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MOLECULAR CHARACTERIZATION OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* ISOLATES REVEALS THE SPREADING OF A NEW CLONE IN BUENOS AIRES CITY

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Abstract We performed a hospital-acquired methicillin-resistant *Staphylococcus aureus* molecular study in Buenos Aires city. Four clones were found harboring the transposable elements Tn*4001* and Tn*5405* and the erythromycin resistance determinants *ermA* and *mef(E)*. Of the isolates, 73% belonged to a clone found previously in the city of Córdoba, which showed an epidemic behavior initially attributed to the widely disseminated South American clone.

Key words: methicillin resistance, erythromycin resistance, HA-MRSA, transposable elements

Resumen ***La caracterización molecular de aislamientos de* Staphylococcus aureus *meticilino-resistente revela la diseminación de un nuevo clon en la ciudad de Buenos Aires.*** Realizamos un estudio molecular en aislamientos de *Staphylococcus aureus* adquiridos en el ambiente hospitalario de la ciudad de Buenos Aires. Se hallaron cuatro clones que albergaban los elementos transponibles Tn*4001* y Tn*5405* y los determinantes de resistencia a eritromicina, *ermA* y *mef(E)*. El 73% de los aislamientos pertenecían a un clon hallado previamente en la ciudad de Córdoba con características epidemiológicas atribuidas inicialmente al clon sudamericano ampliamente diseminado en el mundo.

Palabras clave: meticilino-resistente, resistencia a eritromicina, HA-MRSA, elementos transponibles

Staphylococcus aureus (SA) is a microorganism of remarkable clinical importance due to the wide variety of infections observed in nosocomial as non-nosocomial environments. In Argentina, the percentage of the hospital-acquired methicillin-resistant SA (HA-MRSA) strains during 2002 was 52%, according to a report from the Antibiotic Resistance Informatic System (SIR), a national health network composed by 22 hospitals. This report also showed that SA was the most prevalent species acquired in our hospitals (21%).

Among all the HA-MRSA epidemic clones described so far, the Iberian and the South American clones have spread extensively[1, 2] and the New York/Japanese clone has been disseminating in Brazil in the last years[3]. The South American clone was prevalent among the HA-MRSA isolates in Argentina since the 62.2% of them showed its characteristic PFGE (pulsed-field gel electrophoresis) pattern according to a previous report dated in 1998[4]. This pattern was related to resistance to a wide range of antibiotics like β-lactams, tetracyclines, aminoglycosides, macrolides, quinolones, trimethoprim-sulfamethoxazole, and chloramphenicol[2]. Moreover, the emergence of a vancomycin and teicoplanin resistant subpopulation among the South American clone-related MRSA strains, has been noticed in Brazil recently[5].

In 2002, researchers from Córdoba City (located at 798 km from Buenos Aires city), found the South American epidemic clone only in 34% of the isolates and identified a novel prevalent clone also with epidemic features in 38% of the isolates[6]. This new clone differed from the MRSA South American clone in that was susceptible to minocycline and trimethoprim-sulfamethoxazole[6].

In regard of the mechanisms of resistance, a limited number of aminoglycoside, and macrolide resistance genes have been described in Gram positive cocci: *aac(6')-aph(2'), aph(3')-IIIa, aadE*[7, 8], *erm* genes, *msrA*, *mef(A)*, *vgaA* and *vgaB*[9] frequently carried in transposons such as Tn*4001* or Tn*5405* (*aac(6')-aph(2'), aph(3')-IIIa, aadE*)[7,10].

The purpose of this study was to perform a molecular surveillance of HA-MRSA clones from five hospitals of Buenos Aires city with the aim to analyze the spreading of prevalent multiple resistant genotypes. We also deter-

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TABLE 1.– *MRSA isolates collected for this study at H1 hospital in August 2000*

Isolate n[0]	Antibiotype[1]	PCR Resistance-type[2]	Antibiotic treatment	PFGE[6] pattern
1	a	1	1.Ciprofloxacin2.Vancomicin	A1
2	b	1	Vancomicin	C1
3	a	1	Vancomicin	A3
4	b	2	Vancomicin plus gentamicin	D1
5[3]	a	1	1.Ciprofloxacin plus gentamicin2.Vancomicin	A4
6	a	1	1.Cefalotin plus gentamicin2.Teicoplanin	A1
7	a	1	Not determined.	A5
8	b	1	Teicoplanin	C2
9	b	1	Teicoplanin	B
10	a	1	1.Ceftazidime, amikacin and metronidazol2.Vancomicin	A2
11[3]	b	2	Vancomicin plus rifampicin	D2

[1] *a: Rifampicin, trimethoprim/sulfamethoxazole, tetracycline, minocycline, vancomycin and teicoplanin susceptible; oxacillin, gentamicin, erythromycin, ciprofloxacin, and clindamicin resistant; b: vancomycin and teicoplanin susceptible; oxacillin, gentamicin, erythromycin, ciprofloxacin, minocycline, rifampin, trimethoprim/sulfamethoxazole, clindamicin and tetracycline resistant.*
[2] *1:* aac(6')-aph(2''); aph(3')-IIIa; aadE; erm(A)*;* sat4; mecA *and* mef(E)*; 2: idem 1 but absence of* mef(E);
[3] *Patients dead by sepsis due to staphylococcal infection.*
[4] *Outpatient 7 was hospitalized before in H1a.*
[5] *1: corresponds to the first antimicrobial treatment choice, and 2 corresponds to the second antimicrobial treatment choice.*
[6] *PFGE: pulsed-field gel electrophoresis.*

mined the mechanisms of resistance involved in some of the MRSA isolated from hospital H1 in the first window period (Table 1).

We performed two one-month molecular surveillances of HA-MRSA epidemiologically unrelated isolates during August 2000 (n=11) from hospital H1 and September 2003 (n=26) from five hospitals including H1 (n=9) (Tables 1 and 2) in Buenos Aires city. All MRSA isolates (n=37) were collected from inpatients with different types of infections (Tables 1 and 2). In the hospital H1, the samples were isolated from two buildings that are separated 3 miles from each other (H1a and H1b) that share several nurses and medical staff as well as patients. However, the patients from our study have never been at the two hospital centers before this study. All strains collected were grown in brain-heart infusion agar with 5% sheep blood and incubated at 37 °C during 24 hs in a 5% CO_2 atmosphere.

The susceptibility to a wide variety of antibiotics was determined by the Kirby & Bauer method according to the NCCLS guidelines[11] using *S. aureus* strain 25923 as control[10]. Genomic DNA was extracted by the guanidinium thiocyanate method[12]. The presence of *mecA*, *aac(6')-aph(2'')*, *aph(3')-IIIa*, *ant(4')-Ia*, *aadA*, *aadE*, *mef(A)*, *mef(E)*, *msrA*, *erm(A)*, *erm(B)*, *erm(C)*, *erm(M)*, *erm(TR)*, Tn*4001*, Tn*5405* and the insertion sequences IS*256*, IS*1181*and IS*1182*, was determined by the standard PCR technique. All the PCRs were carried out as previously

TABLE 2.– *Characteristics of MRSA isolates collected in September 2003.*

Isolate n[0]	Hospital	Antibiotype[1]	PFGE[2]pattern
12	H1	c	A1
13, 14, 16, 17	H1	a	A1
15	H1	a	A1
18-20	H1	a	A3
21-25	H2	a	A1
26-27	H3	a	A1
28,29	H3	b	B
30	H4	a	A2
31	H4	a	A1
32	H4	b	C2
33	H4	b	B
34	H5	a	A3
35	H5	a	A3
36-37	H5	a	A3

[1] *a: Rifampicin, trimethoprim/sulfamethoxazole, tetracycline, minocycline, vancomycin and teicoplanin susceptible; oxacillin, gentamicin, erythromycin, ciprofloxacin, and clindamicin resistant; b: vancomycin and teicoplanin susceptible; oxacillin, gentamicin, erythromycin, minocycline, ciprofloxacin, rifampicin, trimethoprim/sulfametoxazole, clindamicin and tetracycline resistant; c: trimethoprim/sulfamethoxazole, minocycline, vancomycin and teicoplanin susceptible; oxacillin, gentamicin, rifampicin, erythromycin, ciprofloxacin, clindamicin and tetracycline resistant.*
[2] *PFGE: pulsed-field gel electrophoresis*

described[8, 13] using primers designed in our laboratory or by others[7, 13] and automated nucleotide sequence analysis was performed.

PFGE with enzyme *Sma*I was used for the clonal characterization of the 37 MRSA isolates using the CHEF DR-III system (BioRad, Hercules, Calif.). Percent similarity was estimated by the simple matching coefficient, and the matrix was clustered by the unweight pair group method (UPMGA). In this study, the analysis was performed as previously described[13]; an 80% similarity level was considered, corresponding to differences in 7 bands.

The 37 MRSA isolates showed 10 different patterns, which were grouped in 4 clusters (A, B, C, and D, Figures 1A and B). The difference in the number of bands among the clusters varied between 8 and 12 bands and between 2 and 5 within the same cluster. The prevalent cluster (A), was identified as the Córdoba city epidemic clone (73% of the isolates) that showed the antibiotype *a* with the exception of one isolate that showed antibiotype *c* (isolate 12, table 2), while the other clones B, C and D (the first recognized as the South American clone) harbored a more resistant antibiotype (antibiotype *b*, Table 1). The antibiotype *a* showed susceptibility to rifampin, trimethoprim-sulfamethoxazole, tetracycline, minocycline, vancomycin and teicoplanin, the antibiotype *b* was only susceptible to vancomycin and teicoplanin and the antibiotype *c* was susceptible to rifampin, minocycline, trimethoprim-sulfamethoxazole, vancomycin and teicoplanin. The molecular resistance profile analysis of the Córdoba city clone, showed the presence of the loci *aac(6')-aph(2''), aph(3')-IIIa, aadE* and *sat4* (resistance to streptothricin). Two PCR products of 1.5 kb each (right and left junctions) and one of 1.1 kb (IS*256*) confirmed the presence of Tn*4001* and the DNA sequence showed

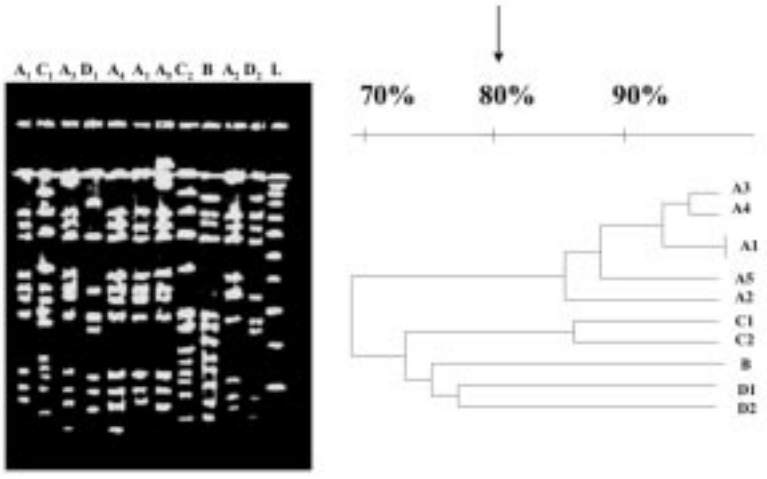

Fig. 1A.– PFGE patterns of *Sma*I digested genomic DNA of the 11 MRSA strains isolated from H1 in 2000. L: phage lambda concatemers used as molecular marker. The dendogram shows the four clones or clusters in the MRSA population. The arrow indicates the cut off used to differ the clusters.

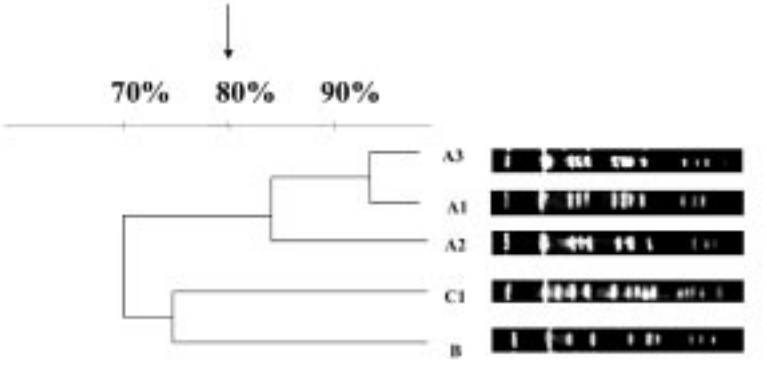

Fig. 1B.– PFGE patterns of *Sma*I digested genomic DNA of the three clones and subtypes. The dendogram shows the three clones or clusters of the MRSA strains isolated from H1, H2, H3, H4 and H5 during the period 2003. The arrow indicates the cut off used to differ the clusters. See also Table 2.

100% homology with this element. The PCR to detect IS*1181*, IS*1182*, *aad*E and *aph(3')-IIIa* sequences rendered amplification products of 1.2 kb (IS*1181*), 1.1 kb (IS*1182*), 3.6 kb (IS*1182-aad*E), 5.7 kb (IS*1182-aph(3')-IIIa*) and 2.2 kb (*aad*E-*aph(3')-IIIa*) that, by nucleotide sequence analysis, belonged to the complete structure of the Tn*5405*. Other resistance determinants detected by PCR in these clones were *erm(A)*(645pb), *mef(A)* (316pb) and *mef(E)*(513 pb) (Table 1).

The percentage of isolation of MRSA strains in our hospitals remained the same (55%), since 1996 when the South American clone was predominant[4]. Our results suggest that since 2000, the South American clone is not having an epidemic behavior in Buenos Aires city, and that the clone from Córdoba city is taking its niche. In Argentina, the dissemination of resistance genes has been increasing in the last decade[4, 14]. Although several works have focused on epidemiological surveys of MRSA[2, 15], to our knowledge, this is the first study where the antimicrobial determinants as well as the clonal spreading have been evaluated at the same time in a molecular surveillance.

The emergence of a novel epidemic clone of MRSA in our hospitals denotes that although the spreading of a MRSA clone may be limited, it does not imply the eradication of this species in the hospital environment. In regard of MRSA, the molecular identification of virulence factors could be helpful to identify the reason of the predominance of this clone over another because the spreading of an epidemic clone can be explained not only by selective antibiotic pressure but also by its ability to survive within the nosocomial environment and colonize patients and hospital personnel.

It can be concluded that it is necessary to perform a continuously molecular and epidemiological surveillance system to detect rapid changes that modify the genotype and the resistance profile of HA-MRSA clones with epidemic behavior. Such studies would help to evaluate control infection policies with the aim to reduce the incidence of infections caused by MRSA isolates.

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