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Research note

Common skin infection due to Panton–Valentine leucocidin-producing *Staphylococcus aureus* strains in asylum seekers from Eritrea: a genome-based investigation of a suspected outbreak

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ABSTRACT

Since late 2014, multiple cases of abscesses and boils due to methicillin-susceptible *Staphylococcus aureus* (MSSA) expressing the Panton–Valentine leucocidin (PVL) were observed in Eritrean asylum seekers in Lausanne, Switzerland. Strains isolated from infected Eritrean and non-Eritrean patients were compared by whole genome sequencing to determine whether these numerous cases result from an outbreak. The genome of *S. aureus* PVL-producing strains were sequenced and compared. Clinical and epidemiological characteristics of patients infected by PVL-producing strains were investigated. This work reports 15 cases of infections due to PVL-producing strains affecting mostly asylum seekers ($n = 10$), people working with refugees and/or exposed to Africans ($n = 3$). Most infections were due to closely related strains of CC152 ($n = 8$) and CC15 ($n = 3$), two distantly related (>34 000 core single nucleotide polymorphisms) clonal complexes. An epidemiological link between the 15 cases could be ruled out by whole genome sequencing (33 to 172 core single nucleotide polymorphisms between the different strains of a given complex). Altogether, these results reflect the probable high incidence of CC15 and CC152 PVL-producing strains in eastern Africa. Clinicians facing unusual skin infections in African refugees (or in any person returning from this region of high endemicity) should consider *S. aureus* PVL-producer before suspecting rare infections such as leishmaniasis or rickettsiosis. Clinicians should also remember that PVL are frequently expressed by MSSA in some regions of the world and that antibiotics that are efficient on toxin expression, such as clindamycin, represent the best therapeutic option. **L. Jaton, CMI 2016;22:739.e5–739.e8**

Introduction

In recent years, an increasing number of African migrants have been arriving in Switzerland, mainly transiting through Italy [1]. Nearly half of these migrants arriving in Italy come from Syria or Eritrea [1]. Eritreans travel first through Sudan then to Libya [2], where they are often kept, sometimes for months, in detention centres, where they face promiscuity and deficient sanitary conditions [3]. Then, according to the opportunities encountered, they may board a boat to Italy, and arrive in Switzerland.

In the Lausanne area, asylum seekers (including Eritreans) live in refugee centres. Despite satisfactory sanitary conditions, scabies was commonly observed in this migrant population. In addition, we often observed abscesses and boils largely due to infection by Panton–Valentine leucocidin (PVL) -producing *Staphylococcus aureus* strains. The PVL is a pore-forming toxin encoded on a prophage that leads to neutrophil lysis [4]. Regardless of methicillin resistance, PVL-producing strains are strongly associated with skin

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and soft-tissue infections, such as a boil or abscess [5]. PVL-encoding genes can be found in highly diverse *S. aureus* isolates [6], and the prevalence of PVL-positive methicillin-susceptible *S. aureus* (MSSA) varies widely worldwide [7]. Eighty-three per cent of PVL-encoding *S. aureus* strains in European hospitalized subjects are methicillin-resistant *S. aureus* (MSRA) [8]. In Switzerland, the prevalence of PVL-producing MSSA in nasal swabs from children was only 1.6% (9/572) in 2006 [9]. Given the significant pathogenicity of PVL-producing strains and the number of observed cases, we therefore investigated whether a potential outbreak of PVL-producing *S. aureus* skin infections occurred in the migrant population arriving in Lausanne.

Results

A total of 14 MSSA infections and one MRSA infection with positive PVL PCR was observed from January 2014 to August 2015 (see Supplementary materials). The clinical presentation of all 15 patients is reported in Table 1. Fourteen of the 15 patients exhibited a skin infection.

Seven strains belonged to ST152, three to ST15 and one to each of the following sequence types (ST): ST1633, ST121, ST5, ST10 and ST21 (Table 1). ST1633 is a single locus variant (*gmk*) to ST152 and so belongs to the same clonal complex (CC) 152. The eight CC152 cases include four Eritreans and one Ethiopian refugee, two Swiss with recent exposure to the Eritrean/Ethiopian population and one Portuguese child with a Cape-Verdean mother (Table 1). All three patients infected by CC15 strains (Fig. 1, Table 1) were Eritreans.

Core genome single nucleotide polymorphisms (SNPs) between strains from the two main CC were investigated by mapping. As mapping approaches can be sensitive to the reference choice, it was done using two different references (see Supplementary material, Materials and methods). Both gave similar results (see Supplementary material, Tables S1 and S2). CC152 strains exhibited between 33 and 172 SNPs, and CC15 between 112 and 117 SNPs (see Supplementary material, Table S1). Core SNPs were used to reconstruct the phylogeny of the two CC (see Supplementary material, Fig. S1).

A phylogenetic reconstruction based on publicly available complete genome alignments including 35 strains from 28 different ST showed that CC152 is only distantly related to other ST (Fig. 1a). The eight CC152 strains exhibited more than 34 000 SNPs with the reference genomes and 34 668 to 34 724 SNPs with CC15 strains (see Supplementary material, Tables S1 and S2). All three CC15 strains contain a 65-kb prophage encoding both the staphylococcal enterotoxin type A (*sea*) and the PVL (Fig. 1b, see Supplementary material, Fig. S3). CC152 genomes contain between two and three prophages that do not carry any additional known virulence factors (Figs S3, S4, S5).

Discussion

This work investigated PVL-producing *S. aureus* skin infections identified in Lausanne's University Hospital between January 2014 and August 2015. An outbreak was suspected because ten of the fifteen cases involved Eritreans or Ethiopian refugees.

Table 1
Demographic characteristics, clinical presentation, treatment and evolution of the 15 patients included in this work

Case number	Age	Gender	Country	SCCmec	Strain CC/ST	Co-pathogen Scabies infection	Clinical presentation	Treatment	Evolution
1	35	M	Eritrea[a]	–	152/152	No	Boils	Drainage	Favourable
2	21	M	Eritrea[a]	–	152/152	*Streptococcus pyogenes* Scabies	Pustule, abscesses	Drainage, Amoxicillin + clavulanic acid	Favourable
3	29	M	Eritrea[a]	–	152/152		Cellulitis, abscess	Amoxicillin + clavulanic acid, Clindamycin	Hospitalization
4	27	M	Eritrea[a]	–	152/152	Scabies	Adenitis	Drainage, Amoxicillin + clavulanic acid	Hospitalization
5	14	M	Portugal[b] (Mother from Cape Verde)	–	152/152	No	Cellulitis, abscess	Drainage, clindamycin	Hospitalization
6	44	M	Switzerland[b] (Ethiopian ex-wife)	–	152/152	No	Pustules, abscesses	Drainage, Amoxicillin + clavulanic acid	Favourable
7	45	F	Switzerland[b] (Rwandan husband and working with refugees)	–	152/152	No	Folliculitis, abscesses, cellulitis	Drainage, Amoxicillin + clavulanic acid, First-generation cephalosporin, Flucloxacillin, Clindamycin	Hospitalization
8	34	F	Ethiopia[a]	–	152/1633	Scabies	Folliculitis, abscesses	Drainage, Amoxicillin + clavulanic acid	Favourable
9	25	M	Eritrea[a]	–	15/15	*Streptococcus pyogenes* *Corynebacterium diphtheriae* Scabies	Abscess, ulcers	Drainage	Favourable
10	15	M	Eritrea[a]	–	15/15	No	Fibrinous wound	Amoxicillin + clavulanic acid	Favourable
11	28	M	Eritrea[a]	–	15/15	No	Abscesses	Drainage	Favourable
12	23	F	Switzerland[b]	+	72	No	Abscesses	Drainage, Amoxicillin + clavulanic acid	Favourable
13	23	M	Eritrea[a]	–	121/121	Scabies	Abscesses	Drainage, Amoxicillin + clavulanic acid, Clindamycin	Favourable
14	4	F	Switzerland[b]	–	5/5	No	Necrotic pneumonia, septic shock, parapneumonic empyema	Third-generation Cephalosporin, Clindamycin, drainage	Hospitalization
15	17	M	Eritrea[a]	–	80/80	Scabies	Abscesses	Topic treatment	Favourable

[a] Asylum seeker recently arrived in Switzerland.
[b] Living in Switzerland.

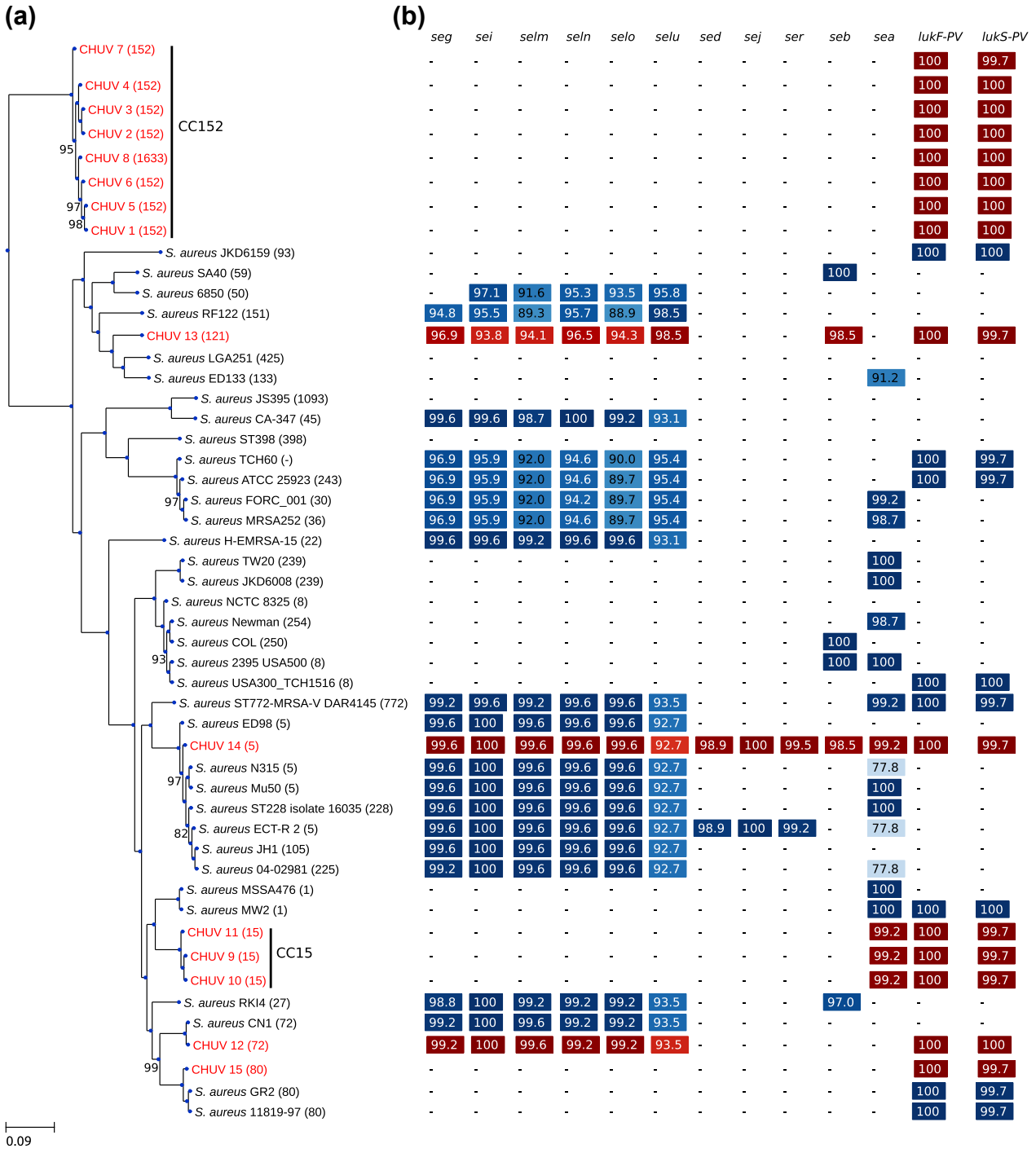

Fig. 1. (a) Phylogenetic tree of the 15 Panton–Valentine leucocidin (PVL) -positive strains isolated in Lausanne together with 35 publicly available *Staphylococcus aureus* genomes. Two clusters of eight CC152 and three ST15 PVL-producing strains could be identified. (b) *Staphylococcus* aureus toxin genes [16] identified in the 15 investigated strains: the two subunits of the PVL (*lukF/S-PV*), the exfoliative toxins a and b (*eta/etb*), the enterotoxins a b and c (*sea/seb/sec*) and the enterotoxin gene cluster (ecg: *seg, sei, selm, seln, selo, selu*). Presence of the toxins in other reference strains is indicated in blue. Numbers indicate the percentage of amino acid identity with the reference toxin (accession numbers are given in the Supplementary material, Table S3). Core genome-encoded toxins and toxins that were not found in any of the 15 genomes are reported in the Supplementary material (Figure S2).

Eleven of the fifteen *S. aureus* strains belonged to only two CC (152 and 15). The largest cluster of eight CC152 cases of skin infections affected mainly Eritrean and Ethiopian refugees ($n = 5$), or European subjects with a history of recent exposure to African people ($n = 3$). The presence of PVL strains in these three people was probably due to household transmission from their relatives originating from Africa. Promiscuity, bad sanitary conditions and injuries during travel may have favoured the dispersal of these strains within the migrant population. Five of the eleven CC152/CC15 cases presented scabies infestation, which may have favoured secondary infection by PVL-encoding *S. aureus* carried by migrants.

Strains that are part of a single outbreak are expected to share very few core SNPs (<12 [10]), with an estimated mutation rate of one SNP per ~6 weeks for *S. aureus* [11]. The two closest strains

investigated exhibited 33 SNPs, suggesting that we are not facing an outbreak. Those strains were probably imported from Eastern Africa.

ST15 MSSA are known worldwide, but toxins such as *sea* and the PVL are rare in this lineage [12]. ST152 PVL-positive was reported to be highly prevalent in western and central Africa [13]. ST15 and ST152 were reported to be common strains in Mali [14]. Import and spread of PVL-positive *S. aureus* was already documented for returning travellers [7]. Moreover, SCCmec acquisition was already reported for both ST15 and ST152 MRSA in European countries [15]. Hence, the increased occurrence of PVL-producing strains in Europe combined to the common presence of MRSA in European countries might lead to the emergence of new clones of PVL-producing MRSA.

This work showed the occurrence of two clusters of *S. aureus* infections involving closely related strains. Most cases affected Eritrean and Ethiopian refugees and few other subjects with recent exposure to Africa. These multiple skin infections probably reflect the high incidence of such strains in East African countries. Hence, when faced with an unusual dermatological infection in African refugees, and before searching for an exotic infection such as leishmaniasis, rickettsiosis or even atypical mycobacteria, clinicians must be aware that it could be an *S. aureus* PVL-producing strain and should ask for the correct test. Clinicians also need to remember that PVL may be produced by MSSA, especially in Africa and that treatment should be an antibiotic that reduces toxin expression (i.e. clindamycin). Finally, this study showed that whole genomic sequencing is a valuable tool to rapidly assess the risk of facing an epidemic and assess the precise relatedness between isolates.

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Transparency declaration

The authors have no specific conflicts of interest.

Appendix A. Supplementary data

Additional Supporting Information may be found in the online version of this article at http://dx.doi.org/10.1016/j.cmi.2016.05.026.

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