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Research paper

Prevalence and characterization of methicillin-resistant *Staphylococcus aureus* among healthy children in a city of Argentina

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ABSTRACT

Community acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) is a major global problem. Healthy carriers of *S. aureus* strains have an important role in the dissemination of this bacterium. The aim of this study was to estimate the prevalence of *S. aureus* and methicillin-resistant *S. aureus* (MRSA) carriage among healthy children in a city of Buenos Aires province, Argentina, and to determine the potential risk factors for its acquisition. We also described the molecular features of MRSA strains circulating in this population. *S. aureus* carriage was investigated in all children attending the last year of kindergarten during the 2008 school- year period. Household contacts of MRSA carriers were also screened. Of 316 healthy children, 98 (31.0%) carried *S. aureus*, including 14 MRSA carriers (4.4%) and 84 methicillin susceptible *S. aureus* (MSSA) carriers (26.6%). All MRSA isolates carried the SCC*mec* type IV cassette. Eight of the fourteen isolates were closely related to the clone responsible for most severe community-acquired MRSA infections caused in our country (CAA: PFGE A, SCC*mec* IV, *spa* t311, ST5). Two subtypes (A_1 and A_2) were distinguished in this group by PFGE. Both had *agr* type II and presented the same virulence determinants, except for PVL coding genes and *sea* that were only harbored by subtype A_1. Our results, based on the analysis of MRSA isolates recovered in the screening of healthy children, provide evidence of a community reservoir of the major CA-MRSA clone described in Argentina.

1. Introduction

Staphylococcus aureus is an important human pathogen causing a wide range of infections. According to the Sistema Informático de Resistencia (Asociación Argentina de Microbiología) in Argentina, methicillin-resistant *S. aureus* (MRSA) strains are among the most prevalent nosocomial pathogens (HA-MRSA) (http://www.aam.org.ar). Historically, patients who developed MRSA infections in the community had traditional risk factors associated with treatment in nosocomial settings. With the recent emergence of MRSA infections in patients lacking contact with a hospital setting the term community-associated MRSA (CA-MRSA) has been introduced (Herold et al., 1998; Hussain et al., 2001; Suggs et al., 1999). Definitions based on epidemiological origin have been generated and genotype testing, and antibiotic susceptibility testing, have also been proposed to distinguish between HA-MRSA and CA-MRSA (Centers for Disease Control and Prevention, 2009; Salgado et al., 2003).

The nasopharyngeal tract is the primary reservoir of *S. aureus* in both adults and children, although it may be found in other body sites as well (Kluytmans et al., 1997). Carriage of *S. aureus* seems to be important because most *S. aureus* infections occur in persons who are previously colonized with this microorganism and may act as vectors for spreading *S. aureus* and MRSA to both community and hospital environments (Cardoso-Lamaro et al., 2009; Torres et al., 2001; Von Specht et al., 2006).

High prevalence of MRSA colonization among hospital patients or in long-term care facilities has been well documented all over the world (Eveillard et al., 2008; Kluytmans et al., 1997). Reports of carriage on healthy people outside the hospital environment are still scarce (Hisata et al., 2005; Lu et al., 2005; Rim and Bacon, 2007; Torano et al., 2001), especially from South America; moreover, there is little information on factors associated with MRSA colonization and transmission of MRSA to household contacts. The purpose of this study was to determine the prevalence of *S. aureus* and MRSA carriage among all the healthy children attending the last year of kindergarten in a small city of Argentina. We also sought to characterize these MRSA colonizing isolates to compare

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them with the main HA-MRSA and CA-MRSA clones causing infections in our country.

2. Materials and methods

2.1. Study design

We conducted a cross sectional study on the total population of healthy children attending the last year of the all eight kindergartens in San Antonio de Areco, Buenos Aires, Argentina, during the 2008 school- year period. A total of 316 kids were sampled during 6 months (May–October). Household contacts were sampled in 39 of them. Written informed consent was obtained from each child's parents before specimen collection. The total of the population accepted to participate.

2.2. Specimens

Samples were obtained from children by using sterile dry-cotton swabs from nasal, inguinal, axial and pharyngeal sites. Samples were immediately inoculated onto mannitol-salt agar (Biokar Diagnostic, Beauvais, France) and Chromagar MRSA plates (bioMérieux, Marcy-l'Etoile, France) and incubated at 37 °C for 48 h, after which morphological and Gram stain examinations were conducted. The isolates were identified using standard biochemical tests.

2.3. Antimicrobial susceptibility testing

In vitro susceptibility testing was performed using disk diffusion tests according to the CLSI guidelines (Clinical Laboratory Standards Institute, 2005). The following twelve antimicrobial agents were tested: oxacillin, cefoxitin, gentamicin, ciprofloxacin, clindamycin, erythromycin, rifampin, vancomycin, teicoplanin, minociclin, levofloxacin and trimethoprim-sulfamethoxazole (Laboratorios Britania, Buenos Aires, Argentina). Susceptibility interpretative criteria used were those established by the CLSI (Clinical Laboratory Standards Institute, 2005).

2.4. PCR amplification of mecA and SCCmec typing

Detection of *mecA* coding gene was performed after extraction of genomic DNA as previously described (Von Specht et al., 2006). Typing of staphylococcal cassette chromosome (SCC*mec*) was performed for all MRSA isolates by the multiplex PCR strategy developed by Oliveira and de Lencastre (Oliveira and De Lencastre, 2002).

2.5. PCR amplification of virulence determinants and agr typing

Detection of PVL coding gene was performed as previously described (Lina et al., 1999). Investigation of toxins and adhesins coding genes (*sea*, *seb*, *sec*, *sed*, *see*, *seh*, *sej*, *seg*, *sei*, *hlg*, *ica*, *fnbA*,*fnbB*, *fib*, *clfA* and *clfB*) was carried out by PCR strategies described by Nashev and Tristan (Nashev et al., 2004; Tristan et al., 2003). Multiplex PCR was applied to determine *agr* group (Gilot et al., 2002).

2.6. PFGE, spa typing, MLST and RAPD-PCR typing

A single MRSA isolate per child was genotyped by using *spa* typing (Harmsen et al., 2003) and pulsed-field gel electrophoresis (PFGE) with SmaI as previously described (Chang et al., 2000). PFGE profiles were clustered by the unweighted pair group method with mathematical averages (UPGMA; Dice coefficient of similarity), followed by tree inference (Treecon for Windows 1.3b; Y. van de Peer). The following epidemic MRSA clones previously described in Argentina were included in PFGE pattern analysis: the pediatric clone (ST5, SCC*mec* IV), the Brazilian clone (ST239, SCC*mec* IIIa), and the Cordobes clone (ST5, SCC*mec* I). The prevalent clone, named CAA clone (PGFE type A, ST5, t311, SCC*mec* type IV), causing community-associated infections in Argentina, was also included. Representative isolates of major pulsotypes were typed by Multilocus Sequence Typing (MLST).

MRSA isolates from different colonization sites in each kid and their house contacts were compared by Random Amplified Polymorphic DNA PCR (RAPD-PCR), using three different primers (RAPD-1, RAPD-7, ERIC-2) (Gardella et al., 2005).

2.7. Questionnaire survey

Individual variables as well as sociodemographic and family characteristics were obtained prior to sample collection by interviews with the parents, using a standardized questionnaire.

Table 1
Characterization of MRSA recovered from healthy children.

Isolates	Kindergarten	SCC*mec*	PVL[a]	Resistance	*spa*		PFGE Patterns	MLST
					Profile	Type		
1[b]	K1	IV	–	ERY, CLIN	4-20-16-34	t2365	B	nd
2[c]	K2	IV	–	GEN	26-23-17-34-17-20-17-12-17-16	t002	C	nd
3[d]	K2	IV	–		–	nd	D	nd
4[b]	K3	IV	–		07-23-21-16-34-33-13	t127	E	nd
5[e]	K3	IV	–		15-12-16-02-16-02-25-17-24-24	t012	F	nd
6[b]	K4	IV	–	RIF	15-12-16-02-16-02-25-17-24-24	t012	G	nd
7[b]	K5	IV	–		26-23-17-34-17-20-17-12-17-16	t002	A_2	ST5
8[f]	K5	IV	–	ERY, CLIN	26-23-17-34-17-20-17-12-17-16	t002	A_2	nd
9[b]	K6	IV	–	GEN	26-23-17-34-17-20-17-12-17-16	t002	A_2	nd
10[c]	K7	IV	–	GEN	26-23-17-34-17-20-17-12-17-16	t002	A_2	nd
11[e]	K8	IV	+		26-23-17-34-20-17-12-17-16	t311	A_1	nd
12[f]	K8	IV	+		26-23-17-34-20-17-12-17-16	t311	A_1	ST5
13[f]	K8	IV	+	GEN	26-23-17-34-20-17-17-16	t2121	A_1	ST5
14[b]	K8	IV	+		26-23-17-34-20-17-12-17-16	t311	A_1	nd

ERY, erythromycin; CLIN, clindamycin; GEN, gentamicin; RIF, rifampin; nd, not determined.
[a] PVL was assessed by PCR amplification of *luk*-PVL genes.
[b] Nasal and pharyngeal.
[c] Pharyngeal.
[d] Axial.
[e] Nasal, pharyngeal and inguinal.
[f] Nasal.