sturdevant, S. M. Mackie, S. R. Gill, and J. M. Musser.** 2001. Evolutionary genomics of *Staphylococcus aureus*: insights into the origin of methicillin-resistant strains and the toxic shock syndrome epidemic. Proc. Natl. Acad. Sci. USA **98:**8821–8826.
12. **Francino, M. P., L. Chao, M. A. Riley, and H. Ochman.** 1996. Asymmetries generated by transcriptional-coupled repair in enterobacterial genes. Science **272:**107–109.
13. **Frenay, H. M. E., A. E. Bunschoten, L. M. Schouls, W. J. van Leeuwen, C. M. van den Broucke-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.
14. **Frenay, H. M. E., J. P. G. Theelen, L. M. Schouls, C. M. J. E. van den Broucke-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.
15. **Harbarth, S., P. Francois, J. Schrenzel, C. Frankhauser-Rodriguez, S. Hugonnet, T. Koessler, A. Huyghe, and D. Pittet.** 2005. Community-associated methicillin-resistant *Staphylococcus aureus*, Switzerland. Emerg. Infect. Dis. **11:**962–965.
16. **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.
17. **Herold, B. C., L. C. Immergluck, M. C. Maranan, D. S. Lauderdale, R. E. Gaskin, S. Boyle-Vavra, C. D. Leitch, and R. S. Daum.** 1998. Community-acquired methicillin-resistant *Staphylococcus aureus* in children with no identified predisposing risk. JAMA **279:**593–598.
18. **Jevons, M. P.** 1961. Celbenin-resistant staphylococci. Br. Med. J. **1:**124–125.
19. **Kahl, B. C., A. Mellmann, S. Deiwick, G. Peters, and D. Harmsen.** 2005. Variation of the polymorphic region X of the protein A gene during persistent airway infection of cystic fibrosis patients reflects two independent mechanisms of the genetic change in *Staphylococcus aureus*. J. Clin. Microbiol. **43:**502–505.
20. **Knox, R., and J. T. Smith.** 1961. Nature of penicillin resistance in staphylococci. Lancet **ii:**520–521.
21. **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.
22. **Kuhn, G., P. Francioli, and D. S. Blanc.** 2006. Evidence for clonal evolution among highly polymorphic genes in methicillin-resistant *Staphylococcus aureus*. J. Bacteriol. **188:**169–178.
23. **Li, Y.-C., A. B. Korol, T. Fahima, and E. Nevo.** 2004. Microsatellites within genes: structure, function, and evolution. Mol. Biol. Evol. **21:**991–1007.
24. **MacKenzie, F. M., M. J. Struelens, K. J. Towner, I. M. Gould, et al.** 2005. Report of the Consensus Conference on Antibiotic Resistance, Prevention and Control (ARPAC). Clin. Microbiol. Infect. **11:**938–954.
25. **Maiden, M. C., J. A. Bygraves, E. Feil, G. Morelli, J. E. Russell, R. Urwin, Q. Zhang, J. Zhou, K. Zurth, D. A. Caugant, I. M. Feavers, M. Achtmann, and B. G. Spratt.** 1998. Multilocus sequence typing: a portable approach to the identification of clones within populations of pathogenic microorganisms. Proc. Natl. Acad. Sci. USA **95:**3140–3145.
26. **Melles, D. C., R. F. J. Gorkink, H. A. M. Boelens, S. V. Snijders, J. K. Peeters, M. J. Moorhouse, P. J. van der Spek, W. B. van Leeuwen, G. Simons, H. A. Verbrugh, and A. van Belkum.** 2004. Natural population dynamics and expansion of pathogenic clones of *Staphylococcus aureus*. J. Clin. Investig. **114:**1732–1740.
27. **Nolan, C. M., and H. N. Beaty.** 1976. *Staphylococcus aureus* bacteremia. Current clinical patterns. Am. J. Med. **60:**495–500.
28. **Oliveira, D. C., I. Crisostomo, I. Santos-Sanches, P. Major, C. R. Alves, M. Aires-de-Sousa, M. K. Thege, and H. de Lencastre.** 2001. Comparison of DNA sequencing of the protein A gene polymorphic region with other molecular typing techniques for typing two epidemiologically diverse collections of methicillin-resistant *Staphylococcus aureus*. J. Clin. Microbiol. **39:**574–580.
29. **Prevost, G., B. Poettecher, M. Dahlet, M. Bientz, J. M. Mantz, and Y. Piemont.** 1991. Pulsed field gel electrophoresis as a new epidemiological tool for monitoring methicillin-resistant *Staphylococcus aureus* in an intensive care unit. J. Hosp. Infect. **17:**255–269.
30. **Robinson, D. A., and M. C. Enright.** 2003. Evolutionary models of the emergence of methicillin-resistant *Staphylococcus aureus*. Antimicrob. Agents Chemother. **47:**3926–3934.
31. **Ruppitsch, W., A. Stöger, and M. Keck.** 2004. Stability of short sequence repeats and their application for the characterization of *Erwinia amylovora* strains. FEMS Microbiol. Lett. **234:**1–8.
32. **Said-Salim, B., P. M. Dunman, F. M. McAleese, D. Macapagal, E. Murphy, P. J. McNamara, S. Arvidson, T. J. Foster, S. J. Projan, and B. N. Kreiswirth.** 2003. Global regulation of *Staphylococcus aureus* genes by Rot. J. Bacteriol. **185:**610–619.
33. **Schnabel, E. L., and A. L. Jones.** 1998. Instability of a pEA29 marker in *Erwinia amylovora* previously used for strain classification. Plant Dis. **82:**1334–1336.
34. **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.
35. **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.
36. **Steinberg, J. P., C. C. Clark, and B. O. Hackmann.** 1996. Nosocomial and community acquired *Staphylococcus aureus* bacteremias from 1980 to 1993: impact of intravascular devices and methicillin resistance. Clin. Infect. Dis. **23:**255–259.
37. **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. Thomson, 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.
38. **Uhlen, M., B. Guss, B. Nilsson, S. Gatenbeck, L. Philipson, and M. Lindberg.** 1984. Complete sequence of the staphylococcal gene encoding protein A. J. Biol. Chem. **259:**1695–1702.
39. **van Belkum, A., N. Riewerts Eriksen, M. Sijmons, W. van Leeuwen, M. van den Bergh, J. Kluytmans, F. Espersen, and H. Verbrugh.** 1996. Are variable repeats in the spa gene suitable targets for epidemiological studies of methicillin-resistant *Staphylococcus aureus* strains? Eur. J. Clin. Microbiol. Infect. Dis. **15:**768–770.
40. **van Belkum, A.** 1999. The role of short sequence repeats in epidemiologic typing. Curr. Opin. Microbiol. **2:**306–311.
41. **van Belkum, A., S. Scherer, L. van Alphen, and H. Verbrugh.** 1998. Short-sequence DNA repeats in prokaryotic genomes. Microbiol. Mol. Biol. Rev. **62:**275–293.
42. **van Belkum, A., W. van Leeuwen, M. E. Kaufmann, B. Cookson, F. Forey, J.**

Etienne, R. Goering, F. Tenover, C. Steward, F. O'Brien, W. Grubb, P. Tassios, N. Legakis, A. Morvan, N. El Solh, R. de Ryck, M. Struelens, S. Salmenlinna, J. Vuopio-Varkila, M. Kooistra, A. Talens, W. Witte, and H. Verbrugh. 1998. Assessment of resolution and intercenter reproducibility of results of genotyping *Staphylococcus aureus* by pulsed-field gel electrophoresis of SmaI macrorestriction fragments: a multicenter study. J. Clin. Microbiol. **36:**1653–1659.

43. **Verstrepen, K. J., A. Jansen, F. Lewitter, and G. R. Fink.** 2005. Intragenic tandem repeats generate functional variability. Nat. Genet. **37:**986–990.
44. **Wernitz, M. H., S. Swidsinski, K. Weist, D. Sohr, W. Witte, K. P. Franke, D. Roloff, H. Rüden, and S. K. Veit.** 2005. Effectiveness of a hospital-wide selective screening programme for methicillin-resistant *Staphylococcus aureus* (MRSA) carriers at hospital admission to prevent hospital-acquired MRSA infections. Clin. Microbiol. Infect. **11:**457–465.