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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*

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The aim of this study was to compare the recently developed typing approach for methicillin-resistant *Staphylococcus aureus* (MRSA) based on the DNA sequencing of the protein A gene polymorphic region (*spaA* typing) with a combination of three well-established molecular typing techniques: *Cla*I-*mecA* vicinity polymorphisms, *Cla*I-Tn*554* insertion patterns, and *Sma*I pulsed-field gel electrophoresis (PFGE) profiles. In order to evaluate the applicability of this typing technique in different types of studies, two groups of MRSA clinical isolates were analyzed: a collection of 185 MRSA isolates circulating in Hungary recovered from 17 hospitals in seven cities during a 3-year period (1994 through 1996), and a selection of 53 MRSA strains isolated in a single hospital in Hungary between 1997 and 1998. The 238 MRSA clinical strains from Hungary were first classified in clonal types (defined as *Cla*I-*mecA*::*Cla*I-Tn*554*::*Sma*I-PFGE patterns), and 65 of the 238 strains, representing major MRSA clones and some sporadic clones, were further analyzed by *spaA* typing. Our results showed that the lineages most recently introduced in the hospital setting showed little variability in *spaA* types, whereas the MRSA clones circulating for a longer period of time and spread among several hospitals showed a higher degree of variability. The implementation of the *spaA* typing method was straightforward, and the results obtained were reproducible, unambiguous, and easily interpreted. This method seems to be adequate for outbreak investigations but should be complemented with other techniques in long-term surveillance or in studies comparing distant clonal lineages.

Three molecular typing techniques (5) have been largely used for the characterization of clones of methicillin-resistant *Staphylococcus aureus* (MRSA) and enabled the detection of widely spread MRSA lineages, such as the Iberian, Brazilian, New York/Tokyo, and pediatric MRSA clones (1, 7, 11, 17, 20, 21, 24). The combined methods consist of (5, 10) Southern blot analysis of chromosomal *Cla*I digests with a *mecA* DNA probe (*Cla*I-*mecA* polymorphisms) and with a Tn*554* transposon probe (*Cla*I-Tn*554* insertion patterns) and restriction fragment length polymorphism analysis of chromosomal DNA generated after cleavage with *Sma*I and pulsed-field gel electrophoresis (PFGE) (*Sma*I-PFGE). *Cla*I-*mecA* polymorphisms are a consequence of the variability in the vicinity of the *mecA* gene, the central element of methicillin resistance, and *Cla*I-Tn*554* patterns reflect the location and copy number of the transposon Tn*554*, present in most MRSA clinical isolates (10). PFGE provides fine fingerprinting of the chromosomal background with high discriminatory power and has been suggested as the gold standard for the molecular typing of MRSA (25, 26).

DNA sequencing-based typing techniques are being developed with obvious advantages in speed, unambiguous data interpretation, simplicity of large-scale database creation, and standardization among laboratories (8). Recently, DNA sequencing of the *spaA* gene (protein A determinant) polymorphic region for typing of MRSA strains was evaluated (22). The polymorphic X region is involved in attachment to the cell wall and consists of a variable number of 24-bp repeats, short sequence repeats, which seem to arise from deletion and duplication of the repetitive units and also by point mutation (9, 27). The existence of well-conserved regions flanking the X region coding sequence in *spaA* allows the use of primers for PCR amplification and direct sequence typing. Shopsin and colleagues (22) have shown that, despite its high degree of polymorphism, the X region of protein A has a variation rate low enough to provide suitable discrimination for outbreak investigations or strain collections restricted to one location and recovered within a short period of time.

In this study we evaluated the discriminatory power of *spaA* typing to differentiate MRSA clones and assessed the correlation between this sequencing typing method and the combined molecular typing methods *Cla*I-*mecA*, *Cla*I-Tn*554*, and *Sma*I-PFGE patterns referred to above. Using these three combined methods, we have studied two groups of MRSA strains with different characteristics recovered from Hungarian hospitals: a representative collection of MRSA strains circulating in Hungarian hospitals during a 3-year period (1994 to 1996) and a collection of MRSA isolates recovered from a single hospital

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during a 2-year period (1997 to 1998). Fifty-six strains representing the most important clones spread in Hungarian hospitals between 1994 and 1998 and also some sporadic clones were further analyzed by *spaA* typing to analyze the correlation between clonal types defined as *Cla*I-*mecA* polymorphisms ::*Cla*I-Tn*554* patterns::*Sma*I-PFGE profiles and *spaA* types.

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MATERIALS AND METHODS

Clinical isolates. The 238 MRSA clinical isolates from Hungary included in this study comprise (i) a collection of 185 isolates recovered between 1994 and 1996 at 17 different hospitals located in seven different cities, which were chosen to be representative of MRSA epidemic clones circulating in Hungarian hospitals, and (ii) 53 isolates recovered at a single hospital from January to October 1997 and from January to December 1998, chosen to illustrate a short-term type of study. Antibiograms were performed by the clinical laboratories using the Kirby-Bauer technique, according to the published recommendations and definitions (14). The panel of antibiotics was different from hospital to hospital, but the great majority of the isolates were multiresistant to the antimicrobial agents tested, such as penicillin, oxacillin, erythromycin, tetracycline, ciprofloxacin, and gentamicin. All isolates were susceptible to vancomycin and teicoplanin.

Molecular typing. *Cla*I-*mecA* polymorphism, *Cla*I-Tn*554* insertion pattern, and *Sma*I-PFGE profile analyses were performed and interpreted as previously described (3, 5, 10, 26).

***spaA* typing.** *spaA* typing was performed essentially as previously described (22). Chromosomal DNA for PCR was prepared (2) and diluted to approximately 0.5 ng/μl. Primers for amplification and sequencing of the X region of the *spaA* gene were designed based on the published sequence (accession no. J01786) and purchased from Gibco-BRL (Life Technologies, Grand Island, N.Y.): SpaF1, GAC GAT CCT TCG GTG ACG, nucleotides 1096 to 1113, and SpaR1, CAG CAG TAG TGC CGT TTG C, nucleotides 1534 to 1516. PCR amplification was performed in a GeneAmp PCR System 9600 thermocycler (Perkin-Elmer Cetus [PE], Branchburg, N.J.), with 2.5 ng of DNA, 5 μl of 10× PCR buffer II (PE), 4 μl of deoxynucleoside triposphate mix at 10 mM (PE), 2.5 U of AmpliTaq Gold DNA polymerase (PE), 1.5 mM $MgCl_2$ (PE), and 10 mmol of each primer in a final reaction volume of 50 μl in 0.2-ml PCR tubes (PE). Thermal cycling parameters were as follows: predenaturation for 10 min at 95°C; 30 cycles of 95°C for 30 s, 60°C for 30 s, 72°C for 45 s, postextension for 10 min at 72°C; and soaking at 4°C. PCR products (2 μl) were visualized by conventional minigel electrophoresis and purified with the Wizard PCR-prep DNA purification system (Promega, Madison, Wis.). DNA cycle sequencing reactions were performed with the ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction Kit (PE) in a final reaction volume of 10 μl: 1 μl of amplified and purified DNA (20 to 30 ng); 2 μl of SpaF1 or SpaR1 at 2 pmol/μl; 4 μl of Ready Reaction mix; and 3 μl of H_2O MilliQ. Amplification parameters were as follows: 25 cycles of 96°C for 10 s, 50°C for 5 s, and 60°C for 4 min, and soaking at 4°C. DNA was precipitated at room temperature for 15 min with 50 μl of 95% ethanol–10 μl of H_2O MilliQ–2 μl of 3 M sodium acetate (pH = 4.6), centrifuged in a microcentrifuge for 20 min at 13,000 rpm, washed with 250 μl of 70% ethanol, centrifuged for 10 min at 13,000 rpm, and dried for 1 min at 90°C. DNA sequences were determined by electrophoresis in an ABI Prism 377 DNA sequencer (PE) according to the manufacturer's instructions at the DNA sequencing facility located at Instituto Gulbenkian de Ciência, Oeiras, Portugal. The assembly of both sequences was performed with SeqMan software (DNAStar software package; Lasergene, Madison, Wis.). Consensus sequences were sought for the previously defined 24-bp repeat polymorphisms (22), using specific software (GeneSearch, designed by Ludwig Krippahl). The output (*spaA* type) consists of a sequence of letters that correspond to the succession of the different 24-bp repeats within the polymorphic region of the *spaA* gene.

RESULTS

Clonal analysis of Hungarian MRSA. Tables 1 and 2 summarize the clonal types found in the 1994 to 1996 and 1997 to 1998 collections, respectively. The clonal types were compared to those of a previous study in which the application of these techniques to a collection of 48 MRSA clinical isolates recovered from six provincial hospitals located hundreds of kilometers apart in Hungary between 1993 and 1994 demonstrated the existence of a unique epidemic MRSA clone, the Hungarian MRSA clone (clone III::B::A), which was present in 81% of the isolates (6). In the present study, this lineage, characterized by PFGE pattern A, was still present in both collections, but its prevalence had decreased to 70% in 1994 to 1996 and its variability, expressed by the panoply of PFGE subtypes (37 subtypes were found for PFGE pattern A), *Cla*I-*mecA* types, and *Cla*I-Tn*554* insertion patterns, had increased, as shown by the presence of clonal type III::B::A in only 33% of the isolates in 1994 to 1996. The observed variability in the *Cla*I-*mecA* polymorphisms (patterns III, IX, XI, and III′) was characterized by small shifts in the hybridization fragment size of the *mecA* downstream vicinity which were detected in strains isolated after 1993 and that were recently explained (16) as being caused by different copy numbers of the direct repeat unit (*dru*) within the hypervariable region downstream of *mecA* (19). Pattern IX has 11 *dru* copies, pattern XI has 10 copies, pattern III has 9 copies, and pattern III′ has 8 copies (16). Therefore, it is reasonable to consider that clonal types III::B::A, IX::B::A, XI::B::A, and III′::B::A are equivalent, and altogether they accounted for 42% of the clonal types in 1994 to 1996. *Cla*I-Tn*554* insertion patterns are a consequence of the location and copy number of a transposon, by definition a mobile element, and within the PFGE pattern A cluster as many as 16 different insertion patterns were found, all characterized by the presence of multiple copies of transposon Tn*554*. However, since PFGE is the technique with the highest discriminatory power, strains sharing PFGE pattern A were classified as closely related and accounted for 70% of the clonal types in 1994 to 1996 and 40% in the 1997 to 1998 collection. This high degree of variability within an MRSA clone has never been reported before and seems to be a particular characteristic of the Hungarian clone. It is not observed, for example, in the highly epidemic Iberian and Brazilian clones (H. de Lencastre, unpublished observations).

In the 1994 to 1996 collection, a new family of clones were found in Hungarian hospitals, PFGE pattern D-related clones II::q::D, II::A1::D, and II::D::D (15% of the isolates), which were also detected in 9% of the isolates recovered from the single hospital studied in 1997 to 1998. In this same hospital in 1997 to 1998, a previously undetected lineage (II::E1::S) was present in 28% of the isolates. Both lineages showed significantly less variability than PFGE pattern A-related clones, which may be explained by its more recent introduction in Hungarian hospitals and, in the case of clone II::E1::S, by the fact that it was detected in isolates from a single hospital. These lineages do not appear (by the typing methods used) to be related to other MRSA clones spread in other countries and previously identified in this laboratory. Figure 1 shows some subtypes of the most important PFGE patterns found in this study, PFGE patterns A, D, and S.

***spaA* typing.** Fifty-six strains representing the most important clonal types detected in Hungarian hospitals in 1994 to 1996 and 1997 to 1998 were studied by *spaA* typing. For each lineage (defined according to PFGE patterns A, D, and S), several strains with different *Cla*I-*mecA* and *Cla*I-Tn*554* patterns were selected from different hospitals, cities, and periods of isolation. Nine strains belonging to sporadic clones were

TABLE 1. Molecular typing of 185 MRSA clinical strains isolated between 1994 and 1996 at 17 hospitals located in seven cities in Hungary

Clone type	City (county) or county	Yr of isolation	No. of isolates	Clonal type[a]	Total no. of isolates (% of total)
Epidemic A	Szekesfehervar (Fejer)	1994, 1996	12	III::B::A	61 (33.0)
	Dunaujvaros (Fejer)	1994	3		
	Debrecen (Hajdu)	1994	2		
	Miskolc (Borsod-A. Z.)	1996	15		
	Somogy County	1994, 1996	22		
	Budapest	1995, 1996	7		
	Dunaujvaros (Fejer)	1996	9	III′::B::A	12 (6.5)
	Debrecen (Hajdu)	1994	2		
	Budapest	1995	1		
	Somogy County	1994, 1996	2	IX::B::A	2
	Debrecen (Hajdu)	1994	1	XI::B::A	2
	Miskolc (Borsod-A. Z.)	1995	1		
	Miskolc (Borsod-A. Z.)	1995	1	III::M::A	26 (14.1)
	Somogy County	1996	6		
	Budapest	1995	19		
	Dunaujvaros (Fejer)	1996	3	III′::M::A	4 (2.1)
	Budapest	1995	1		
	Miskolc (Borsod-A. Z.)	1995	3	III::W::A	4 (2.1)
	Somogy County	1996	1		
	Budapest	1995	1	III::DD::A	
	Budapest	1995	1	III::F::A	
	Budapest	1996	2	III::A::A	
	Budapest	1996	1	III::B2::A	
	Budapest	1996	1	III::k::A	
	Budapest	1996	1	XI::k::A	
	Miskolc (Borsod-A. Z.)	1995	1	III::W1::A	
	Miskolc (Borsod-A. Z.)	1995	1	III::i::A	
	Miskolc (Borsod-A. Z.)	1995	1	III::m::A	
	Miskolc (Borsod-A. Z.)	1995	1	III::n::A	
	Miskolc (Borsod-A. Z.)	1995	1	III::t::A	
	Miskolc (Borsod-A. Z.)	1996	1	III::λλ::A	
	Somogy County	1994	1	IX::E::A	
	Somogy County	1994	1	III::i::A	
	Somogy County	1996	1	XI::B1::A	
	Somogy County	1996	1	III::M1::A	
	Szekesfehervar (Fejer)	1996	1	III::M::A	
	Szekesfehervar (Fejer)	1996	1	III::i::A	
	Total				130 (70.2)
Epidemic D	Budapest	1994, 1996	18	II::q::D	18 (9.7)
	Somogy County	1994	1	II::D::D	9 (4.9)
	Budapest	1995, 1996	8		
	Budapest	1996	1	II::A1::D	1
	Total				28 (15.1)
Sporadic	Budapest	1995	1	III::B::Q	
	Budapest	1995	1	II::A1::R	
	Budapest	1996	4	II::A::E	
	Budapest	1996	1	II::D::E	
	Budapest	1995	1	XI::B2::O	
	Budapest	1996	2	VI::F::P	
	Miskolc (Borsod-A. Z.)	1995	1	III::W::H	
	Miskolc (Borsod-A. Z.)	1996	1	III::B::B	
	Miskolc (Borsod-A. Z.)	1996	1	III::W::B	
	Miskolc (Borsod-A. Z.)	1996	1	III′::M1::F	
	Miskolc (Borsod-A. Z.)	1996	1	III′::αα::G	
	Szekesfehervar (Fejer)	1994	1	III::B::M	
	Szekesfehervar (Fejer)	1994	1	III::j::M	
	Szekesfehervar (Fejer)	1994	2	III::B::N	
	Szekesfehervar (Fejer)	1994	1	III::j::N	
	Szekesfehervar (Fejer)	1996	1	III::B1::B	
	Dunaujvaros (Fejer)	1994	1	III::n::B	
	Dunaujvaros (Fejer)	1994	1	IX::B::K	
	Somogy County	1996	1	III::B::I	
	Somogy County	1996	1	III::x::J	
	Debrecen (Hajdu)	1994	1	III::p::L	
	Szombathely (Vas)	1994	1	III::M::N	
	Total				27 (14.6)

[a] Clonal types were defined on the basis of *Cla*I-*mecA* polymorphisms::*Cla*I-Tn*554* patterns::*Sma*I-PFGE restriction profiles. A total of 185 strains were examined. *Cla*I-*mecA* polymorphisms with a prime and *Cla*I-Tn*554* insertion patterns with number codes (e.g., B1) contain small variations from previously described patterns. *Cla*I-Tn*554* insertion patterns with lowercase letters are new patterns not described in previous studies.

TABLE 2. Molecular typing of 53 MRSA clinical strains isolated from a single hospital in Hungary between 1997 and 1998

PFGE pattern type	Yr of isolation	No. of isolates	Clonal type[a]	Total no. of isolates (% of total)
A	1997	7	III::M1::A	9 (17.0)
	1998	2		
	1997	1	III::B2::A	5 (9.4)
	1998	4		
	1997	2	III::B1::A	6
	1998	2	XI::B::A	
	1998	1	III′::B3::A	
	1998	1	III′::q::A	
	Total			20 (37.8)
D	1997	4	II::q::D	5
	1997	1	II::q1::D	
	Total			5 (9.4)
S	1997	12	II::E1::S	15 (28.3)
	1998	3		
Varied	1997	2	III′::B2::B	13 (24.5)
	1997	1	III::B2::B	
	1997	1	VIII::aa::M	
	1997	1	III::M4::T	
	1997	1	III::M1::Y	
	1998	1	III′::n::B	
	1998	2	I::NH::W	
	1998	1	III::B2::N	
	1998	1	VII::ρ1::V	
	1998	1	V::W1::X	
	1998	1	III::ββ1::Z	
	Total			13 (24.5)

[a] See Table 1, footnote *a*.

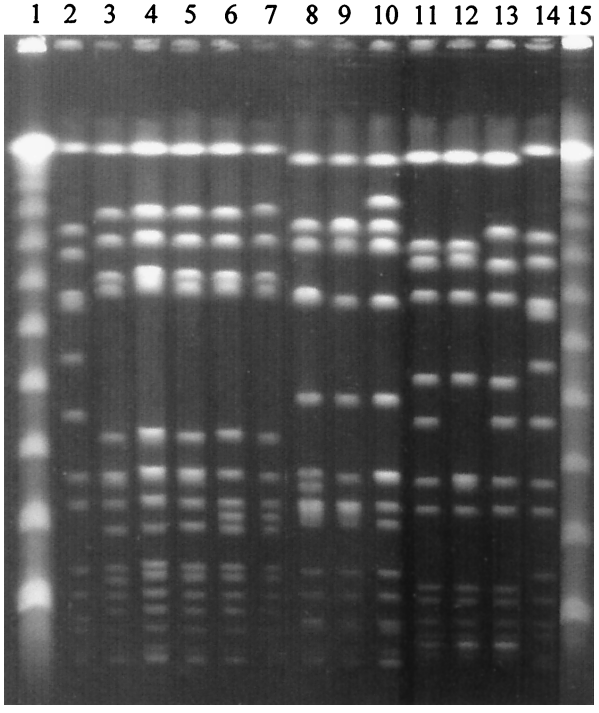

FIG. 1. Representative gel of some subtypes of the most frequent *Sma*I-PFGE profiles and one sporadic clone. Strain codes indicate the period of isolation: HUSA, 1993 to 1994 (4); HU, 1994 to 1996; and HUR, 1997 to 1998. Samples are as follows: lanes 1 and 15, molecular size markers (lambda DNA ladder; New England Biolabs); lanes 2 and 14, reference strain NCTC8325; lanes 3 to 5, pattern A1 (HUSA67, HU1, and HUR36); lane 6, pattern A36 (HU221); lane 7, pattern A37 (HUR1); lane 8, pattern D1 (HU181); lane 9, pattern D2 (HU164); lane 10, pattern D3 (HU150); lane 11, pattern S1 (HUR9); lane 12, pattern S4 (HUR95); lane 13, pattern S5 (HUR94).

also included. Nine different *spaA* types were found among the 65 five strains studied (Table 3).

Four related *spaA* types sharing the KAOMQ motif (*spaA* types WGKAKBAOKAOMQ, WGKAKAOKAOMQ, WGKA OKAOMQ, and XKAOKAOMQ) were found among the isolates belonging to PFGE pattern A-related clones. However two of these *spaA* types (WGKAKAOKAOMQ and WGKA OKAOMQ) were also found among strains belonging to the sporadic clones characterized by PFGE patterns B, T, and H.

The *spaA* type WGKAQAQQ was specific for the 12 isolates studied belonging to PFGE pattern D-related clones. The *spaA* type TIMBMDMGMK was specific for 9 of 10 isolates studied belonging to clonal type II::E1::S, and the other isolate was characterized by the related *spaA* type TIMBME. The remaining two *spaA* types, YHFGFMBQBLO and WGKAOMQ, were specific for strains belonging to the sporadic clones I::NH::W and III::ββ1::Z, respectively.

DISCUSSION

We have applied the recently developed MRSA typing technique based on DNA sequencing of the protein A gene polymorphic region, *spaA* typing (22), to characterize two distinct MRSA collections representing two different kinds of studies: a representative collection of MRSA clinical isolates circulating in Hungarian hospitals during a 3-year period and a collection of clinical strains recovered from a single hospital during a 2-year period, also in Hungary. *spaA* types were compared to the results obtained using other molecular typing techniques (*Cla*I-*mecA*, *Cla*I-Tn*554*, and *Sma*I-PFGE patterns) in order to evaluate the discriminatory power of *spaA* typing and its use to characterize MRSA clones circulating in a particular country or hospital. In addition, we wanted to assess the ease of implementation and execution of this method in our laboratory, since we have been interested on the molecular typing of clinical strains of MRSA, and therefore, *spaA* typing was potentially useful in our studies.

Implementation of *spaA* typing technique. The implementation of the *spaA* typing technique, essentially according to the published procedure (22), was straightforward and easy to establish in our laboratory, requiring only some expertise in PCR and DNA sequencing techniques, which are easily achieved with modern reagent kits, thermocyclers, and sequencers. To evaluate the reproducibility of the *spaA* types, four strains were typed twice, and in all cases the *spaA* types obtained were exactly the same. The stability of *spaA* types was also evaluated for one strain, which was daily diluted in fresh medium over a 3-week period (approximately 15×10^9 generations), and no changes in *spaA* type were detected. In order to make a first evaluation of *spaA* typing, a small collection of 20 MRSA isolates representative of well-characterized and internationally spread clones, such as the Iberian (7, 21), Brazilian (24),

TABLE 3. *spaA* typing of selected MRSA strains isolated in Hungary in 1994 to 1996 and 1997 to 1998

spaA type[a]	Clonal type[b] (no. of strains)
WGKAKBAOKAOMQ	III::B1::A (2), IX::B::A (2), III::M::A (1)
WGKAKAOKAOMQ	III::B::A (5), III′::B::A (2), III::B2::A (1), III′::B2::A (2), XI::B::A (1), III::M::A (2), III::M1::A (6), III::W::A (2), III::B′::B (1), III::N::B (1), III::W::B (1)
WGKAOKAOMQ	III::B::A (1), III′::B3::A (1), III::M::A (1), III′::q::A (1), V::W1::A (1), III::B::B (1), III::M4::T (1), III::M′::H (1)
XKAOKAOMQ	XI::B::A (2), III′::n::A (1)
WGKAQAQQ	II::q::D (7), II::q1::D (1), II::D::D (3), II::A′::D (1)
TIMBMDMGMK	II::E1::S (9)
TIMBME	II::E1::S (1)
WGKAOMQ	III::ββ1::Z (1)
YHFGFMBQBLO	I::NH::W (2)

[a] *spaA* types were assigned according to Shopsin and colleagues (22). Each letter corresponds to a different polymorphism of the 24-bp repeat; the sequence of letters correponds to the sequence of the 24-bp repeats within the *spaA* gene polymorphic X region.
[b] Clonal types were defined on the basis of *Cla*I-*mecA* polymorphisms::*Cla*I-Tn*554* patterns::*Sma*I-PFGE restriction profiles.

and New York/Tokyo (1, 11) clones, was tested, and it was found that *spaA* typing was able to discriminate among the different MRSA lineages: Iberian clone *spaA* type YHFG FMBQBLO; Brazilian clone *spaA* type XKAOMQ; and New York/Tokyo clone *spaA* type TJMBMDMGMK (Oliveira et al., unpublished data).

Comparison between *spaA* typing and other molecular typing techniques. In this study, *spaA* typing was excellent in discriminating the clonal lineages more recently introduced in Hungarian hospitals (PFGE pattern D clones and clone II::E1::S), which were characterized by the specific *spaA* types WGKAQAQQ and TIMBMDMGMK/TIMBME, respectively.

However, the isolates belonging to the clonal lineage characterized by PFGE pattern A, circulating in Hungarian hospitals at least since 1993, were characterized by a cluster of four *spaA* types with the KAOMQ motif, and two of these *spaA* types were also found among strains belonging to the sporadic clones characterized by PFGE patterns B, T, and H. These findings suggest that these sporadic clones (III::B::B, III::B′::B, III::W::B, III::M4::T, and III::M′::H) may have evolved from clone III::B::A, so that a less discriminative technique like *spaA* typing might not be able to differentiate them. This hypothesis is supported by the fact that these sporadic clones show the same *Cla*I-*mecA* and *Cla*I-Tn*554* types as PFGE pattern A-related clones. Moreover, another sporadic clone analyzed by *spaA* typing (clone I::NH::W), with nothing in common with clone III::B::A, displayed the unrelated and specific *spaA* type YHFGFMBQBLO, whereas the sporadic clone III::ββ1::Z (sharing *Cla*I-*mecA* type III) was characterized by *spaA* type WGKAOMO, with the KAOMQ motif. The variability of *spaA* types among the PFGE pattern A-related clones parallels the variability also detected by the other typing techniques, suggesting that the mutation rate of the *spaA* gene polymorphic region is comparable to the variability rate of *Cla*I-*mecA* polymorphisms, *Cla*I-Tn*554* insertion patterns, and PFGE subtypes.

The application of *spaA* typing also provided interesting clonal relationships among MRSA. The sporadic clone I::NH::W found in Hungary in 1998 (Table 2) showed exactly the same *spaA* type as the Iberian clone-related strains isolated since the mid-1980s and also strain DEN2125 isolated in Denmark in 1964 (results not shown), confirming the previous finding (4) that the Iberian clone, first described in Spain (7) and since then shown to be widely spread throughout Europe (12, 13, 21) and the United States (17, 18), may have in strain DEN2125 a evolutionary precursor. The I::NH::W clone may also be an Iberian clone derivative that has just been introduced in Hungary and will eventually disseminate among Hungarian hospitals. Analysis of Fig. 2, in which the PFGE profiles of these strains are displayed, showed that these strains do not

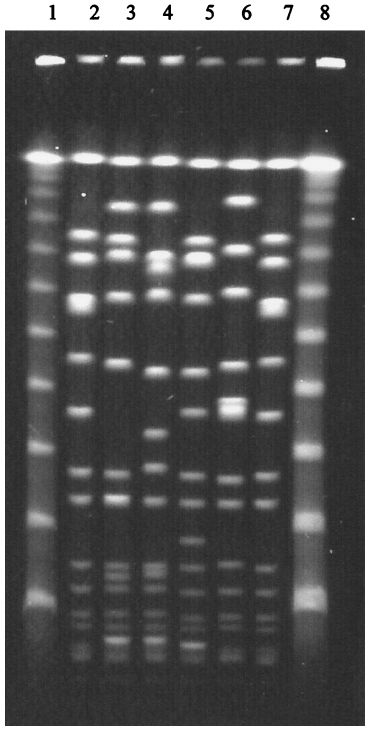

FIG. 2. *Sma*I-PFGE profiles of strains with the Iberian *spaA* type. Strains are as follows: lanes 1 and 8, molecular size markers (lambda DNA ladder; New England Biolabs); lanes 2 and 7, reference strain NCTC8325; lane 3, PER34 (Iberian clone representative strain [7]); lane 4, HUC191 (Iberian clone closely related strain [15]); lane 5, DEN2125 (archaic clone [1]); and lane 6, HUR97 (sporadic clone isolated in Hungary).

have the same PFGE pattern, although these patterns seem to be related (10 band differences). The stability in the *spaA* type of Iberian clone-related strains over a span of at least three decades contrasts with the variability found in the *spaA* type related to the Hungarian clone strains and was somehow unexpected, since the molecular basis for *spaA* type is precisely the polymorphic X region within the *spaA* gene. These findings suggest that there are highly stable alleles of the X region, presumably very efficient in determining pathogenesis and/or adherence mechanisms.

As previously suggested by Shopsin and colleagues (22) and also confirmed by Tang and colleagues (23), DNA sequencing of the protein A gene polymorphic region as a typing technique seems to be a powerful technique for MRSA typing. This is especially true for MRSA isolates restricted to one location and to a short period of isolation (that is, for outbreak investigations), as was shown in this study by the excellent discrimination of the clonal lineages more recently introduced in Hungarian hospitals (clones related to PFGE patterns D and S). *spaA* typing also had a satisfactory capacity for discrimination in more diverse collections, as illustrated in this study with the PFGE pattern A clonal lineage. However, in these cases, other typing techniques and a more careful interpretation of the *spaA* types may be needed. In our study, we found *spaA* types to be stable and reproducible, and the *spaA* typing technique was easy to implement and provided unambiguous results.

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