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STAR gene restriction profile analysis in epidemiological typing of methicillin-resistant *Staphylococcus aureus*: description of the new method and comparison with other polymerasechainreaction (PCR)-based methods

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Abstract

A method based on restriction profile analysis of the STAR repetitive element PCR (STAR-RP PCR) product obtained by digestion with *Alu*I and *Tru*9I was developed for typing methicillin resistant *Staphylococcus aureus* (MRSA). We evaluated a well defined collection of MRSA from Argentina, previously characterized by PFGE (pulsed field gel electrophoresis) of chromosomal *Sma*I digests and hybridization with DNA probes for probes *Cla*I-*mec*A and *Cla*I-Tn*554*. We comparatively evaluated STAR-RP analysis with other PCR based methods such as Inter IS*256*-PCR, Rep-MP3 PCR and Coa-RP. The discriminatory power (*D*) of STAR-RP (0.86) was similar to that of PFGE (0.84) at the type level. Comparable results were obtained with Inter IS*256* PCR (0.85) and Rep-MP3 PCR (0.80). A lower value (0.74) was obtained for Coa-RP. An excellent reproducibility (100%) of STAR-RP was observed. Good concordance between STAR-RP and other molecular typing methods was found for MRSA isolates ($n = 39$). STAR-RP typing showed 87% concordance with *mec*A::Tn*554*::PFGE, 87% with Inter IS*256* PCR and 71% with Rep-MP3 typing. STAR-RP is suggested as an adequate molecular typing assay for MRSA epidemiologic assessment.

Keywords: Methicillin-resistant *Staphylococcus aureus;* Molecular epidemiology; Molecular typing

1. Introduction

Staphylococcus aureus is one of the most relevant pathogens causing nosocomial infections, and the emergence of methicillin resistant *S. aureus* (MRSA) has become a major problem worldwide (Ayliffe et al., 1997; Blanc et al., 2001; Chambers, 1997; Leski et al.,1998; Roman et al., 1997; Townsend et al.,1987). Different MRSA clones have been described in diverse geographical locations. The international Brazilian clone, also called the South American clone, which was first detected in Brazilian hospitals (Teixeira et al., 1995), was subsequently found in other countries of South America (Argentina, Uruguay and Chile) and Europe (Portugal and the Czech Republic) (Aires de Sousa et al., 1998; Aires de Sousa et al., 2001; Corso et al.,1998; Da Silva Coimbra et al., 1999; Melter et al.,1999). In Argentina, the Brazilian clone is the most prevalent one in several cities, including Buenos Aires, Posadas, and Tucumán. More recently, a novel epidemic clone which coexists with the South American clone was recently described in hospitals of Córdoba (Argentina) (Solá et al., 2002). A second international MRSA clone, the Pediatric clone, recently described in pediatric children populations of Portugal, Colombia and USA, was also found in Argentina (Corso et al., 1998, Sá-Leao et al., 1999).

Monitoring and limiting intra and inter-hospital spread of MRSA strains require the use of efficient and accurate epidemiologic typing systems. A large number of DNA-based methods have been developed for typing MRSA strains. Pulsed-field gel electrophoresis (PFGE) analysis is a reliable and discriminatory method used by many hospitals and reference laboratories, but it is technically demanding and time consuming (Bannerman et al., 1995; Deplano et al., 1997; Tenover et al.,1995). When compared with PFGE

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analysis, PCR-based typing methods offer the advantages of rapidity and simplicity. In particular, repetitive-element PCR (rep-PCR) analysis based on multicopy elements of the staphylococcal genome has shown good reproducibility and discriminatory power in single center studies (Del Vecchio et al., 1995; Deplano et al., 1997, Van Der Zee et al., 1999). A new repetitive element called STAR has been identified in two intergenic regions in *S. aureus* (Cramton et al., 2000). Because this element was found in multiple copies and exhibited sequence diversity at the same locus, we suggest its use as a potential epidemiologic typing method. We described here the optimization and discriminatory abilities of this novel method for typing MRSA by using a well defined collection of strains, which were previously characterized by standard procedures (Corso et al., 1998).

2. Materials and Methods

2.1. Bacterial isolates

A collection of 148 MRSA was recovered from 11 different hospitals of Argentina over a period of several years (Corso et al., 1998). All isolates were previously characterized using the combination of three typing methods: *Cla*I *mec*A and *Cla*I *Tn*554 polymorphisms, and PFGE typing at the ITQB, Universidade Nova de Lisboa, Oeiras, Portugal (Corso et al., 1998). From this collection, two sets of isolates were selected. The first set included 24 epidemiologically unrelated isolates representative of the MRSA *S. aureus* population in Buenos Aires and vicinity hospitals (Table 1). The first 16 strains described in Table 1 represent different subtypes of the epidemic clones and included the most prevalent one in Argentina: the Brazilian clone (clonal type XI::B::B) together with its closely related variants (XI::B'::B and XI::AA::B), and two minor epidemic clones, namely II::NH::D and II::E::C. The remaining 9 isolates represent sporadic strains that were resolved into 8 clonal types by the combination of the three methodologies. The second set ($n = 14$) included epidemiologically related MRSA isolates from two outbreaks which occurred in Buenos Aires ($n = 9$) and Tucumán ($n = 5$) representing the Brazilian clone and one of its variants. Reference strains *S. aureus* NCTC 8325 and PER34, a representative strain of the Iberian clone were obtained from the ITQB collection and included as quality controls. Isolates were stored in Brain heart infusion (Difco Labs., Detroit, Mich.) medium with 20% glycerol at −20°C until used.

2.2. Preparation of genomic DNA

DNA was extracted according to the method of Hall et al., (1992), with a modification that consisted of the addition of lysostaphin to 5 mg/ml.

2.3. Rep-MP3 PCR typing

Rep-Mp3 PCR was carried out in 50 μL reaction volumes. Each reaction mixture contained 75 pmol of primer Rw3A (Van Der Zee et al., 1999), 1 U of *Taq* DNA Polymerase (Promega Corporation, Madison, Wis.), 2.5% dimethyl sulfoxide, 1.5 mM $MgCl_2$ and each deoxynucleoside triphosphate at a concentration of 200 μM in PCR buffer (20 mM Tris-HCl pH 8.0, 100mMKcl, 0.1 mM EDTA, 1 mM dithiothreitol (DTT), 50% glycerol, 0.5% tween 20 and 0.5% Nonidet P-40). Subsequently, 200 ng of genomic *S. aureus* DNA was added to the PCR mixture. Reactions were performed in a Perkin-Elmer Thermocycler 9600 under the following program: 3 min at 94°C, followed by 30 cycles of 1 min at 94°C, 1 min at 45°C, and 2 min at 72°C. The program ended with an additional 5 min extension step at 72°C.

2.4. STAR element-based PCR procedure

The intergenic sequences between *uvrA* and *hprk* were amplified using the primers described by Cramton et al. (2000). These were: 5′ GGTCCTGAAGGTGGTAGTGGCGGTGG and 3′ GGCTTCGATAATCCTTCTTCACCAGCG. One-hundred nanograms of DNA was added to the PCR mixture in a final volume of 50 μL containing 0.5 μM of each primer, 5 μL of 10X PCR buffer (see above), 200 μM each deoxynucleoside triphosphate, 2.5 mM of $MgCl_2$ and 1 U of Taq DNA polymerase (Promega). Each sample was heated at 95°C for 5 min, followed by 40 PCR cycles consisting of 30 sec at 95°C, 1 min at 62°C, and 1 min at 72°C. The primers utilized in this study allowed amplification of an expected 876 bp fragment (AF195958), which represents the STAR 2 product of the *S. aureus* 8325 control strain (Fig. 1 and Fig. 2A).

2.5. STAR restriction profile (STAR-RP) analysis

Ten microliters of the solution containing this PCR product was digested overnight at 37°C with 10 U of *Rsa*I, *Alu*I or *Tru*9I (Promega), according to the manufacter's directions. The schematic representation of the restriction sites of the intergenic region between *uvrA* and *hprk* is shown in Fig. 1. Both the PCR products and the restriction digest fragments were detected by electrophoresis in a 1.5% agarose gel at 75 V. Restriction analysis of the PCR product was performed with the Webcutter program from the Goteborg University web page (www.medkem.gu.se).

2.6. Inter-IS256 PCR typing

Inter-IS256 PCR typing was performed as described by Deplano et al. (Deplano et al., 1997; Deplano et al., 2000). Amplification was performed in 50 μL of reaction buffer containing 200 μM each deoxynucleoside triphosphate, 2.5 mM MgCl2, 0.5 μM of each primer and 1.25 U Taq DNA

Table 1
Molecular typing of related and unrelated MRSA isolates from Argentina (n = 39)

Category	Strain code (Arg)	Type as determined by:				
		Clonal type[a] *mec*A::Tn*554*:: PFGE	STAR-RP[b]	Rep-MP3 PCR	Inter-IS*256* PCR	Coa-RP[c]
	113	XI::B::B1	1/1	I	A	e
	114	XI::B::B2	1/1	I	A	b
	166	XI::B::B3	1/1	I	A	a
	76	XI::B::B5	1/1	I	A	b
	78	XI::B::B10	1/1	I	A	b
Unrelated	336	XI::B::B16	1/1	I	A	b
Epidemic	75	XI::B′::B1	1/1	I	A	b
clones	12	XI::B′::B5	1/1	I	A	b
	266	XI::AA::B4	2/2	I	B	b
	173	II::E::C1	3/4	II	C	c
	138	II::E::C2	3/4	II	C	c
	294	II::E::C7	4/5	II	D	c
	302	II::NH::D1	5/5	III	E	a
	221	II::NH::D2	5/5	III	E	a
	218	II::NH::D3	5/5	III	E	a
	301	II::NH::D8	5/5	III	E	a
	127	II::NH::E1	5/5	IV	F	e
	230	II::NH::E3	5/7	V	G	b
	225	II::NH::F	5/4	III	E	a
Unrelated	229	I::NH::G	5/9	III	E	a
Non	199	I::NH::H	6/6	VI	H	d
epidemic	53	II::NH::I	8/8	III	I	a
clones	64	II::E::J	3/4	VII	J	c
	105	II::E::K1	3/3	III	C	a
	231	II::DD::K4	7/7	VIII	K	a
Outbreaks						
I	273	XI::B::B1	1/1	I	A	b
	279	XI::B::B1	1/1	I	A	b
	281	XI::B::B1	1/1	I	A	b
	186	XI::B::B1	1/1	I	A	b
	276	XI::B′::B1	1/1	I	A	b
	288	XI::B′::B1	1/1	I	A	b
	271	XI::B::B5	1/1	I	A	b
	272	XI::B::B5	1/1	I	A	b
	284	XI::B::B5	1/1	I	A	b
II	251	XI::AA::B4	2/2	I	B	b
	258	XI::AA::B4	2/2	I	B	b
	259	XI::AA::B4	2/2	I	B	b
	260	XI::AA::B4	2/2	I	B	b
	261	XI::AA::B4	2/2	I	B	b

[a] Clonal type were defined on the basis of *Cla*I-*mec*A polymorphisms::*Cla*I-Tn*554* patterns::S*ma*I PFGE (pulsed field gel electrophoresis profiles).
[b] Star gene restriction profiles obtained by the enzymatic digestion of *Alu*I and *Tru*9I.
[c] Coagulase gene restriction profile obtained by the enzymatic digestion of *Alu*I.

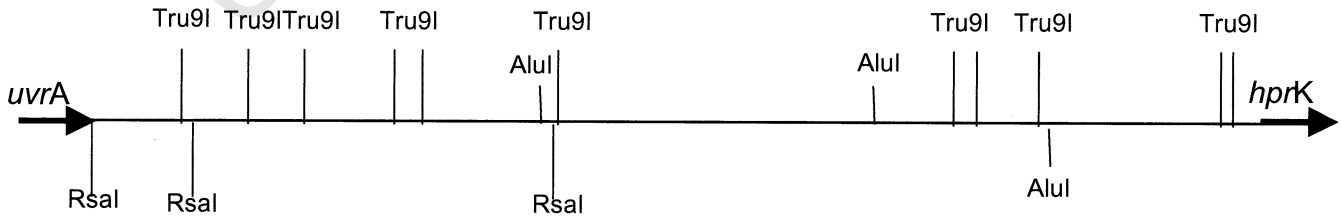

Fig. 1. Schematic representation of the STAR 02 sequence structure. The intergenic sequence between *uvrA* and *hprk* coding regions show the restriction sites of *Alu*I, *Tru*9I and *Rsa*I.

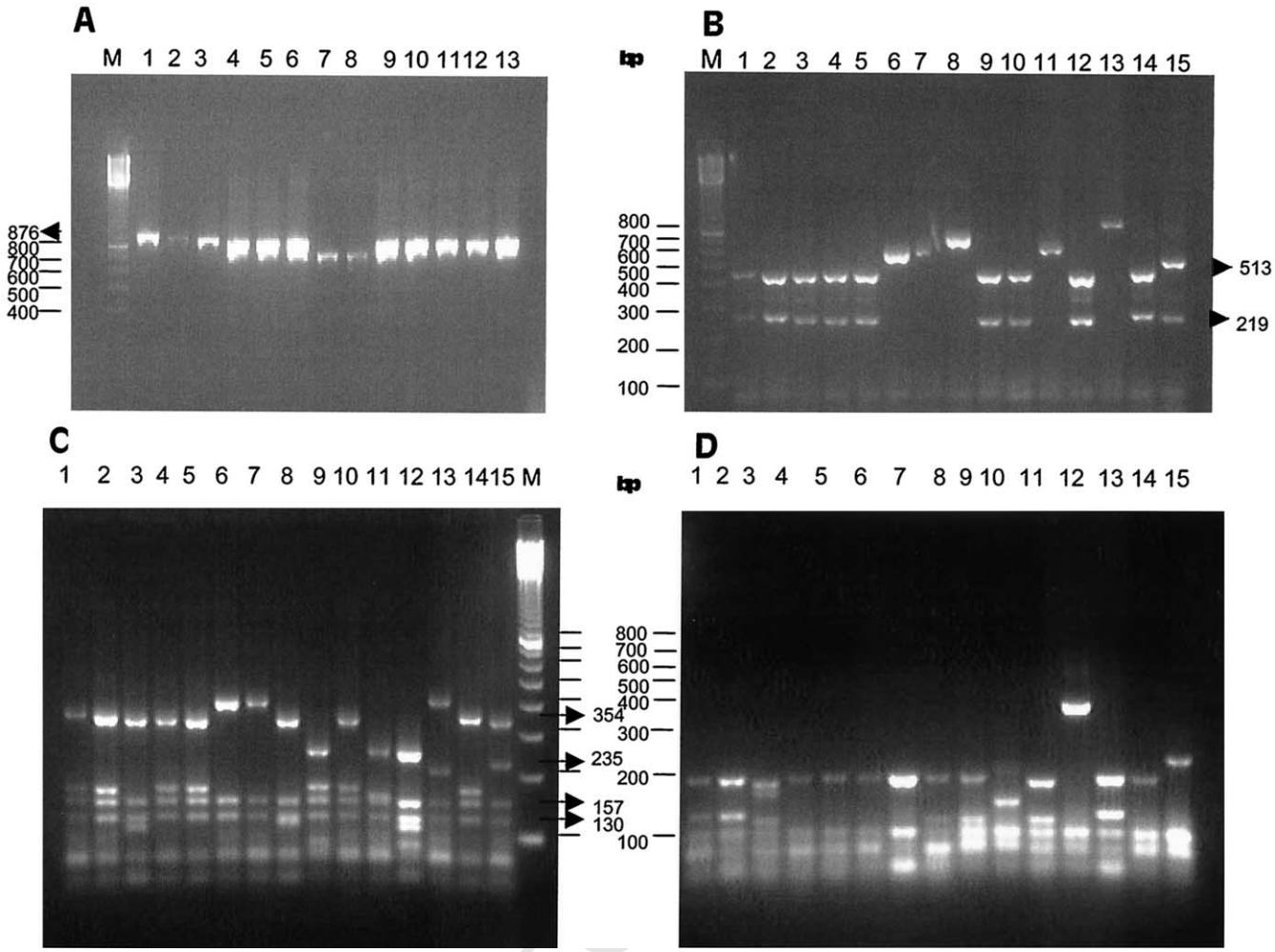

Fig. 2. A. Examples of STAR PCR products. M: 100 bp Ladder (Amersham Pharmacia Biotech, Roosendal, The Netherlands). B. *Rsa*I restriction enzyme digest of the PCR STAR products. M: 100 bp Ladder. C. *Alu*I restriction enzyme digest of the PCR STAR products. M: 100 bp Ladder. Lanes 1, 2, 4, 5 and 10: type 1; lane 3: type 5; lanes 6 and 7: type 3; lane 8: type 7; lane 9:PER 34; lane 11: type 6; lane 12: type 8; lane13: type 4; lane 14: type 2 and lane 15 *S. aureus* NCTC 8325 (arrows). D. *Tru*9I restriction enzyme digest of the PCR STAR products. M: 100 bp Ladder. Lanes 1, 2 and lane 13: type 5; lane 3: type 9; lanes 4, 5 and 6: type 4; lane 7: type 3; lane 8: type 2; lane 9: type 7; lane 10: type 8; lane 11: type 1; lane 12: type 6; lane 14: PER-34; and lane 15: *S. aureus* NCTC 8325 (arrows).

Polymerase (Promega). Subsequently, 200 ng of chromosomal *S. aureus* DNA was added to the PCR mixture. Each sample was subjected to an initial 3 min denaturation step at 94°C followed by 40 PCR cycles consisting of 30 sec at 94°C, 1 min at 45°C and 1 min at 72°C. PCR products were separated by electrophoresis of 15 μL of the reaction mixtures in 1.5% agarose gels in 1 X TBE (Tris-borate-EDTA) buffer for 3 h at 85 V.

2.7. Coagulase gene restriction profile. (Coa-RP) analysis

Coagulase gene typing was performed by a modification of the method described by Goh et al. (1992). The 3′ end region of the coagulase gene was amplified with two primers: COAG2 (5′CGAGACCAAGATTCAACA3′) and COAG3 (5′AAAGAAAACCATCACATCA 3′). One-hundred nanograms of DNA was added to the PCR mixture containing 1 μM of each primer, 5 μL of 10X buffer, 200 μM each deoxynucleoside triphosphate and 1 U of Taq DNA polymerase (Promega). Each sample was subjected to 30 PCR cycles consisting of 30 sec at 95°C, 2 min at 55°C and 2 min at 72°C. Ten microliters of the PCR product was digested overnight at 37°C with 10 U of *Alu*I (Promega) according to the manufacter's directions. Both the PCR products and the restriction digest fragments were detected by electrophoresis in a 1.5% agarose gel.

2.8. Analysis of PCR banding patterns

The gels were analyzed by both visual and computer-aided methods. The bands used to determine the STAR-RP type were arbitrarily chosen to range from 80 bp to 420 bp (*Alu*I) and from 50 to 400 bp (*Tru*9I). Strains that had

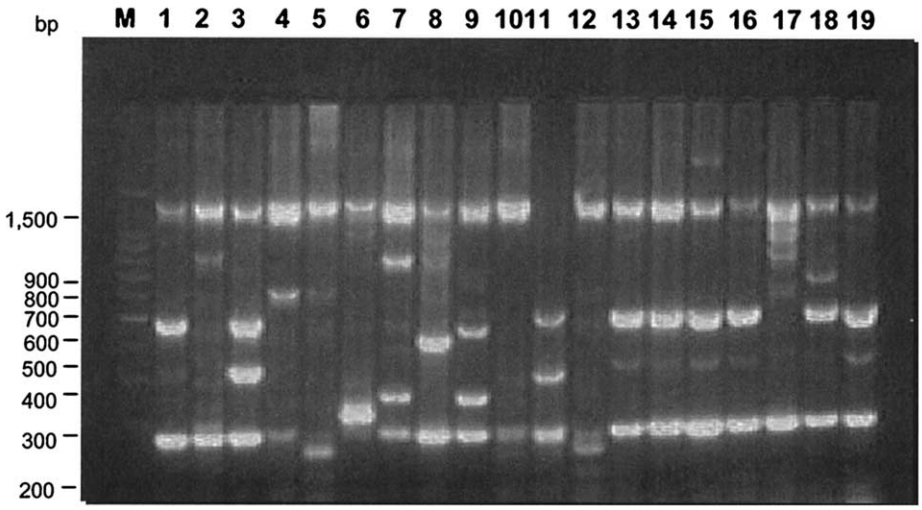

Fig. 3. Representative Inter IS*256* PCR patterns of MRSA strains.

PCR-generated DNA banding patterns that had more than one band difference in terms of size or intensity were considered distinct types. All DNA banding patterns were analyzed with the GelCompar software (version 4.1, Applied Maths, Kortrijk, Belgium). Degrees of homology were determined by the Dice similarity coefficient. Cluster analysis was performed with the unweighted pair group method with arithmetic averages (UPGMA).

2.9. Discriminatory power (D)

The discriminatory power was evaluated by determination of the discrimination index (*D*) (Hunter, 1990). This index depends upon the number of types and/or the frequency of distribution of strains of each type. The discrimination index was determined with the following formula:

$$D = 1 - \frac{1}{N(N-1)} \sum_{j=1}^{S} nj(nj-1)$$

where N is the number of unrelated strains tested, *S* is the number of different types, and *nj* is the number of strains belonging in the *jth* type. To assess the *D* value 25 epidemiologically unrelated isolates were utilized (Table 1).

2.10. Concordance analysis

The concordance of results obtained by STAR-RP analysis of the MRSA collection described in Table 1 ($n = 39$), with those obtained by other methods was evaluated. Concordance was determined as the maximum proportion (percentage) of strains grouped together into unique types by STAR-RP analysis and by each other method tested (Deplano et al., 1997).

3. Results

3.1. Genotyping by STAR-RP analysis

Analysis of MRSA strains by STAR-RP yielded different 3 to 6band patterns according to the enzyme utilized. The STAR-RP banding pattern after digestion with *Rsa*I identified only 2 types and a number of isolates remained non-typeable (Fig. 2B). Due to these results, *Rsa*I was not utilized in further experiments. Analysis of STAR-RP after DNA restriction with *Alu*I and *Tru*9I, however, permitted identification of 8 and 9 types respectively and produced DNA fragments of sizes from 80 to 420 bp (*Alu*I) and from 50 to 400 bp (*Tru*9I) (Fig. 2C and 2D). Combined use of the *Alu*I and *Tru*9I increased the level of discrimination to 12 types. This finding supported the combined use of both enzymes in further experiments. In order to assess the reproducibility of the STAR-RP procedure, the PCR product was obtained at the temperature of annealing (62°C). The DNA extracts from 25 unrelated isolates, which represent distinct types and subtypes, were run in three independent PCR runs. Subsequently, PCR products were digested with *Alu*I and *Tru*9I and, under these conditions, reproducibility of the method was 100% (data not shown).

3.2. Comparison of STAR-RP with other typing methods

To establish the usefulness of STAR-RP, the first set of MRSA *S. aureus* strains (epidemiologically unrelated) previously characterized by *mec*A::Tn*554*::PFGE (Table 1) (Corso et al., 1998) was investigated by STAR-RP and the other PCR-based methods selected for this study, i.e., Inter-IS*256* PCR (Fig. 3), rep MP3-PCR and CRP (data not shown). Twelve genotypes were detected by *Alu*I-*Tru*9I STAR-RP and patterns were identified with two digits, one for the *Alu*I pattern and the other for the *Tru*9I pattern.

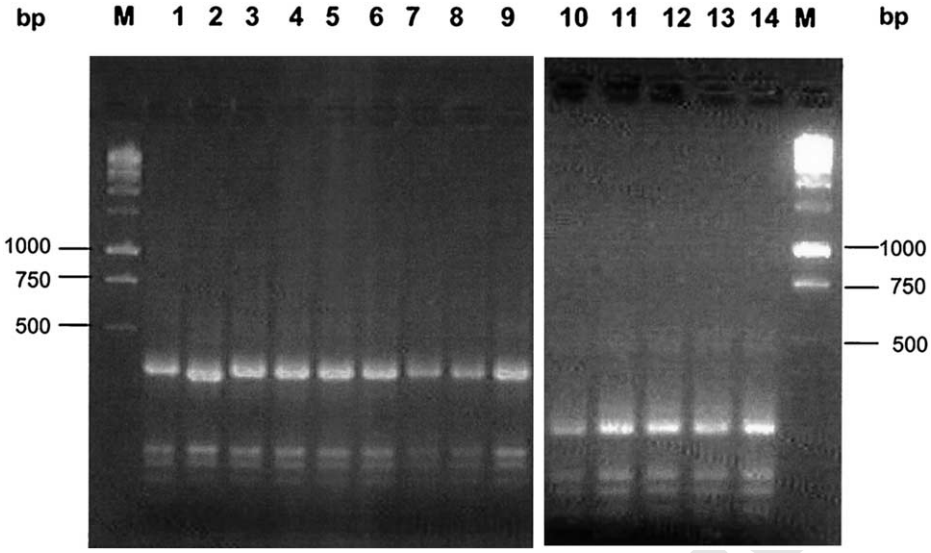

Fig. 4. STAR restriction profiles of epidemiologically related MRSA strains. Lanes 1-9: outbreak I (clonal type XI::B::B1) and lanes 10-14:outbreak II (clonal type XI::AA::B4).

Analysis of MRSA *S. aureus* isolates related to epidemic clones (Table 1) revealed that STAR-RP, Inter-IS*256* PCR and rep-MP3 PCR typing had identical discrimination power to identify Brazilian clone *S. aureus* (clonal type XI::B::B) and its related variants (XI::B′::B and XI::AA::B) at the type level. At the subtype level, PFGE discriminated 7 subtypes. *S. aureus* clonal type XI::AA::B4 was identified not only by STAR-RP but also by Inter-IS*256* PCR typing. Similar discrimination was achieved by analysis of the epidemic clone type II::NH::D, which was similarly identified by STAR-RP (5/5), Rep-MP3 PCR (III) and Inter-IS*256* PCR (E) (Table 1). The three MRSA *S. aureus* strains clonal type II::E::C were discriminated into two coincident types, 3/4-4/5 and C-D by STAR-RP and Inter-IS*256* PCR typing, respectively. Rep-MP3 PCR, however, assigned these three strains to the same rep-MP3 type (II). The second set of isolates ($n = 14$, Table 1) (outbreaks) was used to evaluate the capacity of STAR-RP typing to generate identical band patterns from epidemiologically related MRSA. To this purpose we analyzed isolates from the most prevalent epidemic clone (XI::B::B1) and one of its variants (XI::AA::B4) (Table 1). In this regard, the epidemiologically related isolates showed an identical banding pattern (Fig. 4). Similar results were obtained when only one endonuclease was utilized in identification of epidemiologically related MRSA. Considering the sporadic clones, which were mainly identified by PFGE, two discrepancies were found. STAR-RP typing permitted univocal identification of PFGE types and subtypes E3, H, I, K1 and K4, but identified PFGE subtypes E1 and J as epidemic clones D and C. This misidentification was resolved by rep-MP3 PCR and Inter-IS*256* PCR typing, which permitted discrimination of these subtypes. PFGE types F and G, however, were discriminated as different clones by STAR-RP typing and could not be differentiated by rep-MP3 PCR or Inter-IS*256* PCR typing. Analysis of coagulase restriction profiles have an acceptable capacity to detect the epidemic clones. In this regard, most strains of the Brazilian clone were detected as well as the epidemic clone II::E::C. These types, however, were found not only among the epidemic clones (type a was found in clonal types XI::B::B and II::E::D) but also in the nonepidemic clones as well. Consequently, the validity of coagulase restriction profiles for MRSA typing remains arguable.

3.3. Discriminatory power and concordance of AluI–Tru9I STAR-RP vs. other typing methods.

Clonal type definition of MRSA isolates from Argentina confirmed that *mec*A::Tn*554*::PFGE is the combination of methods that provides the highest discriminatory power (0.91) at the type level. (Table 2). The discriminatory power of the PCR-based typing methods utilized in this study was comparable with those of other methods described above, and ranged from 0.80 to 0.86. In this regard, the STAR-RP analysis showed a discriminatory power of 0.80 and 0.83 when endonucleases *Alu*I and *Tru9*I were used, respectively. A similar value was obtained by rep-MP3 PCR typing (0.80). Inter-IS*256* PCR showed a discriminatory power of 0.85, which was very close to that obtained by STAR-RP typing when the two enzymes (*Alu*I and *Tru9*I) were combined (0.86). The discriminatory power of coagulase restriction profile analysis was 0.74. Although this result was not so distant from those of the other rep-typing methods, its validity is under discussion (see above). Concordance of the methods evaluated in this study with *Alu*I-*Tru*9I STAR-RP ranged from 61 to 87% (Table 2) when epidemiologically related and unrelated isolates were studied. Typing by *mec*A::Tn*554*::PFGE showed the best concordance with *Alu*I-*Tru*9I STAR-RP typing (87%). A sim-

Table 2
Comparison of discriminatory power (*D*) and concordance of *Alu*I-*Tru*9I PCR with other molecular typing methods

Typing method	N° of types	*D*[a] (%)	Concordance with *Alu*I-*Tru*9I-STAR-RP (%)[c]
*mec*A	3	57	61
*Tn*554	6	76	71
PFGE	10	84	74
*mec*A::*Tn*554::PFGE	13	91	87
Rep-MP3 PCR	8	80	71
Inter-IS*256* PCR	11	85	87
Coa-RP	5	74	61
STAR-RP[b]	12	86	NA

[a] Discriminatory index (*D*). The discriminatory index was determined with the strains described here. *S. aureus* NTCC 8325 and PER34, a representative strain of the international Iberian clone, were included in the determination.

[b] STAR gene restriction profiles obtained by the enzymatic digestion of *Alu*I and *Tru*9I.

NA: Not applicable.

[c] Concordance analysis was determined as described in Materials and Methods. *S. aureus* NTCC 8325 and PER34, a representative strain of the international Iberian clone, were included in the determination.

ilar level of concordance was obtained for Inter-IS*256* PCR (87%), followed by PFGE (74%), Rep-MP3 PCR (71%) and *Cla*I-Tn*554* (71%) typing. Typing by *Cla*I-*mecA* and *Alu*I-Coa-RP exhibited the same concordance value (61%).

3.4. DNA pattern similarities

Banding patterns were scanned and analyzed as described in Materials and Methods. All strains with similar STAR-RP type, as assessed visually, were recognized as identical by GelCompar analysis (Figs. 5A and 5B). The estimated relationships of *Tru*9I-STAR-RP types are indicated in the dendrogram depicted in Fig. 5A. At the 80% similarity level 10 clusters were found, which corresponded to each type that this method identified. Control strain NCTC 8325 was grouped in a separate cluster whereas control strain PER 34 was included in a cluster that contained a clinical isolate. At the same percentage of similarity, *Alu*I-STAR-RP types showed a higher degree of relatedness between strains (Fig. 5B) when compared with those obtained by *Tru*9I-STAR-RP typing. Indeed 7 clusters were found among clinical isolates. The two control strains were grouped separately into two different clusters. At a similarity level close to 90% 3 clusters were found which corresponded to groups of *Alu*I-STAR-RP types 1-2, 3-4 and 5-7, respectively. Our results showed that *Alu*I types 1 and 2 corresponded to 2 variants of the Brazilian clone. In addition, types 3 and 4 were found as different subtypes of the clonal epidemic type II::E::C.(Table 1). However, *Alu*I typing used isolatedly, as described above, did not differentiate at the 80% level MRSA strains Arg 302 and Arg 231 (types 5 and 7 respectively) of dissimilar *Sma*I PFGE type. Differences were indeed apparent at the 90% level.

4. Discussion

This study was performed to assess the efficacy of STAR-RP for identification of MRSA clones as an alternative to currently used methods for research and clinical applications. STAR-RP is based on the amplification and subsequent enzymatic digestion of a new repetitive element called STAR (*Staphylococcus aureus* repeat) that is highly variable and is found in many copies in *S. aureus*. (Cramton et al., 2000). A collection of well defined MRSA strains (Corso et al., 1998) was utilized to establish the value of this novel typing system, as suggested previously (Solá et al., 2002; Struelens et al., 1996)

The ability of STAR-RP to discriminate the MRSA strains selected for this study depended upon the enzyme utilized. Whereas the use of *Rsa*I was disregarded because it did not permit any discrimination, the use of *Alu*I and *Tru*9I permitted an acceptable similar level of discrimination (8 and 9 types respectively). In addition, the use of *Alu*I and *Tru*9I exhibited increased discrimination when the results obtained by using both enzymes were combined (12 types). From the epidemiologic viewpoint, STAR-RP PCR distinguished the three Argentinean epidemic clones evaluated in this study, originally defined with the combination of *Cla*I-mec*A* and *Cla*I-Tn*554*, the Brazilian clone (XI::B::B) and its variants (XI::B'::B and XI::AA::B4), the clonal type II:E:C, which was also found in the Czech republic (Melter et al., 1999), and the Pediatric clone (II::NH::D). This last clone, originally found in Portugal (Sá-Leao et al., 1999), was described as the prevalent one in Colombia but it was completely absent from Brazil (Gomes et al., 2001).

STAR-RP typing is a method that can be useful to study non-epidemic MRSA clones. In this regard in the present study we only found two discrepancies using this method. Macrorestriction of DNA with *Sma*I and further analysis of PFGE band patterns provides high accuracy and discrimination, and is currently utilized as reference method for typing MRSA strains (Bannerman et al., 1995; Deplano et al., 1997, Olive et al., 1999, Shmitz et al., 1998, Tenover et al., 1995). Other useful typing approaches include the chromosomal probe fingerprinting techniques. Combination of *Cla*I-*mec*A and *Cla*I-*Tn*554 probes with PFGE, a procedure widely accepted for MRSA typing, was utilized in the present study to define MRSA clonal types. Whereas it is the most discriminative method, further epidemiologic information has been obtained by combination of *Sma*I PFGE with these two probes. In this regard, we were able to evaluate with this method the variability of the mec*A* vicinity or the loss of transposon Tn*554* (Oliveira et al., 1998). Although STAR-RP does not have the resolving power of *Sma*I PFGE subtyping, it has demonstrated good concordance with *Sma*I PFGE at a type level (74%). Moreover, there is evidence with regard to subtyping of PFGE that *Sma*I-IS*256* probe fingerprinting permitted detection of variants of a subtype of the Brazilian clone. (Dos Santos Soares et al., 2001). Comparative analysis of different meth-

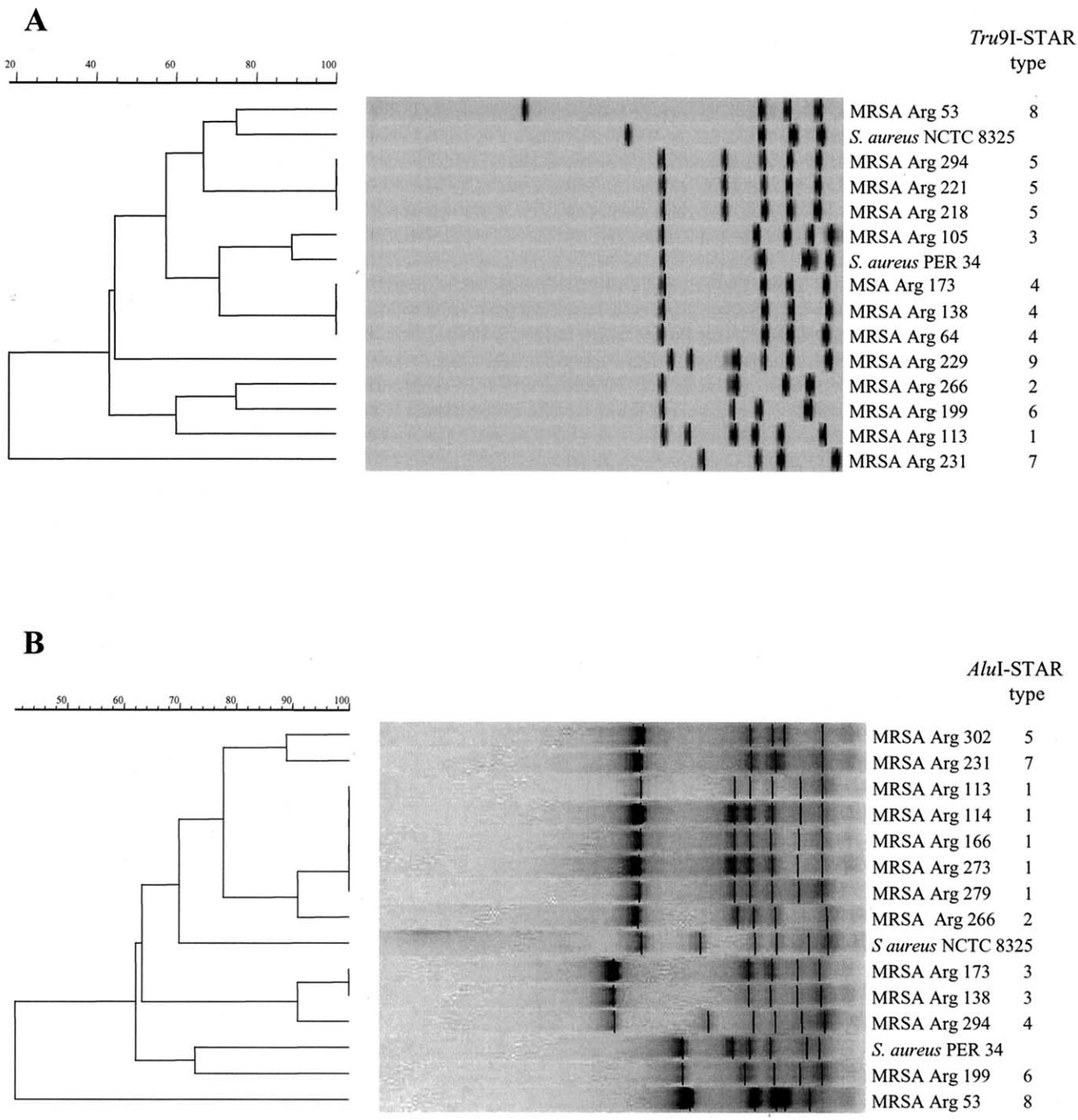

Fig. 5. GelCompar software analysis of the representative STAR RP patterns from MRSA strains. A: Dendrogram of similarity of the *Alu*I-SRP patterns. B: Dendrogram of similarity of *Tru*9-SRP patterns. The column on the right in both panels indicates the STAR-RP types as assessed visually.

ods revealed that clonal types obtained by STAR-RP PCR agreed with those obtained with the gene probes *Cla*I-mec*A*, *Cla*I-Tn*554* and PFGE analysis.

Comparison of STAR-RP PCR with other PCR-based methods revealed that analysis of coagulase restriction profiles for epidemiologic typing of the Brazilian clone provides more discrimination than the rep-PCR typing methods (3 types). In this regard, one of the types found in this clone was also found as the unique type in the clonal type II::NH::D. Moreover, most non-epidemic MRSA where found within epidemic clones, in agreement with previous results (Deplano et al., 1997; Hoefnagels-Schuermans et al., 1997; Schwarzkopf et al., 1994; Tenover et al., 1994). On the other hand, STAR-RP PCR showed concordance and reproducibility comparable to those of the rep-PCR methods, namely Rep MP3 and Inter-IS256 PCR, evaluated in this study. Our results also demonstrated that *Alu*I-*Tru*9I STAR-RP PCR provides good discriminatory power for

evaluation of the Argentinean clones (0.86), similar to that achieved by IS*256* PCR typing (0.85). In addition, both methods had a slightly higher discriminatory power when compared with that of the rep-MP3 PCR (0.80). Moreover, higher discrimination was attained by combining results of the three rep PCR typing methods tested here, especially for detection of sporadic clones.

In this study we have evaluated one of the two recently described repetitive STAR elements (Cramton et al., 2000). The STAR-based method utilizes an element that does not have a defined short repeated sequence in tandem a variable number of times, or possess known insertion elements. Our results show that it is suitable for MRSA discrimination as other methods based on the analysis of repeating unit sequences. The use of the STAR repeat element as the basis for a typing method exhibited excellent correlation with other rep-PCR-based methods described here (Inter-IS256 PCR and MP3 PCR) for epidemiologic typing of MRSA clones found in Argentina. The strain collection used in this study was obtained in Argentina. However, the clones which compose this collection are widely dispersed throughout Latin America (Gomes et al.,2001; Aires de Sousa et al., 2001), a fact that adds importance to our finding. Moreover, the method described here may also have future relevance in other geographical regions of the world since prevalent clones in Latin America were recently found in Europe (Melter et al., 1999; Sá-Leao et al., 1999). Finally, cross-hybridization was observed with DNA of certain species such as *S. epidermidis*, *S. saprophyticus*, *S. haemolyticus* and *S. hominis* when the STAR-element described here (*uvr*A-*hprK*) was used as a probe. This finding merits further research since it may provide information about the use of the STAR element for evaluation of staphylococcal species other than *aureus.*

In summary, we have compared STAR-RP PCR with several currently recommended techniques for their ability to differentiate a well-defined collection of MRSA strains from Argentina. The STAR-RP PCR method is rapid and easy to perform and offers very good reproducibility, ease of Interpretation and standardization. It has also demonstrated good discriminatory power, comparable to those of other rep-PCR based methods such as Inter-IS*256* PCR and MP3 PCR. The current adequacy of STAR-RP PCR to distinguish epidemiologically related strains makes it particularly well suited for the initial screening of MRSA from clinical settings.

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