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

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

4

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23 **ABSTRACT**

24 In order to evaluate the incidence of CA-MRSA in Portugal, we analyzed a collection
25 of 38 *S. aureus* isolates recovered from 30 children attending the paediatric
26 emergency department of a central hospital in Lisbon due to skin and soft tissue
27 infections. Molecular characterization identified seven clonal lineages among the 35
28 MSSA isolates, out of which the major lineage PFGE A/t159/ST121 included 63% of
29 the isolates. The three MRSA isolates belonged to the Paediatric clone - PFGE
30 D/t535/ ST5-IV (n = 2) and to the European CA-MRSA clone - PFGE G/ t044/ ST80-
31 IVc. All isolates harboured several virulence factors, namely leukocidins. PVL was
32 produced by isolates from five MSSA lineages and by the ST80 MRSA. Of interest
33 this is the first reported isolation of CA-MRSA ST80 in Portugal.

34 **(127 words, max 200)**

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36 (Body text: **1386 words**)

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

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

56 Although reports of CA-MRSA prevalence are increasing worldwide, there is no
57 description of the actual scenario in Portugal, a country with >50% of HA-MRSA
58 infection, currently the highest in Europe [8]. Previous studies dated from 1996 to
59 2009, including isolates from nasal swabs of young healthy individuals and
60 nasopharyngeal swabs of children attending day care centres, reported a MRSA

61 prevalence lower than 0.25% in the Portuguese healthy community (0.24% in 1996-
62 1998 and 0.13% in 2006-2009) [6, 9, 10].

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

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

68

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

79 *S. aureus* isolates were first identified by conventional coagulase and catalase tests.
80 Additional identification and susceptibility testing with a panel of 10 antibiotics (Fig.
81 1) were performed by the semi-automatic VITEK2 system (bioMérieux, SA, France)
82 and disk diffusion method for clindamycin [11]. All isolates were tested by PCR for
83 the presence of *mecA* [12] and also characterized by 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 *SCCmec* 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 the Fisher's exact test when appropriated, considering *P* values of ≤ 0.05 statistically significant, and Odds Ratio (OD) estimates using SPSS package version 11.5 (SPSS Inc., Chicago, USA).

During the 15-months study period, 30 (73.2%) out of the 41 children that 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 was recovered, out 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. 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 paediatric communities: one MRSA isolate, PVL negative responsible for staphylococcal scalded skin syndrome in China and two MRSA, PVL positive, *SCCmec* 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 in 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 paediatric 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 more frequent type among MSSA isolates recovered in 26 European 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 comparing to MRSA, which have a predominantly regional spread of 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, positive D-test 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 children treatment, 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 Paediatric clone, described for the first time in a Portuguese paediatric hospital [36]. The spread in the community of a typical HA-MRSA lineage raises some concern about the changing epidemiology of MRSA, and the blurring of the boundaries between the hospital and the community.

All 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 controversial 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 already reported in recent studies in children infections [38, 39]. Conversely, 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 as 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 to predict and control the emergence of methicillin resistance and spread of staphylococcal infections in the community.

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FIGURE LEGENDS:

Fig. 1 Antimicrobial susceptibility of the 38 *S. 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 the resistant phenotypes observed for clindamycin mean inducible resistance, determined by D-test [11].

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; SEL, SEP, staphylococcal enterotoxins L and P.

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

No. of isolates	PFGE types (No. of subtypes)	<i>spa</i> type ^a	MLST ^b		SCC _{mec} type	PVL	<i>agr</i> type	Related clone ^c	Clinical presentation (No. of
			ST	CC					

									isolates)
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	South Westh Pacific	cutaneous abcess (6)
3	C (3)	t576	45	45	MSSA	-	I	Berlin	cutaneous abscess (2), celullitis (1)
2	D (2)	t535	5	5	IVc	-	II	Paediatric	impetigo (1), bacteremia (1)
1	D (1)	t311	5	5	MSSA	+	II	Paediatric	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)

344

345 ^a Ridom nomenclature (<http://spaserver.ridom.de/>).346 ^b MLST – multilocus sequence typing, ST – sequence type; CC – clonal complex, defined by
347 eBurst v3 assessed on 12 April 2010.348 ^d Common designation of community or hospital associated MRSA or MSSA clones.

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