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Comparative Genomics of Community-Associated Methicillin-Resistant *Staphylococcus aureus* Shows the Emergence of Clone ST8-USA300 in Geneva, Switzerland

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(See the editorial commentary by van der Mee-Marquet on pages 1362–3.)

Background. Previous investigations of community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) isolates have revealed a wide diversity of genetic backgrounds, with only sporadic occurrence of ST8-USA300, in Geneva, Switzerland. We conducted a molecular epidemiologic analysis to identify the origin of a sudden increase of ST8 PVL–positive isolates in Geneva during 2013.

Methods. On the basis of prospective CA-MRSA surveillance, we collected colonizing and infecting ST8-USA300 isolates and compared them to non-ST8 CA-MRSA isolates. Whole-genome sequencing (WGS) was performed for each isolate of this collection, and discriminating molecular features were linked to patient data.

Results. In 2013, 22 isolates with the ST8-USA300 profile were identified among 46 cases of CA-MRSA. WGS revealed 2 groups of strains that differed by the type of the SCC*mec* IV element encoded and whether they harbored an arginine catabolism mobile element (ACME) locus. ACME-negative strains were mainly isolated from patients traveling in or originating from South America. Single-nucleotide polymorphism positions in isolate groups were used to infer their common ancestor, determine their geographical origin, and trace their relatedness.

Conclusions. WGS allowed the identification of transmission events and revealed that the increased prevalence of USA300 CA-MRSA isolates resulted from multiple importation events from the Americas but not from local clonal expansion of a successful clone.

Keywords. epidemiological survey; methicillin-resistant *Staphylococcus aureus*; USA300; importation event; genome evolution; high throughput sequencing; comparative genomics.

The epidemiology of methicillin-resistant *Staphylococcus aureus* (MRSA) is constantly evolving. After its initial identification in the early 1960s as a nosocomial pathogen, community infections were first reported in the 1990s in young healthy patients [1]. Whereas European countries were confronted with the spread of ST80 isolates originating from North Africa, the United States faced the massive transmission of the USA300 clone [2, 3]. Both of these prevalent clones harbor a SCC*mec* IV element that confers methicillin resistance and encode Panton-Valentine leukocidin (PVL), a pore-forming toxin that triggers recurrent skin and soft tissue infections or lethal necrotizing pneumonia [4–6].

In the United States, USA300 (ST8-SCC*mec* IVa) isolates are the predominant cause of community-associated MRSA (CA-MRSA) infections, accounting for up to 90% of MRSA infections in some areas [7]. Recent investigations have traced the epidemiology and evolution of this unique clone in the United States, showing that USA300 has great potential for household transmission and that specific genomic markers are shaped by antimicrobial exposure [8]. The successful dissemination of the USA300 clone was reported to be associated with mobile genetic elements, such as the arginine catabolism mobile element (ACME) [9]. The ACME cluster carries *arc* deaminase genes that promote the survival of MRSA in the acidic skin environment [10]. Furthermore, *speG*, a polyamine-tolerance gene associated with the ACME, enhances the resistance of USA300 strains to polyamines produced by human skin that are toxic to bacteria [9]. Therefore, these acquired properties endow the USA300 clones with an enhanced ability to colonize the skin of healthy people and thus, more easily disseminate in the community [2].

In Geneva, Switzerland, the first PVL-positive ST80 CA-MRSA isolate was retrospectively identified in 1994 [11]. Since 2004, our institution has performed active surveillance and molecular characterization of CA-MRSA [12]. In a previous report covering 1993–2005, we identified approximately 150 MRSA isolates harboring staphylococcal cassette chromosome *mec* (SCC*mec*) IV or SCC*mec* V elements. The European clone ST80 was predominant, accounting for 35% of isolates. Other

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frequent CA-MRSA clones were ST5 and ST88 isolates. Overall, we identified many genetic backgrounds originating from importation events across the globe [11, 13].

In 2003, a strain belonging to the USA300 lineage was first identified at Geneva University Hospital, in a female patient who had recently traveled to New York City. Since then, our molecular surveillance system revealed a few sporadic USA300 isolates until 2013, when we detected a sudden increase of CA-MRSA isolates (ST8, PVL positive). Owing to the potential virulence and spreading capacity of the USA300 clone, a molecular epidemiologic investigation was initiated to identify the causes of its increased occurrence and to guide public health interventions. This investigation also attempted to provide a better understanding of the origin and dynamics of this clone that is emerging in our population.

MATERIALS AND METHODS

Strains and Definitions

For each new patient identified with MRSA carriage or infection at the Geneva University Hospitals, the MRSA isolate was typed for its SCC*mec* element, *agr* locus, and genes encoding exotoxins [14].

A CA-MRSA case was defined as a patient with an MRSA isolate retrieved on admission or in an outpatient clinic that carried either SCC*mec* types IV or V and genes encoding for the PVL. No additional CA-MRSA cases were identified during hospitalization.

Asymptomatic CA-MRSA carriers were detected at admission in departments with universal MRSA screening policies or during household screening after identification of an infected index patient. USA300 isolates were defined as CA-MRSA isolates showing multilocus ST8 that also harbor the PVL gene and a SCC*mec* IVa element. USA300-LV (Latin American variant) isolates show the same markers except their SCC*mec* element is of the IVc type. For each CA-MRSA isolate, a case report form with the patient's epidemiological and clinical data was completed, and the corresponding CA-MRSA strain was frozen and stored by the Geneva University Hospitals central bacteriology laboratory. An anonymous database for storage of all epidemiological, clinical, and laboratory data was created. Ethical approval for CA-MRSA studies had been obtained previously from the review board of the Geneva Physicians Association, waiving the need for individual consent [13].

Data Collection and Statistical Analysis

The following epidemiological and clinical data were collected: demographic characteristics, sample and site, infection, contact with healthcare settings, potential risk factors for acquisition of MRSA, and comorbidities [13, 14]. In particular, we attempted to distinguish CA-MRSA cases with and those without USA300 strains. For descriptive information, we used proportions for categorical variables and median values and interquartile ranges for continuous variables. For hypothesis testing and comparison of CA-MRSA cases with and those without USA300 strains, we used the Student *t* test for normally distributed variables, the 2-sample Wilcoxon rank sum test for skewed distributions, and the Fisher exact test or χ^2 test for the homogeneity of proportions for categorical data. Statistical analyses were performed using Stata, version 12 (Stata, College Station, Texas).

Genome Sequencing

High-throughput sequencing was used to sequence the genomes of 23 strains, of which 22 were clinical isolates from 2013 and 1 was a USA300 strain from 2003, the oldest in our collection [11]. Purified genomic DNA for each strain was sequenced on the Illumina HiSeq (Illumina, San Diego, California), using 100 base pairs (bp) paired-end reads and bar codes strategy according to the Nextera XT kit (Illumina), following the manufacturer's recommendations.

Sequence and Genotyping Analysis

Read quality was assessed with the Fastqc program (available at: http://www.bioinformatics.babraham.ac.uk/projects/fastqc/) and filtered using the FastqMcf program (Ea-utils; available at: http://code.google.com/p/ea-utils). Genome assembly was performed using the Edena v3 [15] assembler with an overlap of 63. Assembled genomes were annotated using the Prokka v1.10 program [16]. Multilocus sequence typing analysis was performed using annotated genomes and submitted to the Center for Genomic Epidemiology database (available at: http://cge.cbs.dtu.dk/services/MLST). The phylogenetic relationship of all isolates was investigated by genomic single-nucleotide polymorphism (SNP)–based analysis using *S. aureus* USA300_FPR3757 as the reference genome [2] in the Parsnp v1.0 program [17]. The BlastP analysis was used to investigate the presence of specific genes involved in the phenotype, evolution, and virulence of the isolates. Additionally, 25 published sets of sequencing reads from *S. aureus* ST8 isolates [8] were retrieved from the European Nucleotide Archive database, assembled, and included in the genome comparisons (Supplementary Table 1).

Marker Stability and Microevolution

SCC*mec* elements IVa and IVc, and the ACME locus were compared between *Staphylococcus epidermidis* and *S. aureus* to study the stability of these markers and their evolution rates. The type of selection acting on each locus was assessed by analyzing genomic SNPs from our isolates and the reference. We calculated allelic and nucleotide diversity from randomly selected genes differing from the reference genome. The proportions of synonymous (ds) and nonsynonymous (dn) mutations were calculated, and the ratios of dn/ds were compared to theoretical values for the different schemes of selection; values equal to 0 indicate neutral evolution, values of 1 indicate diversifying selection, and values of −1 indicate purifying selection [18].

Table 1. Characteristics of Patients With Community-Associated Methicillin-Resistant *Staphylococcus aureus* (CA-MRSA) Infection or Carriage Identified at Geneva University Hospitals in 2013

Characteristic	All Patients (n = 46)	Patients With ST8 USA300 (n = 22)	Patients With Non-ST8 CA-MRSA (n = 24)	*P* Values[a]
Male sex	23 (50.0)	13 (59.1)	10 (41.7)	.238
Age, y	31 (7–50)	32 (15–43)	30.5 (7–53.5)	.768
Employment status				
Employed	15 (32.6)	10 (45.5)	5 (20.8)	.075
Unemployed	9 (19.6)	4 (18.2)	5 (20.8)	.821
Retired	6 (13.0)	2 (9.1)	4 (16.7)	.667
Student	16 (34.8)	6 (27.3)	10 (41.7)	.306
Nationality				
Switzerland	22 (47.8)	9 (40.9)	13 (54.2)	.369
Other European countries	5 (10.9)	3 (13.6)	2 (8.3)	.659
North America	0 (0.0)	0 (0.0)	0 (0.0)	1.000
South America	8 (17.4)	7 (31.8)	1 (4.2)	.020
Africa	8 (17.4)	3 (13.6)	5 (20.8)	.702
Asia	3 (6.5)	0 (0.0)	3 (12.5)	.235
People living in the same household, no.	3.5 (2–4)	3 (2–4)	4 (2–4)	.525
Adults	2 (2–2)	2 (2–2)	2 (2–2)	.663
Children	1 (0–2)	1 (0–2)	2 (0–2)	.506
Newly identified case of CA-MRSA	41 (89.1)	20 (90.9)	21 (87.5)	.711
Type of sample				
Clinical sample from infected site	32 (69.6)	14 (63.6)	18 (75.0)	.403
Abscess specimen	24 (75.0)	8 (57.1)	16 (88.9)	.040
Other	8 (25.0)	6 (42.9)	2 (11.1)	.040
MRSA screening swab	14 (30.4)	8 (36.4)	6 (25.0)	.403
Hospital stay in the past 12 mo	12 (26.1)	4 (18.2)	8 (33.3)	.242
Outpatient care in the past 12 mo	35 (76.1)	17 (77.3)	18 (75.0)	.857
Professional contact with patients	1 (2.2)	1(4.5)	0 (0.0)	.478
Potential risk factors for CA-MRSA acquisition in past 12 mo				
Contact with family member with similar lesions	3 (6.5)	2 (9.1)	1 (4.2)	.600
Stay in collective housing	3 (6.5)	0 (0.0)	3 (12.5)	.235
Travel outside Switzerland	39 (84.8)	19 (86.4)	20 (83.3)	.775
Place of travel				
North America	7 (17.9)	5 (26.3)	2 (10.0)	.235
South America	14 (35.9)	12 (63.2)	2 (10.0)	.001
Colombia	6 (42.9)	6 (50.0)	0 (0.0)	.473
Ecuador	2 (14.3)	2 (16.7)	0 (0.0)	1.000
Bolivia	1 (7.1)	1 (8.3)	0 (0.0)	1.000
Brazil	2 (14.3)	1 (8.3)	1 (50.0)	.275
Uruguay	2 (14.3)	1 (8.3)	1 (50.0)	.275
Cuba	1 (7.1)	1 (8.3)	0 (0.0)	1.000
Africa	7 (17.9)	2 (10.5)	5 (25.0)	.407
Asia	11 (28.2)	0 (0.0)	11 (55.0)	.001
Recent use of antibiotics	13 (28.3)	6 (27.3)	7 (29.2)	.887
Team or contact sports	3 (6.5)	2 (9.1)	1 (4.2)	.600
Sexual preferences				
Heterosexual	29 (63.0)	14 (63.6)	15 (62.5)	.936
Homosexual/bisexual	2 (4.3)	2 (9.1)	0 (0.0)	.223
Not applicable or unknown	15 (32.6)	6 (27.3)	9 (37.5)	.460

Table 1 continued.

Characteristic	All Patients (n = 46)	Patients With ST8 USA300 (n = 22)	Patients With Non-ST8 CA-MRSA (n = 24)	*P* Values[a]
Presence of any comorbidity	5 (10.9)	2 (9.1)	3 (12.5)	1.000
Immunosuppression	2 (4.4)	1 (4.5)	1 (4.2)	1.000
Diabetes mellitus	4 (8.7)	1 (4.5)	3 (12.5)	.609

Data are no. (%) of subjects or median value (interquartile range).

[a] Comparison between patients colonized or infected by ST8-USA300 vs non-ST8 CA-MRSA strains.

RESULTS

Epidemiological Investigation

The prevalence of isolates belonging to the ST8-USA300 or USA300-like clone was low; in 2012, only 4 isolates belonged to this clone (ST8 and SCC*mec* IV). In 2013, 594 MRSA isolates were characterized, and their SCC*mec* content was found to be distributed as follows: SCC*mec* I, 307 isolates; SCC*mec* II, 94; SCC*mec* IV, 156; and SCC*mec* V, 21; 16 were nontypeable. Overall, 46 of 594 (7.7%) were PVL positive, and the majority were identified among the SCC*mec* IV isolates (n = 43). The CA-MRSA isolation rate for 2013 was 0.48 new cases/10 000 admissions. Among those 46 CA-MRSA cases, only 4 isolates were ST80, and we noticed a sudden emergence of USA300 and USA300-like isolates in nearly half of the cases (22 isolates: clinical infection, 14; asymptomatic carriage, 8). The remaining strains belonged to ST5 and ST22. Sites of isolation of CA-MRSA ST8-USA300 and USA300-like isolates were distributed across the body rather than being localized to a particular body area. In contrast, non–ST8-USA300 strains frequently affected the facial or inguinal regions (Supplementary Table 2).

Table 1 presents clinical and epidemiological features of patients with and those without ST8-USA300 (and USA300-like) strains. Overall, there was an almost equal distribution among the sexes, with a median age of 31 years (range, 0–87 years). Approximately two thirds of patients had a clinical infection. Previous exposure to healthcare was rare, whereas travel outside of Switzerland was frequently documented. Compared with non-ST8 CA-MRSA cases, ST8-USA300 patients were more likely to be of South American origin or to have travelled to Latin America and less likely to have a skin abscess or to have travelled to Asia (particularly the Philippines). No other significant differences were observed.

Genotyping and Main Genomic Content of Isolates

The whole-genome sequences of all CA-MRSA isolates have been deposited in the DDBJ/EMBL/GenBank database, under the following accession numbers: MRSA1, LFUU00000000; MRSA2, LFUV00000000; MRSA4, LFUW00000000; MRSA5,

Table 2. Epidemiologic, Clinical, and Genomic Characteristics of ST8-USA300 or USA300-like Community-Associated Methicillin-Resistant *Staphylococcus aureus* (CA-MRSA) Isolates Subjected to Whole-Genome Sequencing

Strain	Origin or Travel Exposure[a]	Isolation Date	Clinical Presentation	Genome Size, Mb	GC Content, %	*mecA*	SCC*mec* Type	ACME	Sa1int	Sa2int	SaPI5	Sa3int
MRSA_S1	Columbia	Jan 2013	Infection	2861	32.66	+	IVc	–	–	+	+	+
MRSA_S2	Columbia	Jan 2013	Colonization	2863	32.66	+	IVc	–	–	+	+	+
MRSA_S4	Switzerland	Feb 2013	Colonization	2881	32.66	+	IVa	+	–	+	+	+
MRSA_S5	US	Feb 2013	Infection	2890	32.65	+	IVa	+	–	+	+	+
MRSA_S7	Ecuador	Apr 2013	Infection	2894	32.70	+	IVc	–	–	+	–	+
MRSA_S8	Cuba	Apr 2013	Colonization	2885	32.65	+	IVa	+	–	+	+	+
MRSA_S9	Columbia	Apr 2013	Colonization	2861	32.66	+	IVc	–	–	+	+	+
MRSA_S10	Switzerland	Apr 2013	Infection	2913	32.67	+	IVa	+	–	+	–	+
MRSA_S11	Ecuador	Apr 2013	Colonization	2894	32.69	+	IVc	–	–	+	–	+
MRSA_S12	Brazil	Jun 2013	Colonization	2913	32.67	+	IVa	+	–	+	+	+
MRSA_S13	Bolivia	Jun 2013	Infection	2852	32.67	+	IVc	–	–	+	+	+
MRSA_S14	Uruguay	Jun 2013	Infection	2837	32.68	+	IVc	–	–	+	–	+
MRSA_S15	Columbia	Jul 2013	Infection	2831	32.66	+	IVc	–	–	+	+	+
MRSA_S17	Columbia	Aug 2013	Infection	2908	32.69	+	IVc	–	–	+	+	+
MRSA_S18	US	Aug 2013	Infection	2882	32.65	+	IVa	+	–	+	+	+
MRSA_S19	Columbia	Aug 2013	Infection	2831	32.66	+	IVc	–	–	+	+	+
MRSA_S20	US	Aug 2013	Colonization	2947	32.71	+	IVa	+	–	+	+	+
MRSA_S21	Cameroon	Sep 2013	Infection	2908	32.63	+	IVa	+	–	+	+	+
MRSA_S22	Switzerland	Oct 2013	Infection	2929	32.66	+	IVa	+	–	+	+	+
MRSA_S23	Sierra Leone	Oct 2013	Colonization	2853	32.67	+	IVa	+	–	+	+	+
MRSA_S24	US	Nov 2013	Infection	2852	32.67	+	IVc	–	–	+	+	+
MRSA_S26	US	Nov 2013	Infection	2848	32.67	+	IVc	–	–	+	+	+
MRSA_S28	US	Dec 2003	Infection	2923	32.67	+	IVa	+	–	+	+	+

Abbreviations: –, absent in the genome; +, present in the genome; ACME, arginine catabolic mobile element; *mecA*, methicillin resistance gene; PVL, Panton-Valentine leukocidin; SCC*mec*, staphylococcal cassette chromosome *mec*.

[a] Data denote the country where the patient was originally from or where they traveled during the year before CA-MRSA isolation.

LFUX00000000; MRSA7, LFUY00000000; MRSA8, LFUZ00000000; MRSA9, LFVA00000000; MRSA10, LFVB00000000; MRSA11, LFVC00000000; MRSA12, LFVD00000000; MRSA13, LFVE00000000; MRSA14, LFVF00000000; MRSA15, LFVG00000000; MRSA17, LFVH00000000; MRSA18, LFVI00000000; MRSA19, LFVJ00000000; MRSA20, LFVK00000000; MRSA21, LFVL00000000; MRSA22, LFVM00000000; MRSA23, LFVN00000000; MRSA24, LFVO00000000; MRSA26, LFVP00000000; and MRSA28, LFVQ00000000.

From the genome sequencing, between 7 638 322 and 9 605 888 paired reads were produced for each of the 23 isolates. Following assembly, the average genome coverage was around 270×. Table 2 presents the features of all isolates and the main results of the gene content and WGS analysis. Genome assembly produced between 21 and 40 contigs per genome (Supplementary Table 3). Genomes sizes varied from 2.83 to 2.95 Mb, with an average GC content between 32.6% and 32.7%. The genome annotations confirmed the presence of the *mecA*, *pvlS*, and *pvlF* in all 23 strains belonging to ST8. One or 2 circular plasmids were identified from 8 *S. aureus* strains (Supplementary Table 3). All isolates contained *clfA* (except strain MRSA_S10), *fnbpA*, and *fnbpB* contributing to interaction with fibrinogen or fibronectin. Similarly, all the collected isolates harbored common hemolysins, as well as a functional *agr* locus. All but 1 isolate (MRSA_S9) harbored *clfB* (Supplementary Table 4).

Genome comparisons identified 2 groups of isolates (Table 2): one group with the full ACME cluster integrated in the genome (11 strains; ACME-positive group) and another group lacking the ACME cluster (12 isolates; ACME-negative group; Table 2 and Figure 1*A*). The ACME locus was highly conserved in the USA300 strains and was >99% identical to the ACME locus in *S. epidermidis* (Figure 1*A*). Furthermore, the ACME cluster was flanked by transposase genes (*rev*), suggesting a separate acquisition of the ACME in this MGE (Figure 1*A*). In contrast, the comparison of peptide transporter operons (*opp* locus) adjacent to the ACME cluster showed more variability, with 97% nucleotide identity and 144 SNPs, between these 2 species (Figure 1*A*). This is consistent with 2 different acquisition events in *S. aureus*, one for the ACME gene cluster and another for the *opp* locus. The different levels of polymorphisms in the ACME and *opp* regions show that the genes of the ACME locus are highly conserved. Furthermore, as shown in Figure 1*B* the dn/ds values of the ACME genes were very low and were lower than that of the *opp* locus, which is subjected to diversifying selection (Figure 1*B*).

Loci known to contribute to virulence [8] were identified in the majority of strains (Table 2). Pathogenicity islands S2int and Sa3int were identified in all isolates, whereas SaPI5 was found in all but 4 isolates (1 from the ACME-positive group and 3 from the ACME-negative group).

The profiles of susceptibility to ciprofloxacin and erythromycin were different between isolates harboring or devoid of the ACME locus (Figure 2). Indeed, all ACME-negative isolates were susceptible to these antibiotics, whereas in the ACME-positive group, 2 isolates were susceptible to erythromycin, and 1 isolate was susceptible to ciprofloxacin. All isolates were susceptible to vancomycin, fosfomycin, fusidic acid, teicoplanin, linezolid, cotrimoxazole, and mupirocin (Supplementary Table 5).

SNPs Between Whole Genomes

SNPs in the conserved core genomes of our isolates were compared to the reference genome of USA300_FPR3757 to reveal 2

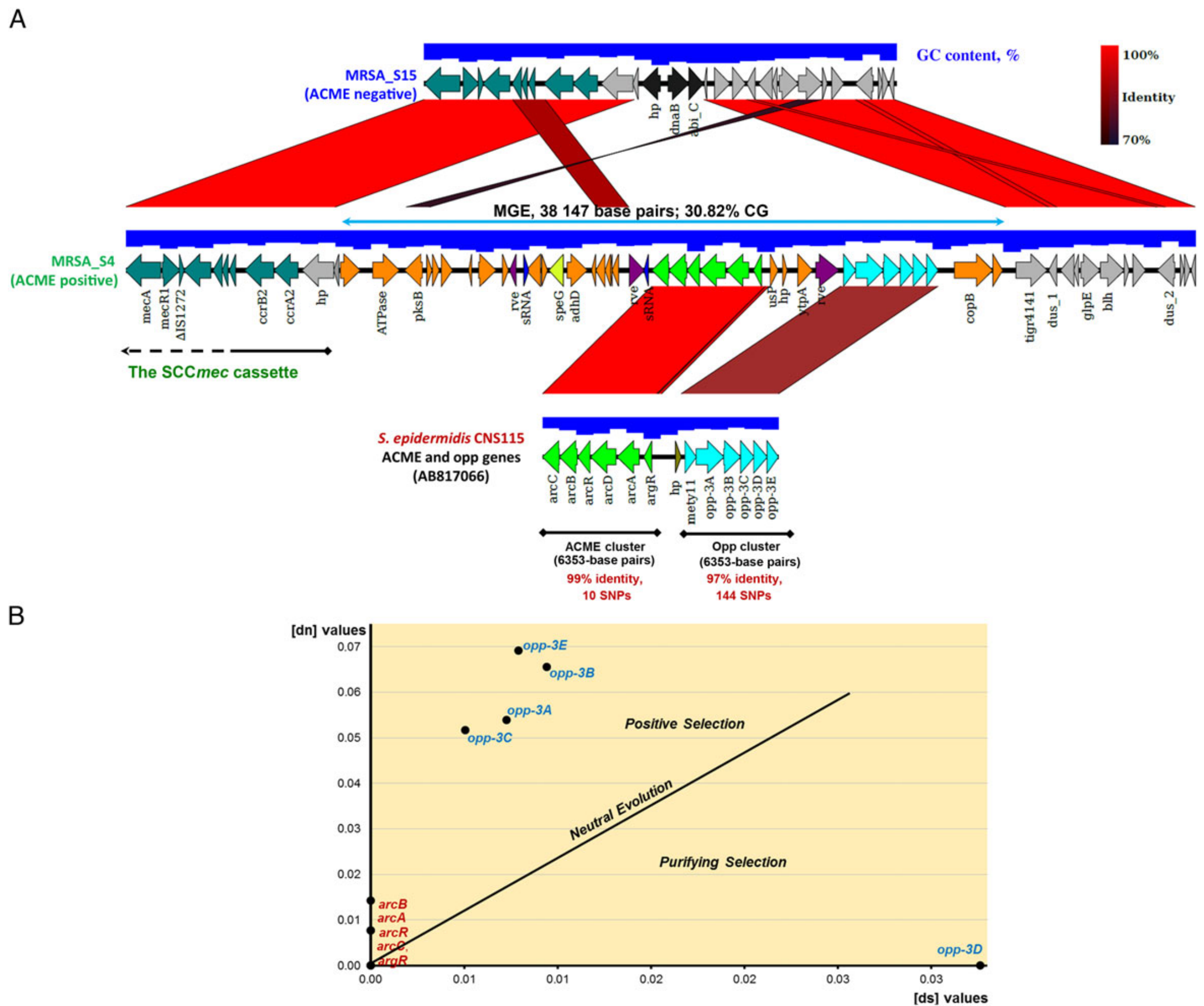

Figure 1. Evolution scheme. *A*, Genomic comparison of the arginine catabolism mobile element (ACME) locus in methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-susceptible *S. aureus* (MSSA) and between *Staphylococcus epidermidis* and *S. aureus*. Integration site of the mobile genetic element (MGE) containing the ACME and *opp* cluster genes in the MRSA isolates and comparison with *S. epidermidis* CNS115. From left to right, genes are as follows: *mecA*, penicillin-binding protein PBP 2a; *mecR1*, methicillin resistance mecR1 protein; ΔIS1272, transposase DDE domain protein; *ccrB2*, cassette chromosome recombinase B2; *ccrA2*, cassette chromosome recombinase A; *ATPase*, transporter; *rve*, resolvase; *sRNA*, small RNA; *arcC*, carbamate kinase; *arcB*, ornithine carbamoyltransferase; *arcR*, HTH-type transcriptional regulator; *arcD*, arginine ornithine antiporter; *arcA*, arginine deiminase; *argR*, arginine repressor; *usP*, universal stress protein family protein; *hp*, hypothetical protein; *ytpA*, phospholipase; *mety*11, tRNA (mo5U34)-methyltransferase; *opp*-3A, peptide ABC transporter peptide-binding protein; *opp*-3B, oligopeptide permease; *opp*-3C, oligopeptide permease; *opp*-3D, ABC transporter ATP-binding protein; and *opp*-3E, ABC transporter ATP-binding protein. *B*, Proportions of synonymous and nonsynonymous mutations in the ACME locus and the flanking peptide transporters between *S. aureus* and *S. epidermidis* are shown. The aligned sequences of each locus were used to calculate the proportion of synonymous (ds) or nonsynonymous (dn) mutations and for plotting dn/ds ratios for each gene of the ACME and the *opp* loci. The ratio of dn/ds indicates a diversifying selection for the *opp* genes, whereas ACME genes showed similar results than housekeeping genes. Abbreviation: SCC*mec*, staphylococcal cassette chromosome *mec*.

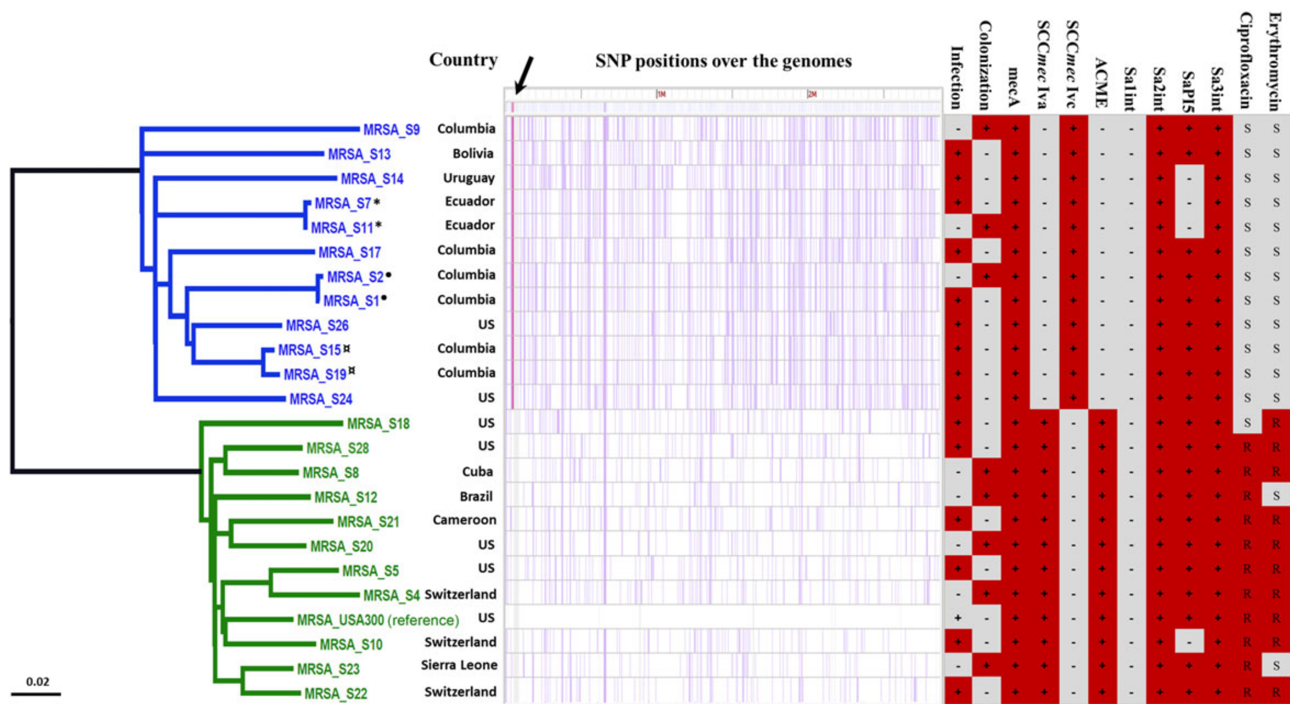

Figure 2. Phylogenetic comparison of strain collection. The USA300 genome is linearized horizontally, and each vertical bar represents a locus containing nucleotide polymorphism, compared with the reference strain (methicillin-resistant *Staphylococcus aureus* [MRSA] USA300 FPR3757). The country where the patient was originally from or where they had traveled during ≤1 year before strain isolation is shown. Two clear clusters describing arginine catabolism mobile element (ACME)–negative (upper cluster) and ACME-positive (lower cluster) strains are visible. Transmission events are indicated by symbols (*, •, and ¤). Abbreviations: –, negative; +, positive; R, resistant; S, susceptible; SCC*mec*, staphylococcal cassette chromosome *mec*; SNP, single-nucleotide polymorphism.

groups of isolates. These groups were significantly differentiated by the number of SNPs identified in their respective genomes and showed similar distances to the root of the tree. The 2 groups of isolates segregated according to the ACME gene cluster content (Figure 2): one group included exclusively ACME-negative strains and the second group included only ACME-positive isolates. The phylogenic tree depicted in Figure 2 is not consistent with a grouping of strains according to their potential country of origin. However, as shown in Figure 3*A*, the ACME-positive group exhibited fewer SNPs (88–156 SNPs) than the ACME-negative group (298–334 SNPs). Pair-wise comparisons between genomes within each group (Figure 3*B*) showed that the ACME-negative group had significantly more SNPs than the ACME-positive group (186 vs 140 SNPs; $P < .05$). Analysis of SNPs within coding regions showed that open-reading frames were approximately 10-fold less likely to have SNPs than intergenic regions. Detailed SNP analysis identified a hot spot of mutations that corresponded to a highly variable open reading frame (1788 base pairs; 34.45% GC; Figure 2) encoding a conserved hypothetical protein upstream of the SCC*mec* cassette.

Figure 3*B* showed that the ACME-negative group of isolates included 3 pairs of very closely related strains (strains S1/S2, S7/S11 and S15/S19). Epidemiological information revealed that each pair of strains was identified in members of the same family. We identified a moderate number of SNPs, varying from 4 (patients S1/S2 and S7/S11 identified within a period of 4 and 14 days, respectively) to 17 SNPs (patients S15/S19 identified within a period of 5 weeks). Excluding these 3 familial transmission events, all other isolates appeared quite distantly related to each other (88 SNPs between the 2 closest isolates), suggesting the absence of direct relatedness even between strains originating from the same country. These 3 cases of transmission involved ACME-negative isolates. In this group, the majority (9 of 12) was responsible for infection, whereas the ACME-positive isolates were equally responsible for infection or colonization.

Genetic Relatedness and Distance of Isolation

Genomes from the Geneva strains of this study were compared to those from a previous US study [8] (Figure 4 and Supplementary Table 1). A phylogenetic structure similar to that in Figure 2 was observed, with a cluster of ACME-positive strains harboring a SCC*mec* IVa element clearly separated from a cluster of ACME-negative isolates harboring the SCC*mec* IVc cassette (Figure 4). Strains with SCC*mec* element IVc were closer to the root of the tree and showed slightly higher diversity than the cluster of ACME-positive strains (higher distance within cluster). Note that strains harboring the IVb element appeared

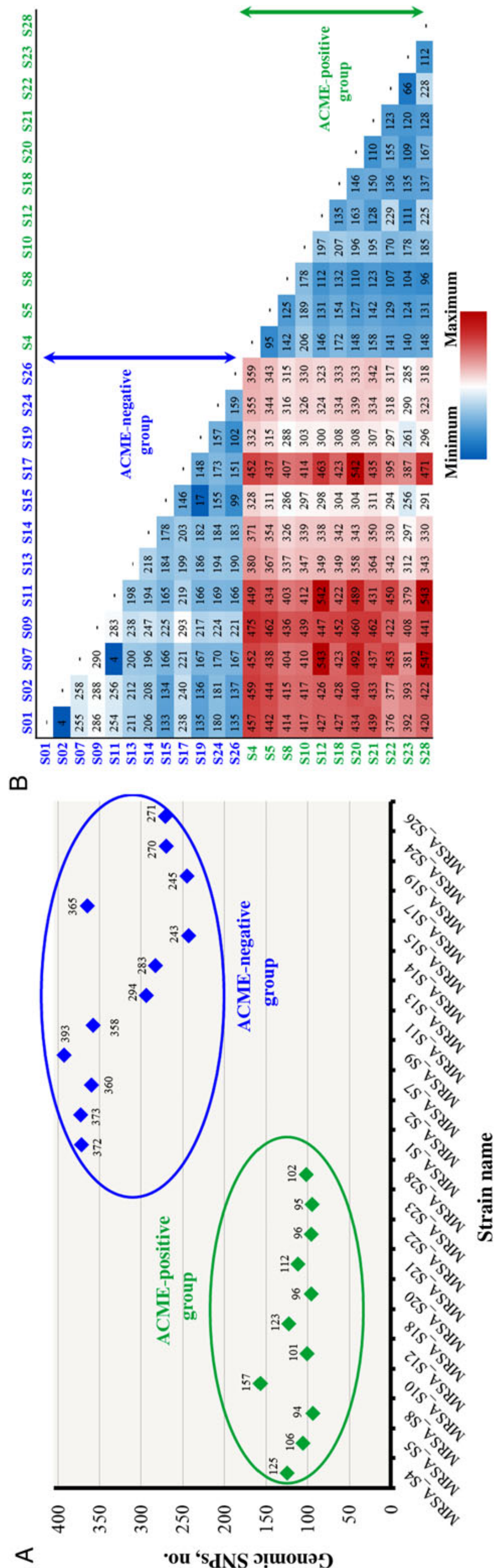

Figure 3. Analysis of single-nucleotide polymorphisms (SNPs) within arginine catabolism mobile element (ACME)–positive and ACME-negative clusters. *A*, The number of genomic SNPs, based on the reference strain, is lower among ACME-positive isolates (green), compared with ACME-negative isolates (blue). These values are determined relative to the reference genome of the reference USA300_FPR3757 strain. *B*, Pairwise comparisons confirmed this trend among the 2 clusters of isolates by identifying lower SNP numbers in the ACME group. This figure is available in black and white in print and in color online.

more distantly related to our 2 clusters of isolates and that isolates harboring the IVg SCC*mec* element were clearly the most distantly related.

Evolution Scheme and Genetic Event in ST8-MRSA

Sequence analysis at the SNP level revealed a population of homogeneous isolates with low levels of plasticity. Within our 2 groups of isolates, comparison of the common parts of the SCC*mec* elements either IVa ACME-positive or ACME-negative for the IVc revealed a high level of conservation (Supplementary Table 6) without sequence alteration.

DISCUSSION

This study showed several interesting findings. First, we documented a worrisome increase in USA300 and USA300-LV isolates in our community, related to repeated importation events from the Americas. Second, WGS revealed no clonal expansion of a successful strain spreading in our community. WGS is the only method able to distinguish between these 2 hypotheses, a parameter of particular importance for decisions based on epidemiological surveillance. Third, the only transmission events identified in our study occurred between family members. Fourth, genomic SNP analysis showed a higher heterogeneity in strains devoid of the ACME locus, despite the genomes in these groups having similar contents of mobile genetic elements. Fifth, the acquisition of different mobile elements colocalized in the chromosome, such as the SCC*mec* element, the ACME locus, and the *opp* genes flanking the ACME locus, probably represent different acquisition events. Finally, clinical infections were more frequently associated with ACME-negative CA-MRSA USA300-LV isolates, but the small number of cases precludes any definitive statement on this observation.

The reasons for the sudden increase in CA-MRSA USA300 isolates in the Geneva region remain obscure. The strong link between affected individuals and the Americas, as well as the results of WGS, suggest the absence of ongoing CA–MRSA USA300 transmission within our community. In particular, our data do not support the hypothesis of the local spreading of a successful clone. Furthermore, patterns of legal and illegal migration between Switzerland and the Americas have not changed during the last 5 years. Thus, the only remaining possibility may be related to an increasing dissemination of successful CA-MRSA USA300 strains in South America, detected randomly in infected or colonized immigrants or travelers after arrival in the Geneva region. Other recent European studies also revealed an increased rate of isolation of USA300 isolates in general hospital and pediatric hospital populations [19, 20]. Our hypothesis about the transmissibility and survival fitness of these strains is in agreement with recent data from a Pan-American genome study of 51 MRSA clinical isolates collected between 1999 and 2012 in the United States, Colombia, Venezuela, and Ecuador [21]. The genomes of these strains are not

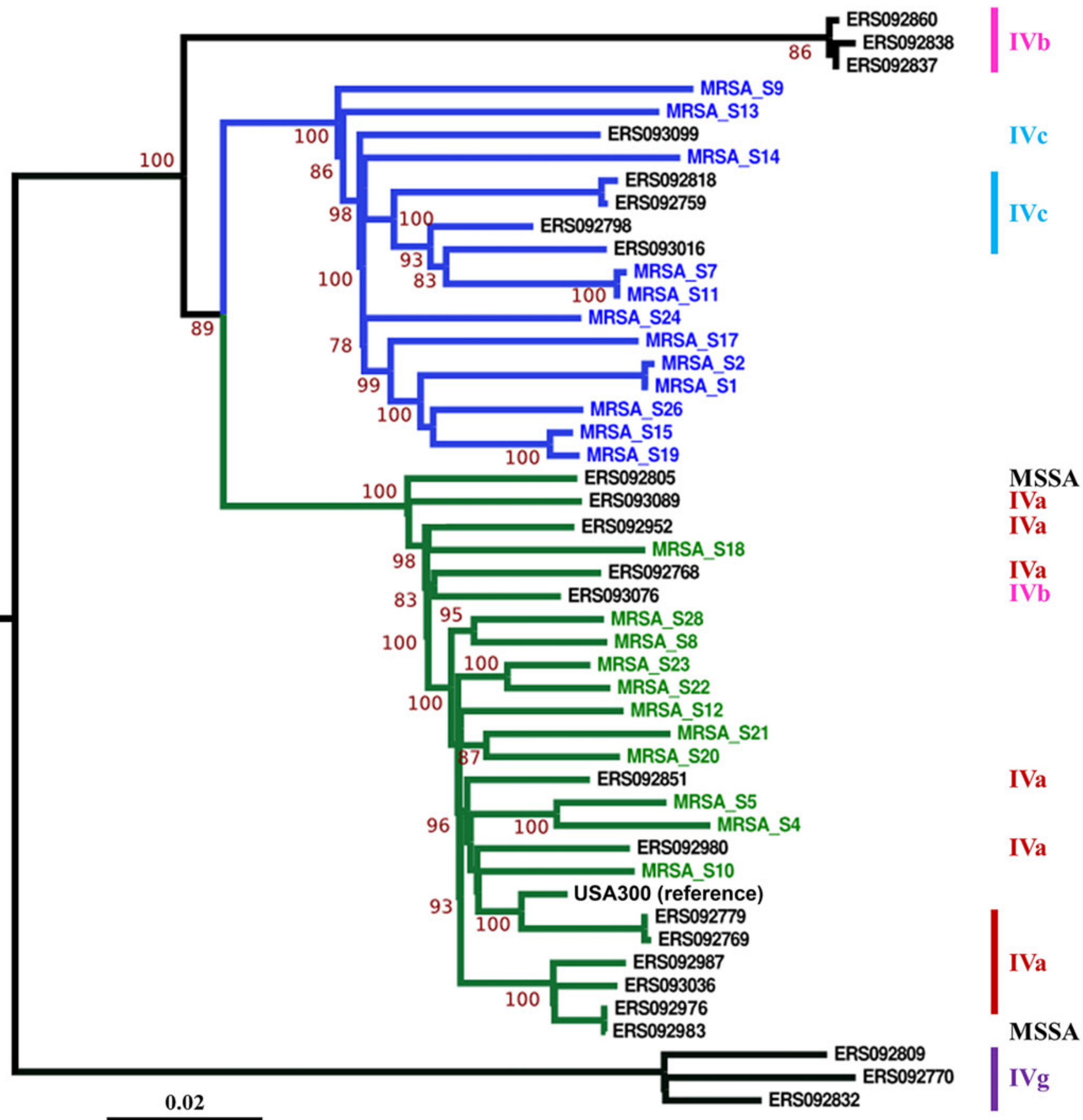

Figure 4. Phylogenetic tree comparison. Publically available genomic sequences of several strains [8] were obtained and compared to those of our strains. Distances between arginine catabolism mobile element (ACME)–positive and ACME-negative strains are consistent with the structure of the staphylococcal cassette chromosome *mec* element. Abbreviations: MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *Staphylococcus aureus*.

yet publicly available; thus, we were not able to assess their relatedness with our isolates. However, the new copper and mercury loci identified by Planet et al [21] were not detected in our strains.

Our collection of 22 CA-MRSA isolates contained 2 groups of strains that differed by the presence of the ACME locus and whether they encoded SCC*mec* IVa or IVc elements. Our strains are similar to isolates collected for a wide comparative genomic study of >387 CA-MRSA ST8 strains from a surveillance network in New York [8]. The comparison of these strains with those in our collection identifies some common features, such as the frequent absence of the ACME element in strains from South America, the SCC*mec* cassette type, or the susceptibility to fluoroquinolones (Table 2 and Supplementary Table 1).

Deciphering the cause of this sudden increase in CA-MRSA USA300 isolates by using epidemiological data and simple molecular characterization (ie, detection of PVL, SCC*mec* element typing, and multilocus sequence typing) would have resulted in the inappropriate conclusion that USA300 strains were significantly spreading in our area. Only the use of WGS allowed us to reveal that, despite 3 punctual familial clusters, USA300 strains present in our area resulted from multiple importation events. Furthermore, since a few patients were in contact with the healthcare system in the previous 12 months prior to CA-MRSA isolation, we could reasonably exclude any healthcare-associated

transmission of CA-MRSA, which remains extremely rare in our setting [22].

Our collection of isolates included 2 groups: one group containing SCC*mec* IVa and the ACME locus and a second group of ACME-negative strains harboring the SCC*mec* IVc element, also named USA300-LV [23]. A majority of strains without the ACME locus (9 of 12) were responsible for infection, whereas only one half of those containing the ACME locus were responsible for infection. The contribution of the ACME locus to virulence has been previously debated [24, 25]. Our data, obtained from a relatively limited number of isolates, do not support a special role for the ACME locus in virulence. As suggested in previous reports, the ACME locus likely confers adaptation to specific niches [7]. Interestingly, the 3 transmission events observed in our study involved only ACME-negative strains. Among these transmission events, only 4–17 SNPs were recorded at the genome scale, on >2.5 million nucleotides. Considering the date of isolation of each event, the rate of spontaneous mutation was approximately 1–2 SNPs/week, a frequency in accordance with previous observations [20]. Recent work on a large number of isolates [8] suggested a cutoff of 23 SNPs for establishing an epidemiological link between isolates, a number that is in agreement with our observation. In our study, the minimal difference observed between 2 isolates was 88 SNPs, which argues against an epidemiological link between the 2 patients. The number of SNPs detected in our 2 related groups, specifically between the most distant isolates, is also in total agreement with that determined by Planet et al, who estimated the common ancestor of USA300 and USA300-LV to have appeared in the mid-1970s [21].

Transmission of mobile elements contributing to virulence is an important event for the adaptation and spread of *S. aureus* [26, 27]. Our results showed that our 2 groups of isolates harbored the same pathogenicity islands (Sa2int, SaPI5, and Sa3int). In addition, we established that the ACME element was probably initially transferred to MRSA by *S. epidermidis* [10, 28]. In contrast, the flanking oligopeptide transporter, *opp*, appeared to be subjected to different selective pressure or to have resulted from a different insertion event. Another important difference between our 2 clades resides in their different susceptibility profiles for erythromycin and ciprofloxacin and the genes they encode for copper and mercury resistance. We speculate that the evolution and expansion of a macrolide- and fluoroquinolone-resistant USA300 clone may be related to a different antibiotic selection pressure between North and South American countries and other extrinsic features [29].

In summary, in Geneva, the epidemiology of MRSA is evolving rapidly. In only a decade, the prevalence of the European ST80 clone in community-acquired MRSA infections decreased substantially [20, 30]. Almost absent from our epidemiological studies in the past, USA300 is now the most commonly identified clonotype. WGS provides unprecedented power to identify transmission events within an apparently clonal population of strains. The absence of close relatedness in our collection clearly suggests that our area is exposed to numerous importation events, rather than to clonal spreading of an adapted clone. Active surveillance of these epidemiological parameters appears important to limit the spread of these isolates known to be particularly successful in the Americas.

Supplementary Data

Supplementary materials are available at http://jid.oxfordjournals.org. Consisting of data provided by the author to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the author, so questions or comments should be addressed to the author.

Notes

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Potential conflicts of interest. All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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