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Non-Multidrug-Resistant, Methicillin-Resistant *Staphylococcus aureus* Causing Infection in Health-care Facilities in Southern Brazil

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Abstract

Community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) is usually susceptible to a variety of non-beta-lactam drugs. These isolates commonly display SCC*mec*IV and are associated with community-acquired infections. More recently, CA-MRSA has been isolated from health-care-associated diseases. We categorize isolates resistant only to oxacillin or oxacillin plus no more than 3 non-beta-lactam antibiotics according to clinical and epidemiological characteristics, from a hospital in Porto Alegre, and performed a combination of molecular techniques including *mec*A, SCC*mec*, Panton-Valentine leukocidin (PVL) detection and Pulsed-field gel electrophoresis (PFGE). A total of twenty-five patients with non-multidrug-resistant MRSA were studied. Nineteen (76%) came from skin and soft tissue infections. All isolates presented SCC*mec* type IV (being 19/25 IVc) whereas the PFGE profile most frequently found was OSCP-like (15/25). The presence of international clones USA400, USA300 was also verified. Comparing the results of clonal type with source, origin, type of SCCmec, presence the PVL gene and antimicrobial resistance we observed that OSCP-like PFGE profile was associated with skin and soft tissue infections (P=0.0012) and that this clonal group was strongly associated with the presence of PVL gene (P<0.001). This study shows a clonal diversity of CA-MRSA and strengths the concept that these isolates emerged globally from different backgrounds.

Keywords: *Staphylococcus aureus*; Methicillin-resistant; CA-MRSA; PVL; OSPC-like

Introduction

Infections caused by *Staphylococcus aureus* especially methicillin-resistant *S. aureus* (MRSA) are emerging as a public health problem [1]. Methicillin resistance in *S. aureus* is conferred by the *mec*A gene, which is itself carried in a mobile genetic element called the staphylococcal cassette chromosome *mec* (SCC*mec*) [2]. In recent years, the epidemiology of infections caused by MRSA is rapidly changing, with an explosive increase in the number of community-associated MRSA (CA-MRSA) causing infection in the absence of classic risk factor for MRSA diseases, making even more complex the understanding of this epidemiology [3-5]. These CA-MRSA strains appear to have rapidly disseminated among the general population and now seem to be endemic in many urban regions, causing most community *S. aureus* infections [6].

CA-MRSA strains used to be distinguished from health care-associated MRSA (HA-MRSA) by its distinct resistance profile and by molecular methods. Contrary to MRSA strains, CA-MRSA carry smaller SCC*mec* elements, most commonly SCC*mec* type IV or type V. Moreover, they frequently carry *lukS-lukF* genes encoding for the Panton-Valentine leukocidin (PVL) [7]. To further complicate the epidemiological framework, some MRSA strains associated with community infection have been noted to cause hospital infections. Outbreaks of hospital infections caused by isolates containing SCC*mec* type IV have been reported [8]. Given the complex epidemiology of CA-MRSA, CDC investigators have used a third category of MRSA infections, "health care-associated, community-onset" MRSA (HACO-MRSA) infection [9]; this category includes cases that would be hospital acquired-MRSA (HA-MRSA) infections by history of health care exposure but have onset in the community.

Previous studies coming from Brazil describe infections caused by CA-MRSA isolates obtained from the community, and from patients with no healthcare-associated infection, in Porto Alegre City [10,11]. Other international clones carrying SCC*mec* IV have also been reported in different Brazilian cities [12]. Scribel and coworkers demonstrated health care-associated infections caused by MRSA type IV [13]. There are not systematic, nationwide studies in Brazil to detect the presence of CA-MRSA, however the presence of these microorganisms is being detected in different cities and regions of Brazil as well as in neighbor countries [14-17].

The aim of this study was to characterize non-multidrug-resistant MRSA strains isolated from hospitalized patients in Southern Brazil.

Methods

The inclusion criteria were *Staphylococcus aureus* isolates showing resistance to oxacillin or oxacillin plus no more than 3 non-beta-lactam antibiotics from May 2007 through September 2009 at Mãe de Deus Hospital, a tertiary-care hospital with approximately 180 beds in Porto Alegre, Southern Brazil. For a given patient, we examined only data from the first positive culture. Information on the following health care risk factors for MRSA was collected: culture obtained approximately 48 hours before or after admission; surgery or hospitalization in previous 12 months preceding the cultures; presence

of an invasive device at time of admission or evaluation; history of MRSA infection or colonization; dialysis or residence in a long-term care facility in the 12 months preceding the culture [9,18-20].

Isolates were defined according to criteria proposed elsewhere [9,19,20]. Isolates were further classified in 1) health care-associated community onset (HACO-MRSA)–case-patients with one or more health care risk factor but with a culture obtained ≤ 48 hours after hospital admission-2) hospital-associated (HA-MRSA)–case-patients with classic healthcare-associated infections whose culture obtained >48 hours after admission with or without other health-care risk factors-3) community-associated (CA-MRSA)–case-patients without documented heath care risk factors recovered at an outpatient setting or < 48 hours of hospital admission.

In vitro susceptibilities were reported as minimal inhibitory concentrations and performed with the MicroScan Walk/Away system (Siemens Healthcare, Sacramento, CA) according to the protocols of the Clinical and Laboratory Standards Institute (CLSI 2010).

Gene mecA characterization and SCC*mec* typing were performed by multiplex polymerase chain reaction (PCR) assay according to the protocol previously developed by Zhang et al. [21] with modifications in the primers concentrations. SCC*mec* type control strains were NCTC10442 (I), N315 (II), 85/2082 (III), CA05 (IVa), 8/6-3P (IVb), MR108 (IVc), JCSC4469 (IVd) and WIS (V). The occurrence of the PVL-encoding gene *luk*S-F was performed by SYBR Green-based real-time PCR. All PCR assays were performed directly from bacterial suspensions obtained after the rapid DNA extraction method. An aliquot of 2 µl of this suspension was added to 23 µl of PCR mixture containing 50 mM KCl, 20 mM Tris-HCl (pH 8.4), 2.5 mM $MgCl_2$, 0.2 mM of each deoxynucleoside triphosphate (dATP, dUTP, dGTP, and dCTP), 1,25 unit of *Taq* DNA polymerase and various concentrations of the primers. Analysis of chromosomal DNA of MRSA isolates were performed by pulse-field gel electrophoresis (PFGE), according to protocol established by the Centers for Disease Control and Prevention (CDC, Atlanta, GA) for *S. aureus* molecular typing [22]. Gels were normalized with reference strain *S. aureus* NCTC 8325 and compared with representative strains of local and global MRSA clones: A1721/HU25 (BEC), WB72 (USA 300), MW2 (USA 400), WB49 (Oceania South Pacific Clone), HAR24 (EMRSA–15), BK2464 (New York/Japan Clone), HDE288 (Pediatric Clone/USA800). DNA profiles were interpreted by visual inspection and by UPGMA (unweighted pair-groups method using arithmetic averages) analysis based on Dice coefficients with Bionumerics software, version 5.0 (Applied-Maths, Kortrijk, Belgium). Strain relatedness was displayed as a dendrogram and a similarity coefficient of 80% was used to distinguish between lineages [22].

Statistical analysis

Statistical analysis was assessed using non-parametric methods. Fisher's exact tests were performed using software Bioestat 5.3 and significance was defined as $P<0.01$.

Results

A total of twenty-five patients with non-multidrug-resistant MRSA were included in this study. Nineteen (76%) came from skin and soft tissue infections, whereas six isolates (24%) came from other sources, including invasive infections (isolates from blood, sputum and cerebral spinal fluid). As expected, all strains were resistant to beta-lactams and sensitive to all antibiotics tested except, occasionally, erythromycin, gentamicin and clindamycin (inducible pattern).

Most of samples, 76% (19/25) were CA-MRSA according to epidemiological data, 3/25 (12%) were HA-MRSA and 3/25 (12%) were HACO-MRSA (Table 1). Genotypic analysis showed that all samples presented with the SCCmec type IV gene. Most of them had the SCC*mec* type IVc (19/25), 4/25 had SCC*mec* type IVb and 2/25 harbored SCC*mec* IVa. The occurrence of the PVL-encoding gene *luk*S-F was observed in 64% of isolates (16/25).

					Antimicrobial	Classification*	Clonal
MRSA isolate	Date of isolation	Infection site	SCCmec Type	PVL	resistance		type
wb94	may/07	Furuncle	IVc	pos	Ery	CA-MRSA	OSPC
wb95	may/07	Abscess	IVc	pos	Ery	CA-MRSA	OSPC
wb100	jun/07	Cellulitis	IVa	neg		CA-MRSA	USA300
wb102	jul/07	Sputum	IVb	neg	Gen	HA-MRSA	USA300
wb103	set/07	Folliculitis	IVc	pos	Ery	CA-MRSA	OSPC
wb104	nov/07	Cellulitis	IVc	pos	Ery	CA-MRSA	OSPC
wb105	nov/07	Abscess	IVc	pos	Ery	CA-MRSA	OSPC
wb106	dez/07	Cellulitis	IVc	neg	Ery	CA-MRSA	USA400
wb108	mar/08	Cellulitis	IVc	pos	Ery	CA-MRSA	OSPC
a4	abr/08	Varicella vesicle	IVa	neg	Cip, Ery	HA-MRSA	USA300
wb116	mai/08	Abscess	IVc	pos	Ery, Gen	CA-MRSA	OSPC
wb117	mai/08	Nasal	IVc	neg	Ery	CA-MRSA	USA400

wb120	jun/08	Blood	IVb	neg	Cli, Ery, Gen	CA-MRSA	USA300
wb121	jul/08	Cellulitis	IVb	pos	Ery, Gen	HACO-MRSA	OSPC
wb122	jul/08	Folliculitis	IVc	pos	Ery, Gen	CA-MRSA	OSPC
wb124	ago/08	Sputum	IVb	neg	Ery, Gen	CA-MRSA	USA300
wb128	set/08	Cellulitis	IVc	pos	Ery	CA-MRSA	USA300
wb129	set/08	Folliculitis	IVc	pos	Ery	CA-MRSA	OSPC
wb130	out/08	Furuncle	IVc	pos		CA-MRSA	OSPC
wb131	nov/08	Cellulitis	IVc	pos	Ery	CA-MRSA	OSPC
wb133	mai/09	Blood	IVc	neg	Cip, Gen	HA-MRSA	***
wb134	mai/09	CSF**	IVc	neg	Cip	CA-MRSA	***
wb135	mai/09	Furuncle	IVc	pos	Cli, Ery	CA-MRSA	OSPC
wb141	ago/09	Abscess	IVc	pos	Ery	HACO-MRSA	OSPC
wb142	set/09	Fistula	IVc	pos		HACO-MRSA	OSPC

*according to criteria proposed by Klevens et al. 2007.

** Cerebral spinal fluid

*** distinct from the clones used for comparison

HACO-MRSA-health care-associated community onset: caseswith a health care risk factor but with a culture obtained ≤ 48 hours after hospital admissiom

HO-MRSA-hospital-onset: cases with culture obtained >48 hours after admission regardless of whether they also had other health-care risk factors

CA-MRSA-community-associated: cases without documented heath care risk factors recovered at an outpatient setting or < 48 hours of hospital admission

Table 1: Phenotypic and genotypic analysis of MRSA isolates

PFGE analysis revealed PFGE profiles similar to the following clones: USA400 (ST1-IV), USA300 (ST-8-IV) e OSPC (ST30-IV) (Figure 1). Two isolates (wb133 and wb134) presented a PFGE profile distinct from the clones used for comparison. Comparing the results of clonal type with source, origin, type of SCC*mec*, presence the PVL gene and antimicrobial resistance we observed a statistical relationship between source and PVL with clonal type (Table 2). A significant difference between the two clonal groups (OSPC vs. others) was in the sites of MRSA infection. Skin and soft tissue were the most common infection sites among OSPC clones (P=0.0012). Furthermore, the presence of PVL gene was shown to have strong correlation with the type clonal OSPC (P<0.001).

		OSCP-like (n=15)	USA 300 (n=6) USA 400 (n=2) Other clonal group (n=2)	Total (n=25)	P
Source	Skin or soft tissue related	15	4	19	0.0012
	Other sources	0	6	6	
Origin	CA-MRSA or HACO-MRSA	14	7	21	0.2668
	HA-MRSA	1	3	4	
Type of sccMec	IVa and IVb	1	5	6	0.0225
	IVc	14	5	19	
PVL	positive	15	0	16	0.0000
	negative	0	9	9	

Antibiotic resistance (other than methicillin)	none	2	1	3	1.000
	Any combination	13	9	22	

Table 2: Characteristics of non-multidrug resistant methicillin-resistant *Staphylococcus aureus* from Porto Alegre, Brazil, according to clonal group

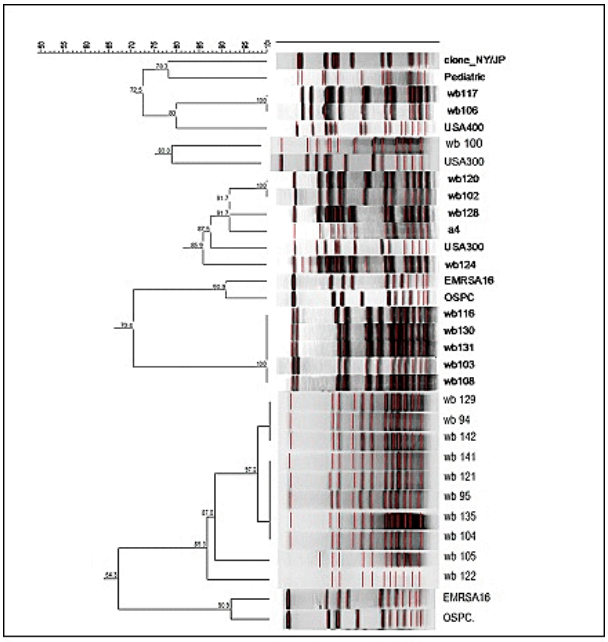

Figure 1: PFGE analysis of non-multidrug resistant methicillin-resistant *Staphylococcus aureus* from Porto Alegre, Brazil

Discussion

The widespread use of antibiotics has accelerated the evolution of *S. aureus*, leading to the emergence of multi-drug resistant isolates in hospitals [23]. On the other hand, a dramatically increase in the incidence of community-associated MRSA (CA-MRSA) was also observed in recent years [24,25]. Besides, this CA-MRSA may cause outbreaks of healthcare-associated infection [26-28], further confounding an epidemiological definition. In our study we first categorized isolates non-multidrug-resistant MRSA, evaluate the epidemiological characteristics and then performed molecular techniques to assess the presence of SCC*mec* IV and PVL-encoding gene *luk*S-F. We noted that even isolates categorized as HA-MRSA harbored SCC*mec* IV gene. Thus, a combination of molecular methods and epidemiological data is required to differentiate CA-MRSA from HA-MRSA.

Certain clones have established themselves as the predominant cause of CA-MRSA infection in certain localities, e.g. USA-300 in the USA [29,30]. Ribeiro and coworkers demonstrated in 2005 a spread of OSPC clone in Porto Alegre [11]. A emergence of USA400 clone was reported in two hospitals in Rio de Janeiro, Brazil [16], and other study showed that OSPC isolates were not only causing community-associated infections but were also involved in health-care associated infections in our country [13]. Our study shows three types of CA-MRSA clones (USA 300, USA400 and OSPC) circulating in Porto Alegre, thus demonstrating a clonal diversity of strains causing infections in both the community and inside the hospital. Furthermore, it was demonstrated that the all isolates belonging to OSPC-like clone caused skin and soft tissues infections, whereas isolates of other clonal groups cause may cause other type of infection (p=0.0012).

PVL has been proposed as a virulence determinant in CA-MRSA [31]; In a model of severe pneumonia, rabbits showed increased morbidity and mortality when infected with PVL-containing USA300 as compared to an isogenic *lukSF* mutant, indicating a significant role of PVL in the development of severe CA-MRSA pneumonia [32]. Furthermore, PVL had a significant effect in experimental CA-MRSA (USA300) osteomyelitis when assayed in rabbits [33]. However, certain studies have questioned the value of PVL as a virulence determinant. Results in rabbit skin infection models with USA300 were controversial [34] demonstrated a moderate yet significant impact of PVL on rabbit skin infection, whereas [35] failed to detect such an effect. There are no studies which test the role of PVL in the presence of infections caused by clone OSPC. In our study most isolates were PVL-positive (16/25; 64%) and interestingly this virulence determinant was found exclusively among OSPC clonal (P<0.001), as previously observed in other Brazilian study [12]. This may indicate that the presence of PVL-encoding gene *luk*S-F may be a marker of OSCP-like clone, not of CA-MRSA.

Our study presents some limitations, including the low number of isolates. This indicate the need in Brazil of a more comprehensive study in order to confirm some points that we observed in this study, notably the association between OSCP-like clone and skin or soft tissue infection; and the presence of PVL-encoding gene lukS-F in this clonal group. The detection and monitoring of non-multidrug-resistant MRSA in both hospital and community environments is crucial to better understand their epidemiology and ultimately control their dissemination.

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