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BRIEF REPORT

High prevalence of ST121 in community-associated methicillin-susceptible *Staphylococcus aureus* lineages responsible for skin and soft tissue infections in Portuguese children

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Abstract In order to evaluate the incidence of community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) in Portugal, we analyzed a collection of 38 *S. aureus* isolates recovered from 30 children attending the pediatric emergency department of a central hospital in Lisbon due to skin and soft tissue infections. Molecular characterization identified seven clonal lineages among the 35 methicillin-susceptible *S. aureus* (MSSA) isolates, of which the major lineage PFGE A/t159/ST121 included 63% of the isolates. The three MRSA isolates belonged to the Pediatric clone PFGE D/t535/ST5-IV (n=2) and to the European CA-MRSA clone PFGE G/t044/ST80-IVc (n=1). All isolates harbored several virulence factors, namely, leukocidins. Panton–Valentine leukocidin (PVL) was produced by isolates from five MSSA lineages and by the ST80 MRSA. Of interest, this is the first reported isolation of CA-MRSA ST80 in Portugal.

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Methicillin-resistant *Staphylococcus aureus* (MRSA) infections in the community have been increasing worldwide and are mainly reported as skin and soft tissue infections (SSTI) in otherwise healthy young individuals [1–3]. The Centers for Disease Control and Prevention (CDC) consider a community-associated MRSA (CA-MRSA) infection when the patient has no previous history of MRSA infection or colonization, surgery, dialysis, hospitalization, residence in a long-term care facility within the year before infection, presence of a percutaneous device or indwelling catheter, or hospitalization >48 h before MRSA cultures [4, 5].

CA-MRSA isolates differ phenotypically and genotypically from hospital-associated (HA-) MRSA, namely, in its non-multiresistant antibiotic patterns, enhanced virulent gene content, including the acquisition of the necrotizing Panton–Valentine leukocidin (PVL) genes or the expression of α-type phenol-soluble modulins (PSMs) [1, 3]. The staphylococcal cassette chromosome *mec* (SCC*mec*) type and the accessory gene regulator (*agr*) alleles are also differentially distributed: SCC*mec* types IV or V and *agr* type III are more commonly present in CA-MRSA, while SCC*mec* types I–III and *agr* type II are more typical of HA-MRSA [1]. In contrast, community-acquired methicillin-susceptible *S. aureus* (CA-MSSA) isolates, responsible for a significant number of mild SSTI, do not differ from HA-MSSA [6, 7].

Although reports of CA-MRSA prevalence are increasing worldwide, there is no description of the actual scenario in Portugal, a country with >50% HA-MRSA infection, currently the highest in Europe [8]. Previous studies dated from 1996 to 2009, including isolates from nasal swabs of young healthy individuals and nasopharyngeal swabs of children attending day care centers, reported an MRSA prevalence lower than 0.25% in the Portuguese healthy community (0.24% in 1996–1998 and 0.13% in 2006–2009) [6, 9, 10].

It is conceivable that the incidence of CA-MRSA in Portugal is underestimated partially because skin infection samples are not routinely cultured.

The aim of the present study was to assess the prevalence and molecular characterization of *S. aureus* in children attending the pediatric emergency department of a central hospital due to SSTI.

The pediatric emergency department of Hospital Fernando Fonseca, a large tertiary-care hospital (670 beds), is the second largest pediatric urgency unit in the urban area of Lisbon, Portugal, and receives approximately 180 children per day. Between August 2005 and October 2006, all children attending this unit due to SSTI were enrolled in the study. Samples were recovered by swabbing the largest area of skin infection or wounds with spontaneous or surgical drainage, or in case of severe SSTI from hemoculture. Whenever possible, nasal swabs were also performed. A questionnaire was filled in order to collect data on basic socio-demographic patient information, risk factors associated with skin infection, and description of the infection.

S. aureus isolates were first identified by conventional coagulase and catalase tests. Additional identification and susceptibility testing with a panel of 10 antibiotics (Fig. 1) were performed by the semi-automatic VITEK2 system (bioMérieux, SA, France) and the disk diffusion method for clindamycin [11]. All isolates were tested by polymerase chain reaction (PCR) for the presence of *mecA* [12] and were also characterized by pulsed-field gel electrophoresis (PFGE) [13]. The resulting SmaI patterns were analyzed by both visual inspection and automatically with BioNumerics software version 4.61 (Applied Maths, Sint-Martens-Latem, Belgium) [14, 15]. Characterization by *spa* typing, multilocus sequence typing (MLST), and the *agr* allele type were performed on representative isolates of each PFGE type [16–18]. The SCC*mec* was typed for all MRSA isolates [19, 20]. Specific staphylococcal virulence determinants, including leukocidins, hemolysins, and super-antigenic toxins of each isolate, were determined by PCR [21, 22] (Fig. 2). Categorical variables were compared using the χ^2 or Fisher's exact test when appropriate, considering p-values of ≤ 0.05 being statistically significant, and odds ratio (OD) estimates using the SPSS software package version 11.5 (SPSS Inc., Chicago, IL, USA).

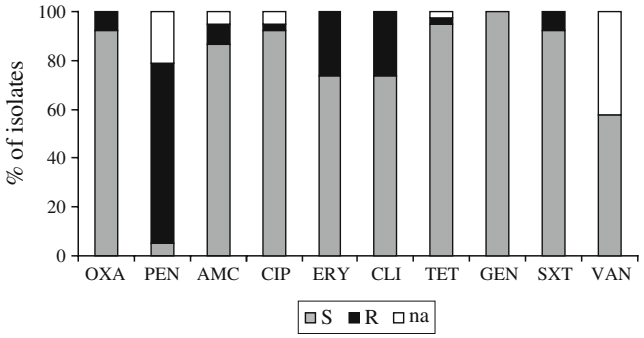

Fig. 1 Antimicrobial susceptibility of the 38 *Staphylococcus aureus* isolates to a panel of 10 antibiotics. Abbreviations: *S*, susceptible; *R*, resistant; *na*, not available; *OXA*, oxacillin; *PEN*, penicillin; *AMC*, amoxicillin–clavulanic acid; *CIP*, ciprofloxacin; *ERY*, erythromycin; *CLI*, clindamycin; *TET*, tetracycline; *GEN*, gentamicin; *SXT*, trimethoprim–sulfamethoxazole; *VAN*, vancomycin. All of the resistant phenotypes observed for clindamycin mean inducible resistance, determined by the D-test [11]

During the 15-month study period, 30 (73.2%) out of the 41 children who attended the emergency department due to SSTI were infected by *S. aureus*. The major clinical infection presentations were cutaneous abscesses (n=12, 40%) and cellulitis (n=10, 33.33%). A total of 38 isolates were recovered, of which three (7.9%) were MRSA. Although eight clonal types (Table 1) were identified among the isolates, 63% (n=19) belonged to a single type: PFGE A, *spa* type t159, sequence type (ST) 121, *agr* type IV. The ST121 lineage was also the predominant lineage found in MSSA isolates through Europe and Russia [23–25] and an identical scenario has been reported among Asiatic children in both carriage and disease isolates, and frequently associated to PVL. Of major concern was the local emergence of methicillin resistance in ST121 isolates in these pediatric communities: one MRSA isolate, PVL-negative responsible for staphylococcal scalded skin syndrome in China and two MRSA, PVL-positive, SCC*mec* type V isolates responsible for osteomyelitis and soft tissue abscess in Cambodia [26, 27]. Moreover, single ST121 MRSA isolates were reported in China (http://saureus.mlst.net/) and the United States [28].

Retrospectively, all STs found in the present study (with the exception of ST152) have already been described in carriage among the Portuguese community in 1996, although with a different prevalence [6]. Despite the small size of the collection, the major CA-MSSA clones described in Europe, ST1, ST5, ST30, and ST45 [29], were found in the pediatric Portuguese community (Table 1). Moreover, all clonal lineages, with the exception of PFGE H/t084/ST582/*agr*IV, were recently reported among a geographical and temporal diverse collection of 211 PVL-positive CA-MSSA [23]. ST1/t127, the sixth most frequent type among MSSA isolates recovered in 26 European

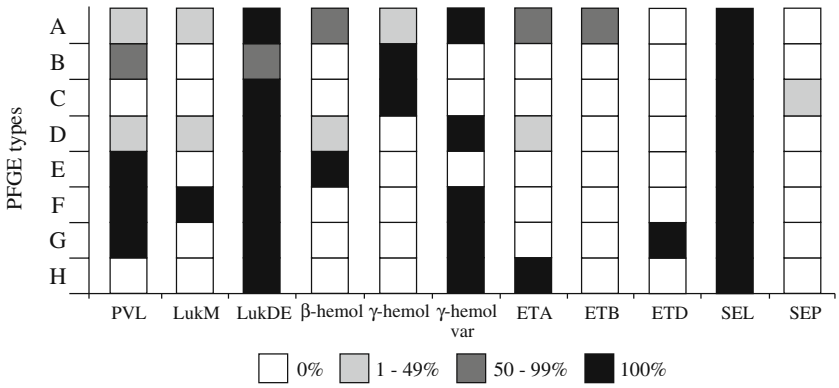

Fig. 2 Virulence gene determinants of the eight clonal lineages. The totality of the isolates was tested for 11 virulence genes. Abbreviations: *PVL*, Panton–Valentine leukocidin; *LukM*, leukocidin M; *LukDE*, leukocidins D and E; *β-hemol*, β-hemolysin; *γ-hemol*, γ-hemolysin; *γ-hemol var*, γ-hemolysin variant; *ETA*, *ETB*, *ETD*, exfoliative toxins A, B, and D, respectively; *SEL*, *SEP*, staphylococcal enterotoxins L and P, respectively

countries [29], was also found in the present collection. In the same work, Grundmann et al. showed that European MSSA isolates belonged to more diverse genetic backgrounds and have a wide geographical distribution compared to MRSA, which have a predominantly regional spread of a few pandemic clones [29].

A non-multiresistant antibiotic pattern is common in the present collection (Fig. 1). Clindamycin and trimethoprim–sulfamethoxazole (SXT) are rational empiric choices for mild-to-moderate CA-MRSA infections [30]. However, a positive D-test result in erythromycin-resistant isolates indicates that clindamycin resistance may emerge during therapy and, therefore, should not be prescribed [31, 32]. The detection of clindamycin inducible resistance in all erythromycin-resistant isolates (n=10) raises some concerns about the antibiotherapy available for the treatment of children, namely, since resistance to STX was found in three isolates from children with no previous hospitalization.

As far as we know, the single tetracycline-resistant strain, HFF189, was the first MRSA ST80 (known as the European CA-MRSA clone), PVL-positive described in Portugal. Nevertheless, since it was recovered from a periumbilical exudate of a neonate, the connection to the nosocomial setting could not be discarded, as ST80 PVL-positive isolates were already described as nosocomial isolates in the late 1990s [33]. Regular surveillance studies in Portuguese hospitals [34], together with the present study, seem to indicate that CA-MRSA-ST80 is not widely spread in Portugal in contrast to what was described in several other European countries [21, 35].

Interestingly, the remaining two MRSA isolates, PVL-negative, showed ST5-SCC*mec* type IV typical of the Pediatric clone, described for the first time in a Portuguese pediatric hospital [36]. The spread in the community of a typical HA-MRSA lineage raises some concern about the changing epidemiology of MRSA, and

Table 1 Molecular characterization and clinical presentation of the clonal lineages found among the 38 *Staphylococcus aureus* isolates recovered during the 15-month study period

No. of isolates	PFGE types (no. of subtypes)	*spa* type[a]	MLST[b]		SCC*mec* type	PVL	*agr* type	Related clone[c]	Clinical presentation (no. of isolates)
			ST	CC					
19	A (8)	t159	121	121	MSSA	−	IV		Cellulitis (9), cutaneous abscess (3), impetigo (1), wound (1), nasal swab (5)
6	B (4)	t318	30	30	MSSA	+	III	Southwest Pacific	Cutaneous abscess (6)
3	C (3)	t576	45	45	MSSA	−	I	Berlin	Cutaneous abscess (2), cellulitis (1)
2	D (1)	t535	5	5	IVc	−	II	Pediatric	Impetigo (1), bacteremia (1)
1	D (1)	t311	5	5	MSSA	+	II	Pediatric	Cutaneous abscess (1)
3	E (2)	t355	152	152	MSSA	+	I		Cutaneous abscess (1), cellulitis (1), adenophlegmon (1)
2	F (2)	t127	1	1	MSSA	+	III		Cutaneous abscess (1), pustule (1)
1	G	t044	80	80	IVc	+	III	European	Pustule (1)
1	H	t084	582	15	MSSA	−	IV		Impetigo (1)

[a] Ridom nomenclature (http://spaserver.ridom.de/)

[b] *MLST*, multilocus sequence typing; *ST*, sequence type; *CC*, clonal complex, defined by eBURST v3, assessed on 12 April 2010

[c] Common designation of community- or hospital-associated MRSA or MSSA clones

the blurring of the boundaries between the hospital and the community.

All of the isolates presented virulence determinants, namely, leukocidins (Fig. 2). Recently, a mechanism of PVL-induced acute lung injury and lung inflammation in rabbit models resolved the controversy about the role of PVL as a key factor in *S. aureus* infection [37]. In our study, isolates producing PVL or γ-hemolysin were mainly associated to cutaneous abscesses ($p=0.003$ and $p=0.000$, respectively), as has already been reported in recent studies in children infections [38, 39]. Conversely, the production of β-hemolysin, ETA, or ETB seemed to be associated with cellulitis ($p=0.010$, $p=0.003$, and $p=0.038$, respectively) (data not shown).

No significant positive association was found between socio-demographic data or possible risk factors for *S. aureus* infection, possibly due to the small dimension of the collection (data not shown).

MSSA infections, independently of the PVL content, frequently show similar epidemiological and clinical characteristics to MRSA, but specific PVL-positive MSSA lineages are dynamically interrelated and recently reported as reservoirs of CA-MRSA [23, 40]. Therefore, a regular surveillance of SSTI, namely, in children, is critical in order to predict and control the emergence of methicillin resistance and spread of staphylococcal infections in the community.

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