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Typing of Methicillin-Resistant *Staphylococcus aureus* in a University Hospital Setting by Using Novel Software for *spa* Repeat Determination and Database Management

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The *spa* gene of *Staphylococcus aureus* encodes protein A and is used for typing of methicillin-resistant *Staphylococcus aureus* (MRSA). We used sequence typing of the *spa* gene repeat region to study the epidemiology of MRSA at a German university hospital. One hundred seven and 84 strains were studied during two periods of 10 and 4 months, respectively. Repeats and *spa* types were determined by Ridom StaphType, a novel software tool allowing rapid repeat determination, data management and retrieval, and Internet-based assignment of new *spa* types following automatic quality control of DNA sequence chromatograms. Isolates representative of the most abundant *spa* types were subjected to multilocus sequence typing and pulsed-field gel electrophoresis. One of two predominant *spa* types was replaced by a clonally related variant in the second study period. Ten unique *spa* types, which were equally distributed in both study periods, were recovered. The data show a rapid dynamics of clone circulation in a university hospital setting. *spa* typing was valuable for tracking of epidemic isolates. The data show that disproval of epidemiologically suggested transmissions of MRSA is one of the main objectives of *spa* typing in departments with a high incidence of MRSA.

Staphylococcus aureus is a major human pathogen causing skin and tissue infections, pneumonia, septicemia, and device-associated infections. The emergence of strains resistant to methicillin and other antibacterial agents has become a major concern especially in the hospital environment, because of the higher mortality due to systemic methicillin-resistant *Staphylococcus aureus* (MRSA) infections (2). Typing of MRSA is used to support infection control measures. While pulsed-field gel electrophoresis (PFGE) is a "gold standard" for strain typing of MRSA (20), DNA sequence-based approaches are becoming more frequently used because sequence data can easily be transferred between laboratories via the Internet. Multilocus sequence typing (MLST), which was developed by using *Neisseria meningitidis* as the model species (9, 18), has been successfully adapted to *S. aureus* (7, 8). However, MLST is not suitable for routine surveillance of MRSA because of the high cost and the necessity of access to a high-throughput DNA sequencing facility.

Although there is evidence for recombination in *S. aureus* (10), it has been shown that point mutations by far exceed recombination events, in contrast to *N. meningitidis* or *Streptococcus pneumoniae* (11). Furthermore, there is only a small number of clonal groupings of MRSA circulating worldwide (7). Therefore, single-locus DNA sequencing of repeat regions of the *coa* (coagulase) gene and the *spa* gene (protein A), respectively, could be used for reliable and accurate typing of MRSA (12, 13, 26–29). *spa* typing is especially interesting for rapid typing of MRSA in a hospital setting since it offers higher resolution than *coa* typing (27). The repeat region of the *spa* gene is subject to spontaneous mutations, as well as loss and gain of repeats. Repeats are assigned an alpha-numerical code, and the *spa* type is deduced from the order of specific repeats. There is a good correlation between clonal groupings determined by MLST and the respective *spa* types (3, 4, 23, 24). Examples have been reported of isolates with the same *spa* type belonging to related MLST sequence types that arose by single-locus variation (5). On the other hand, there seems to be a considerable degree of *spa* gene repeat number variation within a given sequence type, suggesting that *spa* typing in some instances provides greater resolution than MLST (3, 23). Nevertheless, there is a consensus that pulsed-field gel electrophoresis (PFGE) is superior to *spa* typing and probably also MLST in its discriminatory power (14, 15, 19, 26, 29). Therefore, PFGE is still considered a valuable tool for MRSA typing, although it is time-consuming and the interlaboratory comparability of results requires extensive effort for protocol harmonization (20).

For a variety of bacterial species, MLST protocols have been developed during the past 5 years because the method allows the creation of Internet-based curated databases that represent virtual strain collections accessible to the scientific community both for entry of data and for their retrieval (www.mlst.net). Despite being a DNA sequence-based method, *spa* typing, to the best of our knowledge, is hampered by a lack of generally available software tools for repeat identification and by the lack of a consensus on assignments of new repeats and *spa* types. Therefore, *spa* typing cannot be considered a portable tool.

In the present study, the dynamics of MRSA *spa* types at a single hospital was followed in two study periods. A novel

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FIG. 1. Screen shot of the novel Ridom StaphType software featuring a base quality-based sequence editor, a database, and a report generator (not shown) module. This client software synchronizes with an accompanying website to ensure uniform *spa* code terminology usage.

software tool was used for *spa* type determination. This specialized software meets the requirements for modern, Internet-based management of genotyping data.

MATERIALS AND METHODS

Strains. MRSA strains were isolated and identified from various clinical specimens sent to the Institute for Hygiene and Microbiology at the University of Würzburg. Only patients and staff members of the Würzburg University Clinic were included. This hospital is the largest referral center in the southern German region of Lower Franconia, with a population size of about 1,300,000. Copy strains were excluded. Final identification and antimicrobial resistance testing were performed with Vitek 2, an automated bacteriology system that performs bacterial identification and susceptibility testing analyses (BioMérieux, Marci l'Etoile, France). MICs of mupirocin were determined by E test on Mueller-Hinton agar plates after 24 h of incubation as described by the manufacturer (AB Biodisk, Solna, Sweden). Two study periods were included; period 1 (107 isolates) was June 2001 to May 2002, and period 2 (84 isolates) was January 2003 to April 2003. The staff in charge of the diagnostic laboratories was instructed to submit every MRSA isolate first isolated from a patient to *spa* typing. Retrospective examination of the database at the Institute for Hygiene and Microbiology revealed that in period 1, 107 (46.5%) of 230 isolates were subjected to *spa* typing, whereas in period 2, this was the case for 84 (56%) of 150 isolates. *Sma*I macrorestriction patterns were obtained by use of the harmonized European protocol for typing of *S. aureus* by PFGE (20). For cluster analysis, the algorithm described by Claus et al. was used (1).

PCR and DNA sequence analysis. The x region of the *spa* gene was amplified by PCR with primers 1095F (5′-AGACGATCCTTCGGTGAGC-3′) and 1517R (5′-GCTTTTGCAATGTCATTTACTG-3′) (26). DNA sequences were obtained with an ABI 377 sequencer (Applied Biosystems, Foster City, Calif.). *spa* types were determined with the Ridom StaphType software described below (Ridom GmbH, Würzburg, Germany). MLST was performed as described recently (7). Sequence types were determined with the database accessible via http://www.mlst.net/dbqry/saureus.htm.

Ridom StaphType. *spa* types were determined with the novel software Ridom StaphType (Ridom GmbH). Basically, the software consists of three modules: a sequence editor, a database, and a report generator module (Fig. 1). After providing the input sequences (FASTA format or preferably ABI and SCF chromatograms), Ridom StaphType attaches to each called base a quality value that corresponds to a sequence error probability. Taking the quality values into consideration, the software constructs a consensus sequence, automatically detects the *spa* repeats, and assigns a *spa* type. In at least 90% of all cases, no further manual editing is necessary. For the remaining sequences, a versatile graphic user interface allows the user to manually edit sequences with the help of an integrated expert system. No sequence information up- or downstream of the repeat region is taken into consideration for *spa* type coding. However, the software searches for 5′ and 3′ signature sequences at the correct distance to ensure that no leading or ending repeat is missed. Once sequence editing is finished, the *spa* typing results and additional epidemiological relevant data can be stored in a relational database system. Information from the database can be easily retrieved by Boolean searches and exported in tab-delimited spreadsheet format. Furthermore, the database's integrity is checked on a regular basis and the database content can be backed up to protect from data leakage. For privacy,

TABLE 1. Comparison of the two study periods

Study period	Duration (mo)	No. of MRSA isolates	No. of patients	No. of staff members	No. of *spa* types	No. of unique *spa* types	Predominant *spa* types	No. of departments involved	No. of wards involved	No. of outpatient clinics	No. of clusters[a]	No. of wards involved in clusters	No. of patients involved in clusters	*spa* types involved in clusters
1	10	107	106	1	17	7	1, 3	13	29	7	11	7	33	1, 3, 5, 8, 10, 13
2	4	84	82	2	14	9	3, 23	14	29	6	8	6	19	1, 3, 23

[a] A cluster was defined as the identification, within 9 days, of two or more patients on the same ward who harbored MRSA strains with the same *spa* type.

the content is cryptographically secured. Finally, different configurable reports can be created. These reports are stored internally as read-only encrypted tamperproof pdf files. To view and print these files, the freely available Adobe Acrobat Reader software (version 5.0 or higher) must be installed.

Numeric *spa* repeat and type codes are used by Ridom StaphType. To ensure uniform code terminology usage, the software synchronizes either directly via the http protocol or file based (e.g., via e-mail) with an accompanying website that functions as the operative source for all new *spa* repeat and type codes. If wished, all new *spa* repeats and types that meet quality criteria (i.e., *spa* types are deduced from chromatograms and 5′ and 3′ signatures are unambiguously detected) can be transferred during synchronization to the server to obtain a final designation. In exchange, repeats and types detected by others since the last synchronization are transferred to the Ridom StaphType client software. Furthermore, if allowed by the user, the local *spa* type frequencies are also transmitted to the server, which always returns global frequency data. The website (http://www.ridom.de/spaserver/) is accessible to everyone, and *spa* repeat sequences (FASTA format) and *spa* types can be downloaded. Submission of chromatograms of new *spa* repeats and types for inclusion in the reference database is possible. Therefore, users not working with Ridom StaphType have access to the same uniform terminology.

RESULTS

Design of the study. MRSA strains were collected at the University of Würzburg during two study periods (Table 1). In both periods, the number of isolates and the number of departments and wards contributing strains were comparable. There was a twofold difference in the MRSA isolation rate per month between the two study periods, which could not be explained by changes in culture submission criteria or MRSA screening procedures (Table 1). For *spa* type designation, we used newly developed software (Ridom StaphType; for a detailed description, see Materials and Methods).

TABLE 2. *spa* types

spa type	Repeats[a]
01	r26r30r17r34r17r20r17r12r17r16
02	r26r23r17r34r17r20r17r12r17r16
03	r26r17r20r17r12r17r17r16
04	r09r02r16r13r13r17r34r16r34
05	r26r23r13r23r31r05r17r25r17r25r16r28
06	r26r23r13r23r31r05r17r17r25r16r28
08	r11r19r12r21r17r34r24r34r22r25
09	r11r12r21r17r34r24r34r22r24r34r22r33r25
10	r26r17r34r17r20r17r12r17r16
11	r08r16r02r25r34r24r25
12	r15r12r16r02r16r02r25r17r24r24
13	r26r30r17r34r17r20r17r12r17
14	r26r17r20r17r12r17r17r17r16
16	r26r23r13r23r31r05r17r25r16r16r28
17	r15r12r16r16r02r16r02r25r17r24r24
18	r15r12r16r02r16r02r25r17r24r24r24
19	r08r16r02r16r02r25r17r24
20	r26r23r31r29r17r31r29r17r25r17r25r16r28
23	r26r37r17r12r17r16
24	r11r12r21r17r34r24r34r22r25
25	r26r23r23r13r23r29r17r31r29r17r25r17r25r16r28
26	r08r16r34
27	r26r17r13

[a] A numerical code was chosen for Ridom StaphType. A comprehensive collection of *spa* types and repeat sequences is available at http://www.ridom.de/spaserver/.

Typing of MRSA. Seventeen and 14 *spa* types were observed in study periods 1 and 2, respectively (Tables 1 and 2). There were 10 *spa* types that were found only once in all of the 191 strains analyzed (Fig. 2). Five of those occurred in the first period, and five occurred in the second. This finding indicates either permanent import of novel *spa* types or in-house microevolution of *spa* repeats. Microevolution of the *spa* gene was evident for several *spa* types, e.g., deletion-insertion events in *spa-8* and *-9*, and repeat exchange in *spa-1* and *-23* (Table 2). The rank abundance curve in Fig. 2 shows that *spa-1* and *-3* dominated in period 1, whereas *spa-1* was replaced by *spa-23* in period 2. This observation indicates that the circulation of epidemic clones within a hospital is not static but a matter of short-term changes.

The epidemic strains were analyzed further by MLST, antimicrobial resistance patterns, hemolysis, and PGFE. Three strains each of *spa-1*, *-3*, and *-23* were subjected to MLST. All were derivatives of the ST-5 complex. *spa-1* and *-23* were associated with ST-228 (double-locus variant of ST-5; ST-228 strains belong to the southern German epidemic clone) (8). Allele *spa-3*, which was present in both periods, was associated with ST-225. ST-225 is a single-locus variant of ST-5 (EMRSA-3, New York clone; German designation, Rhine-Hesse clone) (8). We also performed MLST on two of five strains with *spa-5* because this *spa* type was exclusively isolated

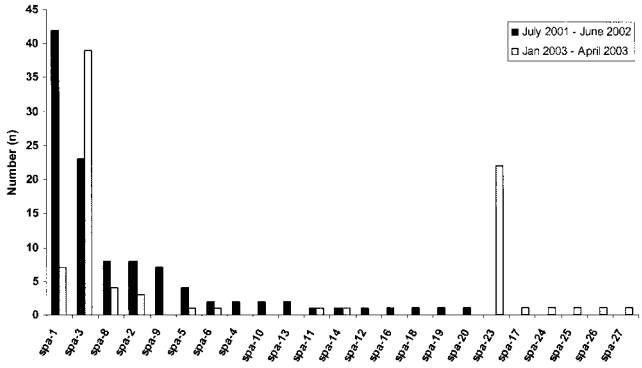

FIG. 2. Rank abundance graph demonstrating the frequencies of *spa* types collected during two study periods.

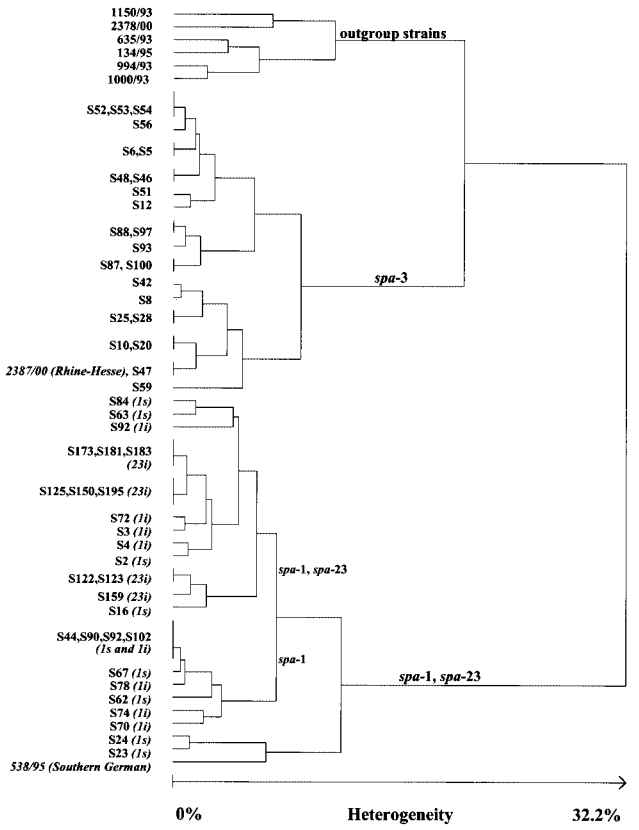

FIG. 3. Dendrogram deduced from the cluster analysis of PFGE macrorestriction patterns of *spa-1*, *-3*, and *-23* strains as determined by the algorithm described by Claus et al. (1). Strains from this study are designated by the letter S followed by a strain number. Additional information was added to *spa-1* and -23 strains as follows: *1s*, *spa-1* and low mupirocin MIC; *1i*, *spa-1* and high mupirocin MIC; *23i*, *spa-23* and high mupirocin MIC. As a control, patterns determined previously from diverse other clones at the German reference laboratory for staphylococci were included for cluster analysis (outgroup strains, i.e., 1150/93, Berlin epidemic MRSA; 2378/00, Barnim epidemic MRSA; 635/93, Vienna epidemic MRSA; 134/93, northern German epidemic MRSA; 1000/93, Hannover epidemic MRSA).

at a surgical department located outside the campus. MRSA with *spa-5* were shown to be ST-22, which corresponds to EMRSA-15 or the Barnim clone (8, 17, 33).

All 60 *spa-3* isolates tested for gentamicin susceptibility were gentamicin susceptible, whereas a total of 70 *spa-1* and *spa-23* isolates tested were gentamicin resistant. Elevated MICs of mupirocin were only observed for *spa-1* and *-23* strains. For *spa-23* strains, the median mupirocin MIC was 24 μg/ml (range, 16 to 48 μg/ml). With regard to mupirocin resistance, two populations of *spa-1* isolates were observed, one for which the MICs were high (median, 28 μg/ml; range, 16 to 48 μg/ml; $n = 14$) and one for which the MICs were low (median, 0.25 μg/ml; range, 0.064 to 0.38 μg/ml; $n = 35$). Forty-nine (79%) of 62 *spa-3* isolates exhibited hemolysis on blood agar plates, whereas hemolysis was observed in only 9 (13%) of a total of 71 *spa-1* and *-23* isolates.

We further analyzed a selection of *spa-1*, *-3*, and *-23* strains by PFGE. A dendrogram deduced from the cluster analysis of PFGE macrorestriction patterns is shown in Fig. 3. *spa-3* strains were clearly distinguished from *spa-1* and *-23* strains by PFGE. This finding was in accordance with those based on MLST, gentamicin resistance, and hemolysis. As expected, *spa-3* isolates were grouped together with Rhine-Hesse epidemic strain 2387/00 and *spa-1* and *-23* strains were grouped together with southern German epidemic strain 538/95. Fifteen PFGE patterns were detected for the *spa-3* group of strains, and 20 patterns were detected for the *spa-1*–*spa-23* group of strains. This finding confirmed the higher resolution of epidemic strains by PFGE compared to *spa* typing. There was no clear distinction between *spa-1* and *-23* strains by PFGE, underlining their relatedness. Furthermore, strains for which the mupirocin MICs were low could not be distinguished from strains for which the mupirocin MICs were high.

Local epidemiology of MRSA. Table 3 demonstrates the frequent detection of epidemic *spa* types in different departments during the second study period. It is noteworthy that there were six incidences of detection of different MRSA types within 1 week from patients taken care of at the same ward. This finding indicates that simultaneous but independent transmission events are not rarely encountered, which supports the use of *spa* typing in a university hospital setting even in periods of a high frequency of isolation of epidemic strains.

DISCUSSION

We present a novel tool for rapid determination of *spa* repeats in *S. aureus*. An important feature of this software is automated data submission via the Internet. Thus, the server can be used to collate and harmonize data from various geographic regions. Furthermore, DNA sequences are automatically subjected to quality control. This feature greatly facilitates the implementation of centralized servers since data need not to be checked by a curator. The software is designed in a way that it can be adapted to single-locus typing schemes relevant to other genetically variable pathogens responsible for nosocomial infections, e.g., vancomycin-resistant enterococci, for which MLST proved to be highly discriminatory (16, 21). In order to simplify *spa* type nomenclature, a numerical repeat code was established in this study. This approach was chosen despite the current existence of an alpha-numerical repeat nomenclature because numerical codes are now widely used for MLST and because Ridom StaphType is the first Internet-based tool available for assignment of *spa* types. This tool now provides the opportunity to harmonize *spa* type designations.

It has long been established that *spa* typing is less discriminatory than PFGE (26, 29). Tang et al. showed that 20 strains with the same *spa* type that were collected during an outbreak that lasted 107 weeks exhibited several related but distinguishable PFGE patterns. This finding corresponds to the PFGE analysis performed in this study. The significance of subtle changes of one band in the PFGE patterns of related strains, e.g., with allele *spa-3*, may be a matter of debate. In *N. meningitidis*, subtle changes in PFGE patterns could be identified if the nasopharyngeal and clonally identical blood isolates of a patient were compared or if the isolate of a patient and that of the clonally identical one of the closest contact were compared (30; U. Vogel, H. Claus, and M. Frosch, Letter, N. Engl. J. Med. **342:**219–220, 2000). In a study on *S. aureus* carriage, several nasal isolates did not differ from clonally identical iso-

TABLE 3. *spa* types isolated at the university hospital in weeks 1 to 18 of 2003 (study period 2)

Department[a]	*spa* type(s) isolated during wk:																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
A	3			23			3	3, 3			3	3, 3	3, 3					
B1																		27
B2													3					
B3												2, 3						
C1	3, 3					3		3, 23										
C2																	26	
C3						17		3	23		23	23				23		
C4										3						23		
C5		23																
C6		23				3								23	3			
C7							3	3			23, 3			1, 1	23		23	
C8			3			24												
C9																3		
D									11									
E		8							2		6	3				8		
F														14				
G											8							
H1								3										
H2					23													
H3									3									
H4									1	3								
H5				3, 1	1			23, 3	3						25			
H6																3		
H7				2														
I1											23							
I2														1				
J1																8		
K1													23					
K2													23		23			
K3													23					
L1																		3
L2						3						3	3				3	
M1		5																
M2			3, 23		23		3											

[a] Capital letters represent departments of the hospital, and numbers represent different wards.

lates consecutively obtained from the blood, which might indicate a higher stability of PFGE patterns in *S. aureus* than in *N. meningitidis* (31). Peacock et al., on the other hand, in their study comparing PFGE and MLST, found four patients whose *S. aureus* isolates, which were recovered from the same patient, differed by one or two bands. This finding suggests that differences in a single PFGE band may be considered hyperdiscriminatory (25). Despite being less discriminatory than PFGE, *spa* typing will certainly help to disprove epidemiological linkage between MRSA-colonized persons in periods with a high incidence of epidemic strains, as shown for several incidences in Table 3.

We are intuitively aware that the isolation of two MRSA strains with the same *spa* type is highly suggestive of person-to-person transmission if there is a low incidence of MRSA isolation. However, wards with a high rate of epidemic *spa* type isolation will suffer from uncertainties about the possibility of direct transmission. It will therefore be desirable to develop algorithms on the basis of *spa* types and their local isolation frequencies to assess the probability of person-to-person transmission each time two or more MRSA strains with the same *spa* type are recovered. Furthermore, novel DNA-based typing schemes might increase resolution within epidemic *spa* types. A possible candidate fulfilling this requirement might be the clumping factor B (*clfB*) gene, which was recently reported to be a highly stable marker detecting differences between strains with the same *spa* type (L. Koreen, S. Ramaswamy, S. Naidich, E. A. Graviss, and B. Kreiswirth, Abstr. 103rd Gen. Meet. Am. Soc. Microbiol., abstr. C-415, 2003).

We determined by MLST the sequence types of dominant

spa types isolated at the hospital during two study periods. *spa-1* and *-23* turned out to be linked with ST-228, which resembles the southern German clone, a multiresistant clone that spread all over Germany (32). *spa-1* was found predominantly in period 1, whereas *spa-23* expanded at the hospital in period 2. This finding indicates that the allele *spa-23* either derived from pre-existing *spa-1* strains by *spa* gene repeat replacement or entered the hospital as a novel clone after the first study period. The first hypothesis is supported by the finding of highly related PFGE patterns of *spa-1* and *-23* strains (Fig. 3). A similar clonal dynamics has been recently shown by de Sousa et al., who reported a steady decline at a single hospital of ST-30 strains, which were replaced by ST-239 strains (4). In our study, type *spa-1* and *-23* isolates, in contrast to type *spa-3* isolates, were mostly nonhemolytic and resistant to gentamicin. Furthermore, elevated mupirocin MICs were observed only for *spa-1* and *-23* isolates. These findings provide further support for a close relationship between *spa-1* and *-23*. Interestingly, there were two *spa-1* populations with regard to mupirocin sensitivity, a sensitive one and another for which the MICs ranged from 16 to 48 μg/ml. One might speculate that *spa-23* strains, which dominated in the second study period, evolved from the mupirocin-resistant subset of the *spa-1* population. However, PFGE provided no evidence supporting this suggestion.

Both of the epidemic sequence types identified at the hospital were derivatives of ST-5, i.e., ST-225, a single-locus variant of ST-5, and ST-228, a double-locus variant. ST-5, ST-225, and ST-228 have been assigned to clonal complex 5 (8). The close relationship of ST-5 and ST-225 was also shown by PFGE in our study, which clearly identified the ST-225 strains from the hospital as derivatives of the Rhine-Hesse clone, which is prevalent in Germany and was shown to be ST-5 (W. Witte and M. Enright, unpublished observation). In the MLST database (http://www.mlst.net/dbqry/saureus.htm), there is only a single ST-225 isolate, which was collected in the United States (strain cdc12). A more detailed picture of the microevolution of clonal complex 5 isolates circulating at the hospital might be achieved, e.g., by determination of SCCmec types (8, 22). According to these data, *S. aureus* with the ST-5 genomic background had acquired different types of SCCmec.

It was of interest that *spa-5* strains (ST-22) circulated exclusively at a surgical department located outside of the campus. Although the patients there are in close contact with the university hospital, e.g., for postoperative care, these ST-22 strains did not start to circulate in other departments. Interestingly, ST-22 strains (EMRSA-15) have been shown to be ubiquitous and frequently cause outbreaks (17, 33). It may be that the *spa-5* strains in that particular department have been adapted to the special cohort of cancer patients treated there. Reasons for such behavior are unclear, but adaptation processes of MRSA have been demonstrated, e.g., for community-acquired MRSA strains causing skin infections (6).

In conclusion, we reported on two phases of surveillance of MRSA by *spa* typing. The method was valuable for tracking of epidemic isolates, elucidation of the rate of import of sporadically occurring clones, and disproof of person-to-person transmission in hospitals with high rates of epidemic MRSA infection. The presentation of software for automatic repeat identification, together with an accompanying Internet database, will help to disseminate *spa* typing to a larger community. Future comparative studies will be greatly facilitated by this, and national and international surveillance of MRSA will be supported.

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