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Community-associated methicillin-resistant *Staphylococcus aureus*, eastern Argentina

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Abstract

Sixty-nine community-associated methicillin-resistant *Staphylococcus aureus* recovered in 6 healthcare centers from northeastern and eastern Argentina were genotyped by pulsed-field gel electrophoresis. The predominant pulsotype was widely distributed harbored SCC*mec* type IV and Panton–Valentine leukocidin genes. Representative isolates were characterized by multilocus sequence typing and *spa* typing, demonstrating that this clone belonged to ST5 and *spa* type 311.

Keywords: Methicillin-resistant *Staphylococcus aureus* (MRSA); Community-acquired MRSA (CA-MRSA); Hospital-acquired MRSA (HA-MRSA); SCC*mec*

Most hospital-acquired infections caused by methicillin-resistant *Staphylococcus aureus* (HA-MRSA) are associated with a relatively small number of epidemic clones that spread over different continents. In Argentina, according to the Sistema Informático de Resistencia (Asociación Argentina de Microbiología, Buenos Aires, Argentina), MRSA strains are among the most prevalent nosocomial pathogens (http://www.aam.org.ar), corresponding to the Brazilian, pediatric, and Cordobés clones (Corso et al., 1998; Gardella et al., 2005; Sola et al., 2002).

MRSA infections in healthy individuals from the community without established risk factors have been called community-associated MRSA (CA-MRSA). Distinct genetic lineages associated with CA-MRSA infections have been determined by typing and their geographic dissemination evaluated in different countries. In Latin America, CA-MRSA have been described in Uruguay, Brazil, and Colombia, including an outbreak between January 2002 and October 2003 in Uruguay that involved more than 1000 patients with at least 12 deaths (Alvarez et al., 2006; Ma et al., 2005; Ribeiro et al., 2005).

This is the 1st description of CA-MRSA clones circulating in eastern and northeastern region of Argentina. We retrospectively studied a total of 69 CA-MRSA isolates that were obtained from different healthcare centers in the northeastern and eastern region of Argentina from June 2004 to March 2006. The isolates were received from 6 hospitals: Hospital de Pediatría de Posadas, province of Misiones (n = 35); Hospital de Clínicas "José de San Martín", Buenos Aires city (n = 3); Sanatorio Güemes, Buenos Aires city (n = 8); Hospital Interzonal General de Agudos "Eva Perón" San Martín, province of Buenos Aires (n = 11); Corporación Médica de San Martín, province of Buenos Aires (n = 8); and Hospital de Paraná, province of Entre Ríos (n = 4) (Fig. 1).

Patients included in this study were considered to have CA-MRSA infection according to the definition created by the Centers for Disease Control and Prevention Active Bacterial Core Surveillance sites (Minnesota Department of Health, 2004). Isolates included as CA-MRSA were isolated from patients who had no history of 1) positive culture for MRSA from any body site obtained more than 48 h after

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Fig. 1. Map of Argentina showing the provinces of Misiones, Entre Rios, and Buenos Aires and Buenos Aires city.

admission to a hospital (if hospitalized); 2) prior MRSA infection or colonization; 3) hospitalization, surgery, residency in a long-term care facility, hemodialysis, or peritoneal dialysis within the past year; or 4) current indwelling percutaneous devices or catheters. In patients with several clinical syndromes, the most invasive infection was considered as representative. Among them, skin and soft tissue infection (n = 45) such as abscesses, boils, and cellulites were predominant, followed by bone and joint infections (n = 8) and septicemia (n = 8). Among the others, 3 cases of meningitis were documented, with 1 deadly case (von Specht et al., 2006).

The isolates were identified using standard biochemical tests. Antimicrobial susceptibility testing was performed using disk diffusion tests as recommended by the Clinical and Laboratory Standards Institute (2005). Oxacillin resistance was confirmed by detection of the *mec*A gene by polymerase chain reaction (PCR). *S. aureus* ATCC 25923 and *S. aureus* ATCC 43300 were included as control.

Detection of *mec*A and Panton–Valentine leukocidin (PVL) coding genes was performed after extraction of genomic DNA as previously described (von Specht et al., 2006). MRSA isolates were genotyped by using pulsed-field gel electrophoresis (PFGE) with *Sma*I as described by Chung et al. (2000), *spa* typing (Harmsen et al., 2003), and multilocus sequence typing (MLST) (Enright et al., 2000). Typing of the staphylococcal cassette chromosome (SCCmec) was performed by using multiplex PCR (Oliveira and de Lencastre, 2002).

Comparison of the fingerprints produced was performed by the unweighted pair-group method clustering analysis, applying the Dice correlation coefficient. From a total of 69 isolated, 61 were resistant only to β-lactam antibiotics. The associated resistance patterns of the other 8 isolates were gentamicin (n = 4), gentamicin and erythromycin (n = 1), tetracycline (n = 1), chloramphenicol (n = 1), and chloramphenicol and erythromycin (n = 1) (Table 1).

Four major PFGE patterns were detected; isolates with indistinguishable patterns were grouped in the same pulsotype and coded with capital letters (A–D) (Fig. 2). Thirty-three isolates were included in pulsotype A, 4 in pulsotype B, 5 in pulsotype C, and 10 in pulsotype D. Seventeen isolates presented minor pulsotypes.

MLST and *spa* typing were performed for 5 representative isolates for the most frequent PFGE types: 1 isolate per PFGE types B, C, and D, and 2 isolates representing type A. Sequence types were assigned with reference to the MLST database (http://saureus.mlst.net) (Enright et al., 2000), and the *spa* types were assigned by reference to the Ridom SpaServer database (http://spaserver.ridom.de) (Harmsen et al., 2003) (Table 1).

Table 1
Distribution of 69 CA-MRSA isolates recovered in 6 healthcare centers by PFGE, MLST, *spa* typing, SCC*mec*, and antibiotype

PFGE type	No. of isolates	No. of healthcare centers	MLST type	ST	*spa* type	SCC*mec* type by PCR multiplex	No. isolates with resistance to non β-lactam antibiotics
A	33	6	1-4-1-4-12-1-10	5	311	IV	2[a]
B	4	1[b]	22-1-14-23-12-107-31	wa	2393	nt	2[c,d]
C	5	1[e]	2-2-2-2-6-3-2	30	019	IV	0
D	10	1[b]	2-4-2-2-6-3-2	282	021	IV	1[a]
Others	17	6	ND	ND	ND	ND	3[a,f,g]

ND = not determined; nt = not typeable; wa = waiting for assignation.
[a] Gentamicin resistant.
[b] Hospital de Pediatría de Posadas.
[c] Chloramphenicol resistant.
[d] Chloramphenicol and erythromycin resistant.
[e] Sanatorio Güemes, Buenos Aires.
[f] Gentamicin and erythromycin resistant.
[g] Tetracycline resistant.

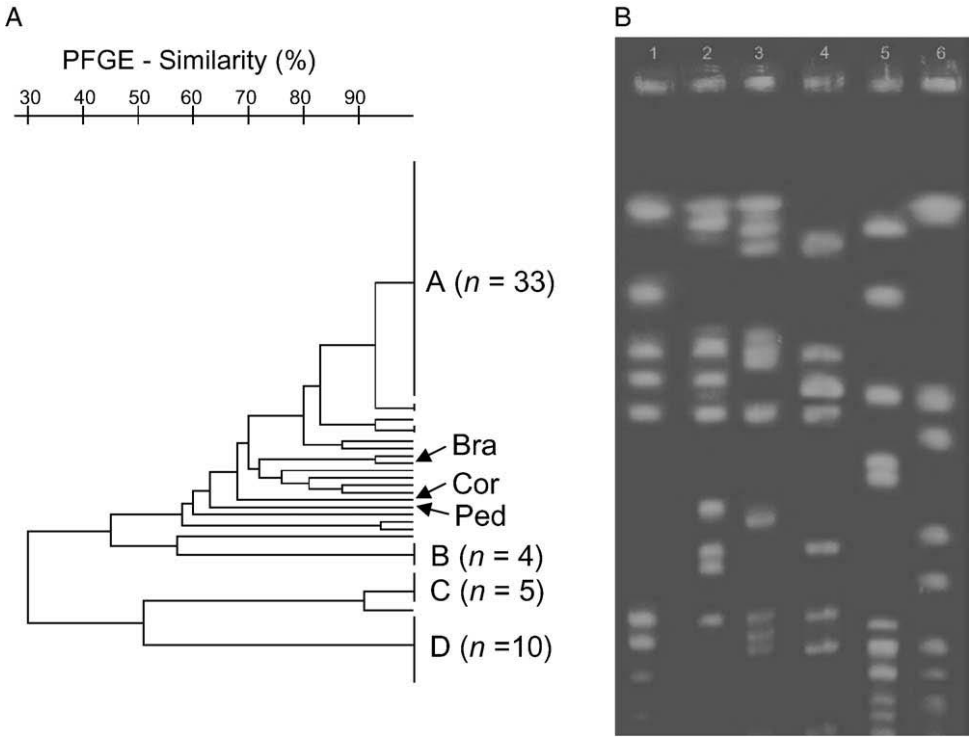

Fig. 2. (A) Dendrogram of pulsed-field electrophoresis banding pattern of 69 CA-MRSA isolates and the 3 clonal types most prevalent in Argentinean hospitals: Brazilian clone (Bra), Cordobés clone (Cor), Pediatric clone (Ped). Similarity coefficient was calculated by using Dice coefficient, and cluster analysis was performed by the unweighted pair-group method. Four major pulsotypes were coded from A to D (n = number of isolates included in each pulsotype). (B) *Sma*I restriction patterns of representative CA-MRSA strains of pulsotypes A, B, and C and representative HA-MRSA strains of prevalent clones in Argentina. Lane 1: Bra; lane 2: Cor; lane 3: Ped; lane 4: pulsotype A; lane 5: pulsotype B; lane 6: pulsotype C.

Although the isolates were distributed into 4 PFGE patterns, pulsotype A was the largest cluster, containing 33 isolates recovered from the 6 healthcare centers. All isolates of this group have PVL genes and SCC*mec* type IV. These isolates were recovered from patients with different types of infections (skin and soft tissue infection, n = 23; "sepsis" syndrome, n = 4; bone and joint infections, n = 3; meningitis, n = 2; pneumonia, n = 1). Representative isolates of this group were characterized further by MLST. This clone displayed the allelic profile 1-4-1-4-12-1-10, which indicates that they belong to ST5.

Pulsotype D included 10 isolates from Misiones recovered from patients with different infection types; all of them were SCC*mec* type IV and PVL positive by PCR. One isolate studied by MLST was characterized as ST282.

Pulsotype C included 5 isolates from a private hospital in Buenos Aires city. All were responsible for skin and soft tissue infections and harbored PVL genes and SCC*mec* type IV; MLST analysis performed on one of these isolates revealed the ST30 allotype.

Four isolates in pulsotype B were from Misiones, 2 of them associated with bone and joint infection, 1 isolated from a septicemia, and 1 isolated from skin and soft tissue infection, being one of them positive for PVL. In these isolates, the SCC*mec* type could not be assigned by multiplex PCR; therefore, we characterized *ccr* (cassette chromosome recombinase) gene as *ccr*AB2 (Ito et al., 2001) and the presence of *mecA* complex class B using primers *IS*1272-NG (5′–ATTTTGGGTTTCACTCGGAT-3′) and *mecR*1-NG (5′-CAAATATTAAAGAACGTGTT) by amplifying a fragment of 565 bp. In contrast, a negative result was obtained for *mecA* complex class A using primers for *mecI* (mI-1 and mI-2) and *mecR1* (mcR-2 and mcR-3) as described by Katayama et al. (2001). In summary, these results confirmed the presence of SCC*mec* type IV in these isolates.

One isolate of group B typed by MLST displayed the allelic profile 22-1-14-23-12-107-31; this combination of allelic variants do not correspond to a previously described ST. It was sent to MLST database for ST assignation.

In this work, we have demonstrated the existence of a predominant clonal type (pulsotype A/ST5) among CA-MRSA isolates recovered in healthcare centers from different geographic areas causing distinct infections types. The predominance of ST5/pulsotype A isolates among patients with CA-MRSA infection in different geographic areas suggest that this strain may carry genetic features that facilitate the persistence in the community. ST5 is one of the major epidemic lineages. Multiple independent introductions of SCC*mec* into ST5-methicillin-sensitive *Staphylococcus aureus* (MSSA) have been suggested, and vancomycin resistance has emerged in one of these genotypes (ST5-MRSA-II) (Enright, 2003). ST5 isolates are frequently associated

to hospital (SCC*mec* type II New York/Japan, SCC*mec* type I Cordobés clone, and SCC*mec* IV pediatric). Moreover, this lineage has been frequently identified among fully susceptible isolates in carriage studies, suggesting that they may harbor genetic traits important for superior epidemicity before acquisition of methicillin and vancomycin resistance (Enright, 2003; Gomes et al., 2006; Sola et al., 2006). Isolates of ST5-SCC*mec* IV harboring PVL coding genes have been reported very recently in a few European locations (Rossney et al., 2007; Sax et al., 2006; Tristan et al., 2007).

The clonal complex 30 currently represents the major group among HA-MRSA and CA-MRSA. ST30-SCC*mec* IV was the most disseminated clone in the large outbreak reported in Uruguay (limiting to the east of Argentina) (Ma et al., 2005). However, in our study, ST30 and ST282—a single locus variant (SLV) of ST30—represented only one-fourth of the studied isolates (pulsotypes C and D). Among all the isolates, an SLV of STs 78–82–88 and 103 involving *tpi* gene was detected, whose final designation is pending.

Our results should be considered an alert for the health system because they show that the main clone circulating in our community possesses a genetic background of ST5 with demonstrated plasticity and efficiency to be established as prevalent in the hospital environment and presents epidemic characteristics.

More surveillance studies are necessary to evaluate the extent of the dissemination of this well-adapted pathogen in community infections in Argentina. Awareness of the local prevalence of these strains and information about the potential severity of infections they can cause are necessary to implement appropriate intervention measures, including prompt and correct empiric antibiotic treatment.

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References

Alvarez CA, Barrientes OJ, Leal AL, Contreras GA, Barrero L, Rincon S, Diaz L, Vanegas N, Arias CA (2006) Community-associated methicillin-resistant *Staphylococcus aureus*, Colombia. *Emerg Infect Dis* 12:2000–2001.

Chung M, de Lencastre H, Matthews P, Tomasz A, Adamsson I, Aires de Sousa M, Camou T, Cocuzza C, Corso A, Cout I, Dominguez A, Gniadkowski M, Goering R, Gomes A, Kikuchi K, Marchese A, Mato R, Melter O, Oliveira D, Palacio R, Sa-Leao R, Santos Sanches I, Song JH, Tassios PT, Villari P (2000) Molecular typing of methicillin-resistant *Staphylococcus aureus* by pulsed-field gel electrophoresis: comparison of results obtained in a multilaboratory effort using identical protocols and MRSA strains. *Microb Drug Resist* 6:189–198.

Clinical and Laboratory Standard Institute (2005) Performance standards for antimicrobial susceptibility testing. Fifteenth informational supplement. M100-S15. Wayne, PA: CLSI.

Corso A, Santos Sanches I, Aires de Sousa M, Rossi A, de Lencastre H (1998) Spread of a methicillin-resistant and multiresistant epidemic clone of *Staphylococcus aureus* in Argentina. *Microb Drug Resist* 4:277–288.

Enright MC (2003) The evolution of a resistant pathogen—the case of MRSA. *Curr Opin Pharmacol* 3:474–479.

Enright MC, Day NP, Davies CE, Peacock SJ, Spratt BG (2000) Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. *J Clin Microbiol* 38:1008–1015.

Gardella N, Picasso R, Predari SC, Lasala M, Foccoli M, Benchetrit G, Famiglietti A, Catalano M, Mollerach M, Gutkind G (2005) Methicillin-resistant *Staphylococcus aureus* strains in Buenos Aires teaching hospitals: replacement of the multidrug resistant South American clone by another susceptible to rifampin, minocycline and trimethoprim–sulfamethoxazole. *Rev Argent Microbiol* 37:156–160.

Gomes AR, Westh H, de Lencastre H (2006) Origins and evolution of methicillin-resistant *Staphylococcus aureus* clonal lineages. *Antimicrob Agents Chemother* 50:3237–3244.

Harmsen D, Claus H, Witte W, Rothganger J, Claus H, Turnwald D, Vogel U (2003) Typing of methicillin-resistant *Staphylococcus aureus* in a university hospital setting by using novel software for spa repeat determination and database management. *J Clin Microbiol* 41:5442–5448.

Ito T, Katayama Y, Asada K, Mori N, Tsutsumimoto K, Tiensasitorn C, Hiramatsu K (2001) Structural comparison of three types of staphylococcal cassette chromosome mec integrated in the chromosome in methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* 45:1323–1336.

Katayama Y, Ito T, Hiramatsu K (2001) Genetic organization of the chromosome region surrounding mecA in clinical staphylococcal strains: role of IS431-mediated mecI deletion in expression of resistance in mecA-carrying, low-level methicillin-resistant *Staphylococcus haemolyticus*. *Antimicrob Agents Chemother* 45:1955–1963.

Ma XX, Galiana A, Pedreira W, Mowszowicz M, Christophersen I, Machiavello S, Lope L, Benaderet S, Buela F, Vincentino W, Albini M, Bertaux O, Constenla I, Bagnulo H, Llosa L, Ito T, Hiramatsu K (2005) Community-acquired methicillin-resistant *Staphylococcus aureus*, Uruguay. *Emerg Infect Dis* 11:973–976.

Minnesota Department of Health (2004) Community-associated methicillin-resistant *Staphylococcus aureus* in Minnesota. *Disease Control Newsletter* 32:61–72.

Oliveira DC, de Lencastre H (2002) Multiplex PCR strategy for rapid identification of structural types and variants of the mec element in methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* 46:2155–2161.

Ribeiro A, Dias C, Silva-Carvalho MC, Berquo L, Ferreira FA, Santos RN, Ferreira-Carvalho BT, Figueiredo AM (2005) First report of infection with community-acquired methicillin-resistant *Staphylococcus aureus* in South America. *J Clin Microbiol* 43:1985–1988.

Rossney AS, Shore AC, Morgan PM, Fitzgibbon MM, O'Connell B, Coleman DC (2007) The emergence and importation of diverse genotypes methicillin-resistant *Staphylococcus aureus* (MRSA) harboring the Panton–Valentine leukocidin gene (pvl) reveal that pvl is a poor marker for community acquired MRSA strains in Ireland. *J Clin Microbiol* 45:2554–2563.

Sax H, Posfay-Barbe K, Harbarth S, Francois P, Touveneau S, Pessoa-Silva CL, Schrenzel J, Dharan S, Gervaix A, Pittet D (2006) Control of a cluster of community-associated, methicillin-resistant *Staphylococcus aureus* in neonatology. *J Hosp Infection* 63:93–100.

Sola C, Gribaudo G, Vindel A, Patrito L, Bocco JL (2002) Identification of a novel methicillin-resistant *Staphylococcus aureus* epidemic clone in Córdoba, Argentina, involved in nosocomial infections. *J Clin Microbiol* 40:1427–1435.

Sola C, Cortes P, Saka HA, Vindel A, Bocco JL (2006) Evolution and molecular characterization of methicillin-resistant *Staphylococcus aureus* epidemic and sporadic clones in Córdoba, Argentina. *J Clin Microbiol* 44:192–200.

Tristan A, Bes M, Meugnier H, Lina G, Bozdogan B, Courvalin P, Reverdy ME, Enright MC, Vandenesch F, Etienne J (2007) Global distribution of Panton–Valentine leukocidin-positive methicillin-resistant *Staphylococcus aureus*, 2006. *Emerg Infect Dis* 13:594–600.

von Specht M, Gardella N, Tagliaferri P, Gutkind G, Mollerach M (2006) Methicillin-resistant *Staphylococcus aureus* in community-acquired meningitis. *Eur J Clin Microbiol Infect Dis* 25: 267–269.