Evolution and Molecular Characterization of Methicillin-Resistant *Staphylococcus aureus* Epidemic and Sporadic Clones in Cordoba, Argentina

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Since 1999, a new, epidemic, methicillin-resistant *Staphylococcus aureus* (MRSA) strain, named the "Cordobes clone," has emerged in Argentina and coexists with the pandemic Brazilian clone. The purpose of this study was to determine the stability over time of the new clone and to investigate its evolutionary relationship with epidemic international MRSA lineages and with other MRSA and methicillin-susceptible *S. aureus* (MSSA) major clones distributed in this region. One hundred three MRSA isolates recovered in 2001 from Cordoba, Argentina, hospitals and 31 MSSA strains collected from 1999 to 2002 were analyzed by their antibiotic resistance patterns, phage typing, and pulsed-field gel electrophoresis. Additionally, representative members of most MRSA defined genotypes (A, B, C, E, K, and I) were characterized by multilocus sequence typing (MLST) and *spaA* and SCC*mec* typing. The most prevalent MSSA pulsotypes were also analyzed by MLST. Our results support the displacement of the Brazilian clone (sequence type [ST] 239, *spaA* type WGKAOMQ, SCC*mec* type IIIA) by the Cordobes clone (ST5, *spaA* type TIMEMDMGMGMK, SCC*mec* type I) in the hospital environment. MRSA and MSSA isolates shared only ST5. The data support the origin of the Cordobes clone as a member of a lineage that includes the pediatric and New York/Japan international clones and that is genetically related to the British EMRSA-3 strain. Interestingly, the pediatric clone, isolated from most community-acquired infections in Cordoba, was characterized by ST100, a single-locus variant of ST5 and a new variant of SCC*mec* type related to SCC*mec* type IVc.

The first European isolate of methicillin-resistant *Staphylococcus aureus* (MRSA) was detected in 1960. Since then, MRSA has become a leading cause of nosocomial infections worldwide and it is beginning to prevail in the wider community as well (7, 29).

In Argentina, as in many other countries, MRSA remains a major cause of nosocomial infections (4, 5, 37). The percentage of MRSA strains causing nosocomial infections ranged from 45 to 58% during 1996 to 2001 (4, 5). In a previous study, we determined an average MRSA prevalence of 37% in Cordoba, the second most inhabited city in Argentina (37).

Over the last few decades, the increase in number and proportion of MRSA infections in different countries has generally been due to the spread of epidemic strains (19, 33). The emergence and spread of epidemic MRSA in Europe was first reported to occur in the United Kingdom in 1980. The three most common MRSA clones present in that country are the epidemic strains named EMRSA-3, -15, and -16 (14).

During the last decade, five major internationally spread MRSA clones, the Iberian, Brazilian, Hungarian, New York/Japan, and pediatric clones, were identified using a combination of ClaI-MecA polymorphisms, Tn*554* insertion patterns, and pulsed-field gel electrophoresis (PFGE) (reference 32 and references therein). Application of multilocus sequence typing (MLST), *spaA* (staphylococcal protein A gene) typing, and SCC*mec* (staphylococcal cassette chromosome *mec*) typing allows the characterization of both the strain phylogeny and the methicillin resistance determinant in order to define the nature of each clone and evolutionary relationships among the pandemic clones. These molecular tools established that there are relatively few major epidemic MRSA clones, which emerged in five phylogenetically distinct lineages, called clonal complexes. The epidemic clones appeared either as descendants of preexisting epidemic MRSA clones or by the horizontal transfer of SCC*mec* into successful methicillin-susceptible *S. aureus* (MSSA) clones (11, 34). To date, five types of SCC*mec* elements (SCC*mec* I, II, III, IV, and V) and different variants of them have been characterized (36). Three types of SCC*mec* (I, II, and III) are carried mostly by health care-associated MRSA strains throughout the world (11). In contrast, SCC*mec* type IV and, more recently, SCC*mec* type V have been widely disseminated among community-acquired MRSA infections (reference 17 and references therein).

A high prevalence of two multiresistant and epidemic clones that coexisted in the hospital environment was documented in 1999 in Cordoba. One of them was the Brazilian clone, and the other was a new clone of MRSA, genetically unrelated to the

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already described pandemic clones, which was named "Cordobes clone" (37). This clone is closely related at the genetic level to a strain showing high prevalence in Chile from 1997 to 1998. However, the evolutionary history of these clones has not been defined.

The purpose of this study was to determine the stability over time of the new clone and to investigate its evolutionary relationship with epidemic international MRSA lineages and with other MRSA and MSSA major clones distributed in this region.

The glycopeptides, particularly vancomycin, have been the mainstays of therapy for MRSA, and the emergence of resistance to these agents is of great concern. Reduced susceptibility to vancomycin (vancomycin-intermediate *S. aureus* [VISA]) emerged from many successful epidemic lineages with no clear clonal disposition (14). Hence, following the characterization of epidemic MRSA clones in this region, it was of outstanding interest to investigate the eventual emergence of vancomycin resistance among these strains.

MATERIALS AND METHODS

Bacterial isolates. One hundred three MRSA isolates collected from single patients from April to June 2001 and 31 MSSA isolates from single patients recovered from 1999 to 2002 were characterized by antibiotic susceptibility, phage typing, and PFGE. The 2001 MRSA collection was obtained from 10 hospitals (H1 to H10), whereas the 1999 MRSA strains were recovered from 6 hospitals (H1 to H6), as previously reported (37). A representative isolate of each subtype of MRSA types A, B, C, E, I, and K were analyzed by MLST and SCC*mec* typing. To increase the phylogenetic resolution, representative isolates of the same MRSA types were characterized by *spaA* typing (28, 34). The MSSA pulsotypes showing more than one isolate and representative strains of some PFGE types having phenotypic similarity to the A pulsotype, or the Cordobes clone, were selected for MLST analysis. *spaA* typing was performed for the M pulsotype. Species identification and antibiograms were performed in the clinical microbiology laboratories of each hospital.

The data obtained from the patient medical records and the criteria defining infections as nosocomial or community acquired were previously described (37). The 2001 MRSA isolates were involved mainly in nosocomial infections (85%, 88/103 isolates) with the following underlying clinical conditions: surgical wound infections (20%), vascular infections or sepsis (16%), urinary tract infections (10%), and respiratory disorders (9%). Forty-one percent of the inpatients were assisted at the surgical service and 24% at the intensive care unit.

The MSSA isolates were recovered from patients assisted at five hospitals (H2, H5, H7, H8, and H9). Thirty-two percent ($n = 10$) and 45% ($n = 14$) of isolates were from nosocomial and community-acquired infections, respectively, whereas the origin of 22% ($n = 7$) of isolates was not available. All strains were susceptible to methicillin and were negative for *mecA* gene detection (data not shown).

The reference strains NCTC 8325 and UK EMRSA-3 (M307) (Harmony Project [24]) were kindly provided by the Nosocomial Infectious Diseases Laboratory, Instituto de Salud Carlos III, Centro Nacional de Microbiología, Majadahonda, Madrid, Spain. The reference strains Mu3 and Mu50 were kindly provided by Keiichi Hiramatsu, Department of Bacteriology, Juntendo University, Tokyo, Japan.

Phenotypic typing. Determination of susceptibility of the isolates from the 2001 collection to different antibiotics was performed by the disk diffusion method according to the CLSI (formerly NCCLS) guidelines (25), with penicillin, oxacillin, minocycline (MIN), erythromycin (ERY), clindamycin (CLI), chloramphenicol (CHL), gentamicin (GEN), trimethoprim-sulfamethoxazole (SXT), ciprofloxacin (CIP), rifampin (RIF), vancomycin (VAN), teicoplanin, and nitrofurantoin (isolates collected from urine). Netilmicin, amikacin (AMK), tobramycin (TOB), and kanamycin (KAN) were used for isolates susceptible to GEN. Detection of the *mecA* gene was performed by multiplex PCR as described previously (37).

ERY and CLI were also tested by the disk diffusion method to differentiate the macrolide-lincosamide-streptogramin B resistance phenotype as inducible or constitutive (20, 45).

Resistance to antibiotics was defined, as described previously, as multiresistant (MR⁺), intermediate phenotype (MR*), or without multiresistance (MR⁻) (37).

TABLE 1. Prevalence of *S. aureus* and MRSA infections in 1999, 2000, and 2001

Hospital	No. of beds	No. of patients infected with *S. aureus* (% with MRSA)		
		1999	2000	2001 (April–June)
H1	270	306 (56)	350 (62)	48 (64)
H2	180	179 (51)	183 (45)	44 (43)
H3	160	94 (38)	116 (58)	16 (19)
H4	180	109 (37)	117 (46)	39 (38)
H5	100	41 (61)	27 (19)	7 (28)
H6	70	49 (33)	42 (48)	8 (38)
H7	70	60 (47)	88 (39)	10 (30)
H8	70	103 (46)	114 (57)	29 (55)
H9	250	87 (30)	75 (36)	28 (38)
H10	100	58 (62)	51 (59)	11 (54)
Total	1,450	1,086 (47)	1,163 (47)	240 (43)

The evaluation of hetero- or homoresistance to methicillin was carried out by the efficiency-of-plating procedure as already described (37).

The screening of VISA was carried out for all MRSA isolates from the 1999 and 2001 collections and for 23 isolates recovered from January to March 1998 from inpatients of two hospitals (H1 and H2). Plates with in-house-prepared brain heart infusion (BHI) agar containing 4 μg/ml of VAN (BHI-V4) or 6 μg/ml of VAN (BHI-V6) and plates with Mueller-Hinton agar (MHA) supplemented with 5 μg/ml of VAN (MH-V5) were used as described previously (13, 15) and following the recommendations of the Centers for Disease Control and Prevention (CDC) (41). Any isolate that grew on these plates was subjected to population analysis, as previously described (13), with the modifications introduced by Hubert and coworkers (15). The MIC of vancomycin was determined for each isolate by the agar dilution method in MHA, according to the CLSI guidelines (26). Broth microdilution and Etest methods (AB Biodisk, Solna, Sweden) with vancomycin and teicoplanin were performed whenever an isolate was positive in any screening test. Etest MICs were determined by using two inoculum densities, 0.5 and 2.0 McFarland standards, on MHA and BHI agar, respectively. The procedure to detect hetero-VISA (h-VISA) was performed as described previously (41, 44). The vancomycin and teicoplanin used in this work were generous gifts of the Lilly and Sanofi-Aventis laboratories (Argentina), respectively.

Staphylococcus aureus ATCC 25923, ATCC 29213, and ATCC 43300 and *S. aureus* Mu3 and Mu50 were used as controls for antibiotic susceptibility tests.

The phage susceptibility typing was carried out and interpreted as described previously (37).

Molecular typing. PFGE of SmaI digests of chromosomal DNA (24, 37), *spaA* typing (1, 35), and MLST (10) were performed using published methods.

In addition, for representative MRSA and MSSA isolates of some pulsotypes, PFGE was carried out using different running conditions as follows. The total run time was 23 h, the first block switch time was 5 to 15 s for 10 h, and the second block switch time was 15 to 60 s for 13 h. PFGE was performed with a 1% gel with 0.5× Tris-borate-EDTA buffer at 6 V/cm and a temperature of 14°C (24). PFGE patterns were interpreted according to the criteria of Tenover et al., which indicate that one to six bands of difference define a PFGE subtype and seven or more bands of difference define a distinct PFGE type (39, 40).

The SCC*mec* types were determined by multiplex PCR (30) or by PCR amplification of the *ccr* (cassette chromosome recombinase) gene and the *mec* gene complex (29) in all isolates classified as SCC*mec* IV and when ambiguous results were obtained with mutiplex PCR assays. Extra primers were utilized to differentiate SCC*mec* types IVa, IVb (29), and IVc (18).

RESULTS

Genotypic characterization by PFGE of the 103 MRSA isolates of the 2001 collection and phenotypic analysis of the different pulsotypes. Three major pulsotypes (A, B, and C) and one minor pulsotype (E) previously described in 1999 (37) and four new minor, or sporadic, pulsotypes (I, J, K, and L) were identified within the 2001 MRSA isolates. As shown in
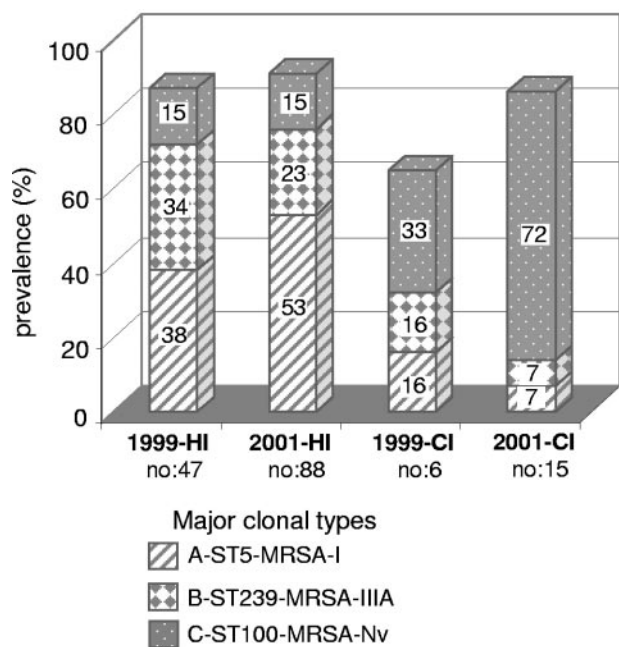
FIG. 1. Prevalence of MRSA major clones over time at Cordoba hospitals. Representative MRSA isolates of each PFGE subtype recovered from 10 hospitals during 3 months in 1999 and 2001 from adult patients affected by hospital-acquired infections (HI) and community-acquired infections (CI) were characterized by MLST and SCC*mec* type. The results are shown as percentages of the total number of strains (no) (indicated below columns) from HI and CI in both 1999 and 2001. Each pulsotype, ST, and SSC*mec* type is indicated at the bottom of the figure.

Table 1, neither the methicillin resistance prevalence nor the total number of *Staphylococcus aureus* infections changed in a significant manner during 1999, 2000, and 2001. There were, however, important shifts in prevalence of major clones between 1999 and 2001 (Fig. 1). A recent surveillance carried out in 12 hospitals in 2004 indicated similar results (data not shown).

Major pulsotype A (*n* = 48, 47%), or the Cordobes clone, was detected in 9 of the 10 hospitals analyzed during 2001, and 47 isolates (47/88, 53%) were recovered from nosocomial infections. Among these isolates, two subtypes were predominant (A1, 13/48 isolates, 27%, and A3, 11/48 isolates, 23%) and 13 additional subtypes were identified (A10 to A22) from those present in 1999 (A1 to A9). Major pulsotype B (*n* = 21, 20%), or the Brazilian clone, was identified in strains from six hospitals, and 20 isolates (20/88, 23%) were from nosocomial infections. This major pulsotype is composed of six subtypes more (B5 to B10) than were present in 1999. Clonal family C (*n* = 24, 23%), or the pediatric clone, was detected in 8 of 10 hospitals, and 11 new subtypes (C8 to C18) were identified. The prevalence of this clonal family (C) as a cause of nosocomial infections remained stable from 1999 to 2001 (7/47, 15%, and 13/88, 15%, respectively), and it was the most prevalent in community-acquired infections during both 1999 (2/6 isolates, 33%) and 2001 (11/15 isolates, 72%) (Fig. 1).

Strains having the E pulsotype were sporadic isolates showing a low prevalence in both 1999 and 2001 (3/53, 6%, and 2/103, 2%, respectively), and all of them were recovered from inpatients affected by nosocomial infections.

Interestingly, a new pulsotype, designated pulsotype K, was identified during the period of April to June 2001. Four isolates identified as subtype K1 were associated with a suspected outbreak in one hospital (H1), and only one isolate identified as subtype K2 was detected, so it was considered a minor clone.

The remaining PFGE patterns (I, J, and L) were single isolates; hence, they were considered sporadic MRSA isolates.

The phenotypic characteristics of major pulsotypes A and B are shown in Table 2, and they remained unchanged during these periods (1999 to 2001).

Two strains having the A17 pulsotype, recovered from patients hospitalized at the intensive care unit in H3, were susceptible to GEN, netilmicin, MIN, CHL, SXT, and RIF and resistant to AMK, TOB, KAN, CIP, ERY, and CLI (constitutive resistance). Major pulsotype A maintained its susceptibility to SXT, in contrast to major pulsotype B, which is resistant (Fig. 2). The I pulsotype was susceptible to GEN and to the other aminoglycosides, and it was resistant only to β-lactam antibiotics, ERY, and CLI (inducible resistance). Four isolates of major pulsotype C showed an MR⁻ phenotype to antibiotics, and they were resistant only to β-lactam antibiotics and GEN. The remainder of the isolates of this pulsotype had small variations in the antibiotic susceptibility patterns (MR*) when they were compared with the phenotypes described above. Several strains showed additional resistance to RIF (72%), CIP (17%), and CLI and ERY (18%) (Fig. 2). The strains having pulsotype C had an important increase of resistance to RIF in 2001 versus 1999 (72% versus 11%) (Fig. 2).

Seventy-one percent (34/48), 90% (19/21), and 58% (14/24) of isolates of pulsotypes A, B, and C, respectively, were typeable with the basic international set of typing phages to *Staphylococcus*, as shown in Table 2.

Genotypic characterization by PFGE of the MSSA isolates and phenotypic analysis of the different pulsotypes. Thirty-one MSSA isolates were distributed into 14 pulsotypes (arbitrarily referred to as types M to Z). On the basis of PFGE patterns, the majority of strains (71%) could be classified into five clones having more than one isolate. Of these clones, the M pulsotype showed the closest similarity in its PFGE DNA pattern to that of the Cordobes clone (Fig. 3). This pulsotype has been detected since 1999 in this region, and it was continuously isolated until 2002.

The M pulsotype showed a mixed lytic pattern to phages of groups I and III and was lysed by the phage 29/52/80/6/84/85/52A+ 79+ (phage type "b"). Importantly, phage type b shares phage 85 with phage type "a," which characterizes both the Cordobes and the Brazilian epidemic clone.

Analysis of MLST, *spaA*, and SCC*mec* types for MRSA and MSSA isolates. The sequence types (ST) and SCC*mec* types determined for the pulsotypes and related subtypes are shown in Table 2.

All subtypes of clonal family C were associated with SCC*mec* type IV by multiplex PCR assays. However, when they were tested by PCR typing for both *mec* and *ccr* gene complexes, a previously undescribed SCC*mec* type was detected. Thus, in a reproducible manner, no amplicons were obtained using specific primers for the class B gene complex (IS*1272*, Δ*mecR1*). The lack of amplified products still persisted with the use of

TABLE 2. Phenotypic and genotypic properties of MRSA strains from patients assisted at 10 hospitals in Cordoba, Argentina

PFGE type (subtype[s])	Antibiotic resistance (no. of isolates)[a]	Methicillin resistance[b]	Lytic phage(s) (no. of isolates); phage type; lytic group	ST; allelic profile[d]	CC[e]	spaA type	SCCmec type[f]
A (A1–A22)	MR$^+$ SXT^s (48)†	Ho-R	85 (30), 85/4 (2), or 85/75 (2); a; III	5; 1-4-1-4-12-1-10	5	TIMEMDMGMGMK	I
B (B1–B10, except B3)	MR$^+$ SXT^r (20)	Ho-R	85 (12), 85/75/42E (2), 85/75 (4), or 85/47 (1); a; III	239; 2-3-1-1-4-4-3	8	WGKAOMQ	IIIA
B (B3)	MR$^+$ SXT^r (1)	Ho-R	NT^c	141; 42-3-1-1-4-4-3	8	WGKAOMQ	IIIA
C (C1–C6; C8–C13; C14–C18)	MR* (16), MR$^-$ (4)	He-R	29/52/80/6/84/85/52A+79+ (2); b; I–III 29/52/80 (8); c; I 95/95 (4)	100; 1-65-1-4-12-1-10	5	TJMBMDMGMK	NV
C (C7)	MR* (3)	He-R	95/95 (1)	142; 1-70-1-4-12-1-10	5	TJMBMDMGMK	NV
C (C14)	MR* (1)	He-R	29/52/80/6/84/85/52A+79+ (1); b; I–III	143; 1-65-1-4-12-63-10	5	TJMBMDMGMK	NV
E (E1–E4)	MR$^+$ SXT^s (2)	Ho-R	NT	85; 31-4-1-4-12-1-10	5	TIMBMDMGMK	I
K (K1 and K2)	MR$^+$ SXT^s (5)	Ho-R	85; a; III (5)	5; 1-4-1-4-12-1-10	5	TIMBMDMGMK	I
I (I1)	MR* (1)†	He-R	77/53/42E (1); d; III	5; 1-4-1-4-12-1-10	5	TJBDMGMK	IVa

[a] MR$^+$, strains resistant to β-lactam antibiotics, CIP, CLI, ERY, and GEN and with variable resistance to MIN, CHL, and RIF; MR$^-$, strains resistant only to β-lactam antibiotics and GEN; MR*, strains resistant to β-lactam antibiotics and GEN and with variable resistance to RIF, ERY, CLI, CIP, and CHL. †, two isolates of the A pulsotype and one of the I pulsotype were susceptible to GEN. Numbers of isolates with the indicated phenotypic characteristics are given in parentheses.
[b] Ho-R, homoresistant strains; He-R, heteroresistant strains.
[c] NT, nontypeable.
[d] Allelic profile assignment (arcC-aroE-glpF-gmk-pta-tpi-yqiL).
[e] CC, clonal complex.
[f] The SCCmec types were determined as described in Materials and Methods. NV, new variant.

specific primers for the J1 region of the IVa or the IVb type, but successful amplification was obtained with the IVc primers, rendering a fragment of the expected size (700 bp). This SCCmec type variant was named new variant (NV) and likely represents a new structural type or structural rearrangement of the mec gene complex. The NV harbored the ccr2 gene complexes and the J1-specific region of the IVc SCCmec subtype. These features strongly suggest that the NV is genetically related to type IV SCCmec, though it lacks typical elements present in other types of SCCmec described until present.

Among the representative isolates of the MSSA major pulsotypes, eight sequence types were identified: ST5 (M pulsotype), ST30, ST188, ST1, ST121, ST8, ST101, and ST144. Interestingly, the last genetic background (ST144, 3-3-1-39-1-1-10) was not previously reported. The M pulsotype (ST5) has the spaA type TJMBMDMGMK.

These results demonstrate that the MRSA strains with PFGE DNA patterns A, K, and I and the MSSA strain with PFGE DNA pattern M share the same ST5 genetic background and a spaA motif (DMGMK) (Table 2). More importantly, ST5 and the spaA motif are also shared with those of the pandemic New York/Japan and pediatric clones as well as one of the main epidemic MRSAs from the United Kingdom, EMRSA-3. As shown in Fig. 3, one isolate belonging to the main subtype of MRSA clones A, K, and I and one representative of the MSSA M clone were compared with a representative strain of the EMRSA-3 clone.

A BURST analysis was used to identify groups of related genotypes among the MRSA and MSSA isolates from Cor-

doba, Argentina. Moreover, data from this study were compared with the S. aureus MLST database to assign isolates to specific clonal complexes. The MRSA and MSSA isolates were distributed in three major clonal complexes (CC5, CC8, and CC1) and four singletons. Thus, five ST (ST5 for MRSA and MSSA, and ST85, ST100, ST142, and ST143 for MRSA), three ST (ST8-MSSA, ST239-MRSA, and ST141-MRSA), and two ST (ST188-MSSA and ST1-MSSA) belong to CC5, CC8, and CC1, respectively.

Finally, the four singletons were identified as ST101-MSSA, ST121-MSSA, ST30-MSSA, and ST144-MSSA. Though these ST were considered singletons in our collection, ST30 is very common in the United Kingdom and it is the putative ancestor of CC30 (12, 34) (http://www.mlst.net).

Analysis of susceptibility to vancomycin. Vancomycin MICs ranged from 0.5 to 2 μg/μl. In total, 17/179 (9%), 9/179 (5%), and 2/179 (1%) isolates grew on BHI-V4, MH-V5, and BHI-V6, respectively. Nevertheless, none possessed the h-VISA phenotype according to the Etest method carried out with a heavy inoculum (McFarland standard of 2) and confirmed by population analysis.

DISCUSSION

The mean percentage of infections produced by MRSA in Cordoba, Argentina, during the years 1999 to 2001 was 46% (Table 1), showing variable prevalence among different hospitals (ranging from 35% to 60%), probably reflecting failures in strategies to control infection and/or differences in antibiotic
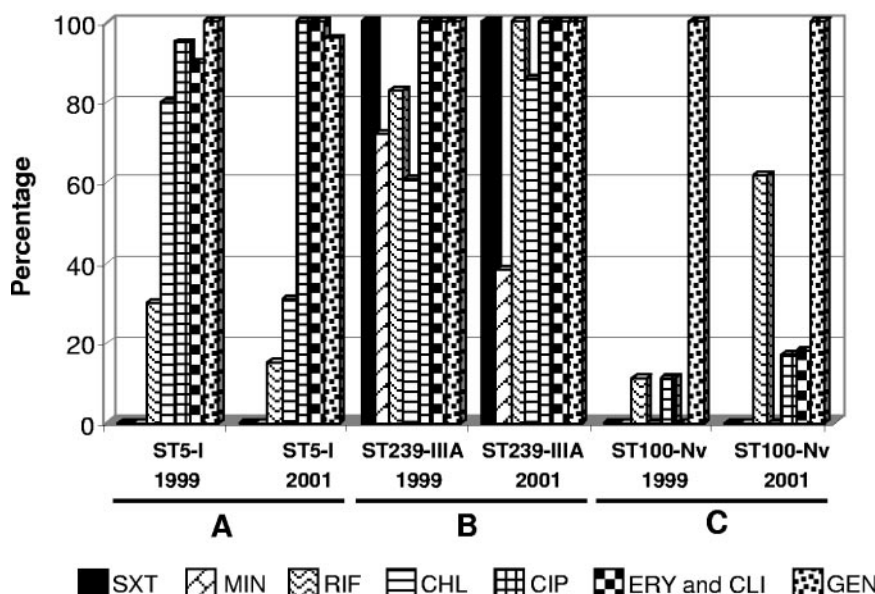
FIG. 2. Resistance patterns over time of the most frequent major clonal types, ST5-I (PFGE type A, ST5, SCC*mec* type I, *spaA* type TIMEMDMGMGMK), ST239-IIIA (PFGE type B, ST239, SCC*mec* type IIIA, *spaA* type WGKAOMQ), and ST100-NV (PFGE type C, ST5, SCC*mec* type NV, *spaA* type TJMBMDMGMK). Resistance to the indicated antibiotics was determined for all isolates, and the obtained resistance profiles for ST5-I, ST239-IIIA, and ST100-NV are shown. The results are expressed as percentages of the total number of strains analyzed. The letters A, B, and C indicate the different PFGE types.

use. Until 1998, the Brazilian clone (PFGE type B, ST239-IIIA, *spaA* type WGKAOMQ) was the only dominant clone in Cordoba (43%) and in other regions of Argentina. However, since 1999 it began to be progressively displaced by a new clone (PFGE type A, ST5-I, *spaA* type TIMEMDMGMGMK), named the Cordobes/Chilean clone (37). Thus, this clone became dominant over the Brazilian clone in 2001 (53% versus 23% [Fig. 1]) in hospital-acquired infections in Cordoba, a situation that still persists, demonstrating the stability of the Cordobes clone over time. Moreover, the susceptibility of the Cordobes clone to SXT is a useful phenotypic marker in the clinical microbiology laboratory as a first criterion to differentiate it from the Brazilian clone in a suspected outbreak, at least in this region (37). Hence, a reduction of resistance to SXT in MRSA isolates would be an early indicator of the dissemination of the Cordobes/Chilean clone. Increasing evidence supports a wide dissemination of the Cordobes clone in Argentina (5) (N. Gardella, R. Picasso, S. C. Pedrari, et al., XVII Congreso Latinoamericano de Micro-biología, abstr. D-238, 2004), displacing the Brazilian clone, which is considered the most successful international epidemic clone of MRSA (1). Similar situations have been observed to occur in other countries, such as the Czech Republic (23), Portugal (3), and Mexico (43), where an epidemic clone was replaced by another one. The reasons explaining these changes are one of the major challenges for the epidemiology field.

Based on MLST alone, the majority of MRSA PFGE patterns were clustered into the lineage CC5, one of the five clonal complexes from which the resistance to methicillin emerged and which includes the New York/Japan (ST5-II) and pediatric (ST5-IV) pandemic clones (11, 34). BURST analysis along with SSC*mec* and *spaA* typing allowed us to propose a model of genetic evolutionary relationship among the five ST (ST5-MRSA and -MSSA, ST85, ST100, ST142, and ST143-MRSA)

belonging to CC5 (Fig. 4). It has been reported that ST5 was associated with four types of SCC*mec*, so ST5-MRSA clones could have emerged by multiple independent introductions of SCC*mec* into the successful ST5-MSSA clone (11). The results obtained in this work are in complete agreement with this hypothesis. Thus, the putative ancestor of this lineage was attributed to ST5-MSSA, pulsotype M, *spaA* type TJMBMD MGMK. Indeed, ST5-MSSA had the largest number of single-step variants and it was associated with three SCC*mec* types: I, IVa, and NV (this work).

Aires de Sousa and de Lencastre reported that ST85 (pulsotype E in this work) probably derived from ST5 and that it could have emerged by independent introductions of two SCC*mec* types (I and IIIA) into a hypothetical ST85-MSSA clone having the *spaA* type TIMBMDMGMK (2). In this study, ST85-MRSA shares a *spaA* type with ST5-MRSA-I (pulsotype K). Therefore, as shown in Fig. 4 it is possible to predict the existence of a successful ancestral clone, ST5-MSSA, *spaA* type T̲IMBMDMGMK, which derived from the clone ST5-MSSA, *spaA* type TJMBMDMGMK, pulsotype M (the change is underlined). The same clone (ST5-MSSA, *spaA* type T̲IM BMDMGMK) could be the ancestor of the successful clones ST85-MSSA, *spaA* type T̲IMBMDMGMK, and ST5-MRSA-I, *spaA* type T̲IMBMDMGMK, pulsotype K. Since pulsotypes M and E were detected earlier than pulsotype K (1999 versus 2001, respectively), the most plausible evolutionary pathway is the one proposed in Fig. 4. On the other hand, pulsotype M has seven band differences with pulsotype A, and pulsotype M is characterized by phage type b (29/52/80/6/84/85/52A+ 79+), which contains phage 85. In addition, pulsotype M has the same ST as the Cordobes clone and a *spaA* type similar to that of the Cordobes clone. These data suggest an evolutionary origin of the Cordobes clone (pulsotype A, ST5-MRSA-I, *spaA*
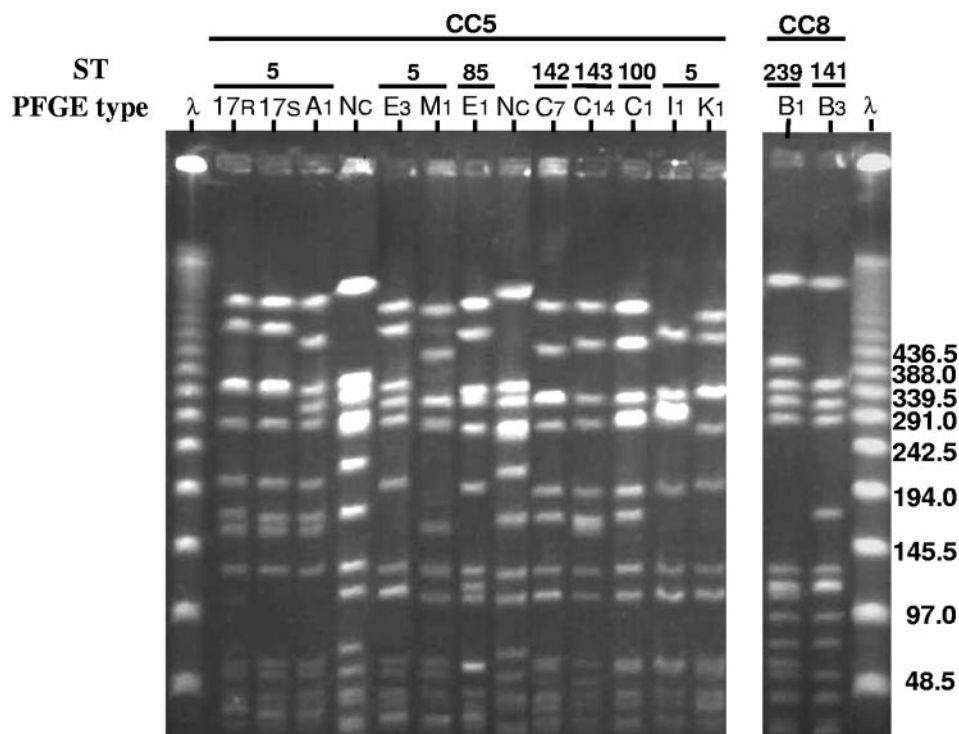
FIG. 3. PFGE of the SmaI-digested genomic DNA of MRSA representative strains of all sequence types belonging to CC5 and CC8 recovered from 10 hospitals in Cordoba, Argentina. Representative isolates of both EMRSA-3 (M307 strain) and a local MSSA (M1 pulsotype) are included for comparison. The ST characterizing the pulsotypes are indicated. 17S and 17R are representative strains of the A17 subtype showing susceptibility and resistance to GEN, respectively. Control strains are as follows: Nc, NCTC 8325; E3, EMRSA-3. The related subtypes are indicated by numbers at the right of the letters. λ, DNA molecular size marker (lambda DNA ladder; Promega). Molecular size markers (in kilobases) are noted at the right of the blots.

type TIMEMDMGMGMK) from the M pulsotype thorough the hypothetical ST5-MSSA clone, *spaA* type TIMBMD MGMK (Fig. 4).

We previously described that an ST5-I clone (known as the Chilean clone), identified in Chile in 1997 and 1998, has a PFGE DNA pattern closely related to that of the Cordobes clone (2, 37). Moreover, a subtype of the Chilean clone (CHL63) shares a *spaA* type with the Cordobes clone. Consequently, the eventual origin of the Cordobes clone in Chile and its further dissemination in Argentina since 1999 cannot be excluded, considering the vast frontier shared between these countries. However, in contrast to the epidemic behavior of the Cordobes clone, the Chilean clone was included within a list of sporadic MRSA clones (2).

ST5-I, an epidemic clone referred to as EMRSA-3 in the literature, was previously identified in the United Kingdom during a survey carried out in 1987 and 1988 (19), and it is one of the three most common MRSA clones distributed in that country, along with the strains EMRSA-15 and EMRSA-16 (14). On the basis of the identical MLST profile (ST5) and SCC*mec* type (I) shared by EMRSA-3 and the Cordobes clone, we hypothesize that the Cordobes clone could have evolved from the EMRSA-3 clone. This evolution entailed genetic changes detected as differences in the PFGE types (seven bands of difference) and in the *spaA* types (TIMBMDMGMK versus TIMEMDMGMGMK) (changes are underlined) as well as in the patterns of susceptibility to phages and antibiotics (24). In conse-

quence, the identification of this clone from 10 hospitals in Cordoba represents the first detection of an epidemic clone genetically related to the EMRSA-3 strain in Argentina.

Our data suggest that pulsotype C might be evolved from pulsotype M by a recent *aroE* point mutation to generate the *aroE65* allele that was first associated with ST100 and by acquisition of the SCC*mec* NV related to SCC*mec* type IVc (through the J1 region) (Fig. 4). Subtype IVc was identified in community-acquired MRSA strains from Japan (18), Uruguay (22), and Sweden (6). In addition, the M and C pulsotypes have seven bands of difference in their DNA PFGE patterns and share the same phage type (type b) and *spaA* type. ST100, associated with a new variant of SCC*mec* (new1), has been previously reported for sporadic isolates (<1%) in Buenos Aires, Argentina, in 1995 (http://www.mlst.net). All ST related to pulsotype C (ST100, ST142, and ST143) share the same *spaA* type, and they were associated with SCC*mec* type NV. These results support that a recent point mutation of ST100 at the alleles *tpi* and *aroE* could be the evolutionary origin of *tpi-63* and *aroE70* alleles present in ST143 and ST142, respectively, both described in this study for the first time. As confirmed here, pulsotype C was the most prevalent in community-acquired infections and the third most frequent MRSA clone in hospital-acquired infections in this region. It has a PFGE pattern closely related to that of the international pediatric clone (ST5-IV), though it is more resistant to antibiotics over time than the latter clone (Fig. 2). Its genetic background
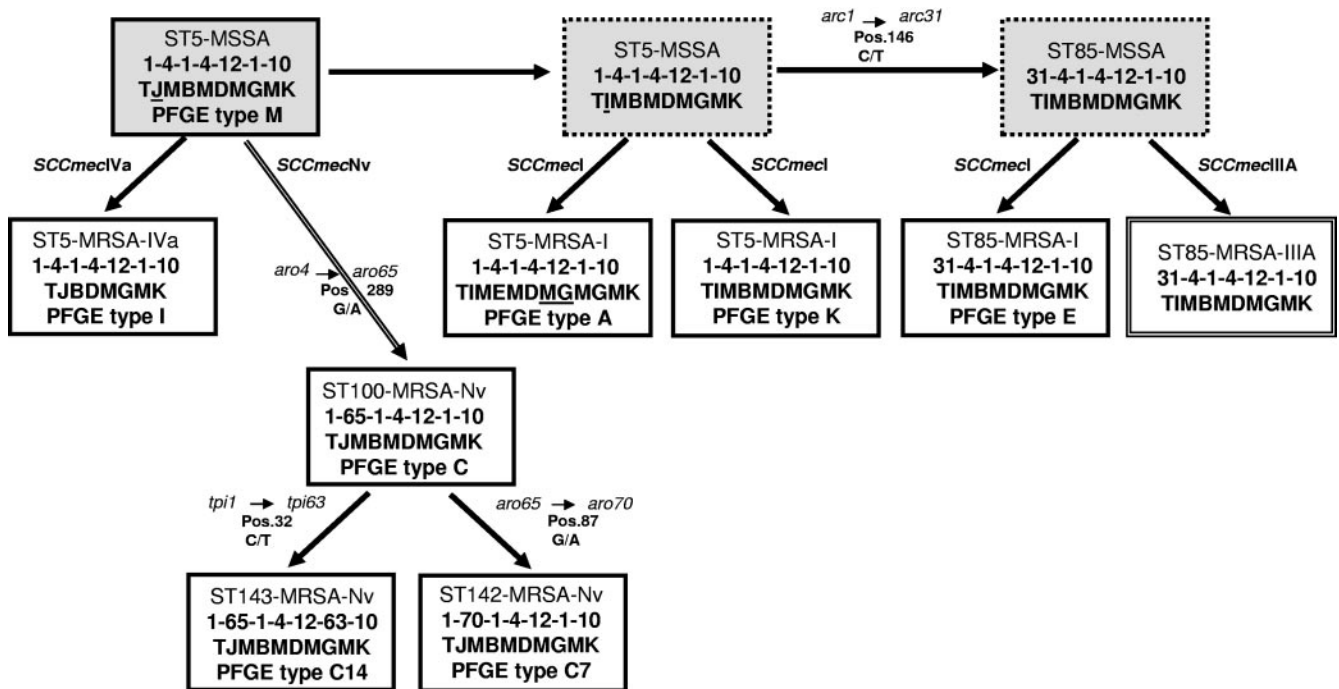
FIG. 4. Proposed evolutionary pathway of different MRSA clones within CC5 in Cordoba, Argentina. The model was based on BURST analysis combined with *spa* typing and acquisitions of the SCC*mec* type. Full genotypes are given, including sequence type, SCC*mec* type, housekeeping gene alleles *arcC-aroE-glpF-gmk-pta-tpi-yqiL*, *spaA* repeats (with change underlined), and PFGE type or subtypes (C7 and C14), respectively. Gray-shaded and white boxes represent MSSA and MRSA, respectively. Arrows indicate the directions of changes between clones. Alterations in the allelic profile, including loci that have changed (top line) and nucleotide positions (Pos.) and nucleotides (middle and bottom lines) that changed, are written next to or above the arrows. Filled and double-lined arrows represent the hypothesis for proposed and unclear evolutionary pathways, respectively. The boxes with dotted borders indicate hypothetical clones not detected in this study. The double-lined box represents a clone previously described to occur (2) in Buenos Aires but not detected in Cordoba.

(ST100) is a single-locus variant of ST5 and is associated with a new variant of SCC*mec* related to SCC*mec* type IVc. According to these observations, it is interesting to propose that pulsotype C (ST100), associated with the SCC*mec* NV, might be a way of evolution of the pediatric clone, allowing for adaptation to the social and medical conditions of this region. A good example could be the pressure exerted by antibiotics, such as RIF, used for treatment of community-acquired MRSA infections, which is supported by the increased resistance of this clone to this antibiotic.

Outside of CC5, the second most frequent MRSA clone of this study was the Brazilian clone, which was characterized by ST239 together with its single-locus variant ST141, identified in this work. The most plausible explanation for the ST141 origin is a recent *arcC* point mutation within the ST239 genetic background, generating a previously nondescribed allele (allele 42 instead of 2 in the allelic profile) (Table 2). All of these strains carried SCC*mec* type IIIA and shared the same *spaA* type, WGKAOMQ.

The phenotypic feature of the Cordobes clone is its susceptibility to more antibiotics than the Brazilian clone (Fig. 2). Moreover, in 2001, two of three strains epidemiologically related by having the A17 pulsotype were susceptible to GEN. The phenotype of the A17 pulsotype correlates with the lack of the *aac6'-aph2''* gene carried by the Tn*4001* transposon and with the conservation of the *ant4'* gene, which confers resistance to KAN, TOB, and AMK (16). The fact that both genta-

micin-resistant MRSA and gentamicin-susceptible MRSA (GS-MRSA) strains share the same pulsotype (A17) strongly suggests that the GS-MRSA strains could have emerged from the gentamicin-resistant MRSA clonal type A17. The emergence of GS-MRSA as either a nosocomial or a community-acquired pathogen is now a worldwide phenomenon (references 8, 21, and 42 and references therein). These strains were characterized by the unexpected reappearance of heterogeneous resistance to oxacillin, and they were more susceptible to other antibiotics than to oxacillin. However, homoresistant strains were also detected in hospital-acquired infections (27). Both GS-MRSA strains with pulsotype A17 analyzed in this study presented homogeneous resistance to oxacillin. On the other hand, pulsotype I (ST5-IVa) showed heteroresistance to oxacillin and susceptibility to all aminoglycosides, including gentamicin, a common feature of GS-MRSA sporadic and epidemic strains of other countries (38, 42). Besides, the A17 and I pulsotypes have different SCC*mec* types (I versus IVa, respectively), explaining, at least in part, the different patterns of susceptibility to other antibiotics.

Several studies demonstrated that genetic modifications conferring antibiotic resistance often occur at the cost of reduced fitness (9). This behavior provides a plausible explanation for the displacement of some epidemic, multiresistant, MRSA strains by other strains more susceptible to antibiotics in several geographic areas. Therefore, it is interesting to propose that the Cordobes clone has lost an antibiotic resistance

determinant favoring its ability to spread. The description of GS-MRSA from two clones with the same genetic ST5 background suggests the ability of this successful lineage to lose resistance determinants in favor of fitness. The systematic follow-up of infections caused by MRSA in Cordoba hospitals could provide new insights to determine if this phenomenon is related to an enhanced ability to spread.

MLST studies of MSSA carried out to investigate the putative existence of the same genetic background as that of the Cordobes clone revealed a great diversity of allelic profiles (eight ST from 31 MSSA strains), which contrasts with the clonal structure of epidemic MRSA, a situation also described in other studies (10, 11, 31).

In summary, our data provide new insights into the epidemiology of MRSA in Cordoba, the second most populated city of Argentina. The results clearly document the displacement of the Brazilian clone (ST239, *spaA* type WGKAOMQ, SCC*mec* IIIA, PFGE type B, resistant to SXT), one of the most successful international epidemic clones of MRSA, by the new Cordobes clone (ST5, *spaA* type TIMEMDMGMGMK, SCC*mec* I, PFGE type A, susceptible to SXT).

The Cordobes and perhaps the Chilean clone are genetically related to the early United Kingdom EMRSA-3 strain, indicating that they share a common ancestor. Moreover they are members of the lineage ST5, which includes the New York/Japan and pediatric clones. Importantly, this genetic background is shared with local MSSA strains that are genetically related to the Cordobes clone (seven bands of difference in PFGE type). Hence, it is interesting to propose that the Cordobes clone emerged from a local MSSA strain, which acquired SCC*mec* and underwent subsequent evolutionary changes leading to the differences in PFGE patterns and *spaA* types. Nevertheless, we cannot exclude an evolutionary pathway occurring in Chile from an MSSA strain (2) and the dissemination of this clone into Argentina, displacing the Brazilian clone.

Our investigation found no evidence of emergence of any h-VISA or VISA strain in Cordoba during this surveillance, and no clinical failure was observed during treatment of any patient despite the high prevalence of h-VISA detected in many countries.

Hospital- and community-acquired infections caused by MRSA strains are a challenge to the health care system, and this picture will become still worse if the strains acquire resistance to additional antibiotics on top of their epidemic capacity. Therefore, the study of MRSA epidemiology is a field of outstanding importance for the design of novel strategies to restrain the spread of MRSA at the local, national, or international level based on the knowledge of how strains spread and how MRSA epidemics occur.

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