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1 **Genomic analysis of *Staphylococcus aureus* sequential isolates**
2 **from lungs of patients with cystic fibrosis**

3

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14

15 **Abstract**

16 *Staphylococcus aureus* is the predominant pathogen in children with cystic fibrosis
17 (CF) in France and, around 80 % of them harbored *S. aureus* in their lungs. This study
18 investigated virulence and antimicrobial resistance-associated genes and within-host
19 evolution polymorphisms in 14 *S. aureus* persistent clones from 14 chronically infected
20 CF children. For each of the 14 patients, we compared genomes of two isogenic
21 sequential isolates separated by 2–9 years. All isolates were methicillin-sensitive and
22 harbored the immune evasion gene cluster, whereas half of them harbored the
23 enterotoxin gene cluster. Most clones were capsule type 8 (8/14) and accessory gene
24 regulator (*agr*)-specificity group 1 (9/14). We identified convergent mutations in genes
25 involved in carbohydrate metabolism, cell wall metabolism, genetic information

26 processing and adhesion, which are likely to play important role in intracellular invasion
27 and persistence. Further explorations relying notably on proteomics will contribute to
28 improve our understanding of the mechanisms at play in the striking long-term
29 persistence ability of *S. aureus*.

30

31 **1. Introduction**

32 *Staphylococcus aureus* is a host-associated bacterium, which primarily colonizes
33 asymptotically human anterior nares of a third of healthy individuals. It is also an
34 opportunistic pathogen causing symptomatic infections ranging from superficial skin
35 and soft tissue infections to life-threatening invasive diseases [1]. *S. aureus* is one of
36 the first pathogens isolated from the airways of young cystic fibrosis (CF) patients, who
37 are chronically infected by lung-adapted *S. aureus* clones [2].

38 *S. aureus* belongs to the 'ESKAPE pathogens' category due to its resistance to
39 antibiotics. In 2018, the prevalence of Methicillin-resistant *S. aureus* (MRSA) and
40 Methicillin-sensitive *S. aureus* (MSSA) in individuals with CF in the United States was
41 25% and 55.3%, respectively [3]. In Europe, the overall proportion of MRSA in *S.*
42 *aureus* infections is below 10% [4]. CF Patients infected with MRSA are characterized
43 by accelerated lung function decline, higher mortality, and increased hospitalization
44 [5]; however, a consensus regarding the appropriate therapeutic approaches for MRSA
45 or MSSA is still lacking [6].

46 Respiratory epithelial cells of CF patients produce a thick and sticky mucus as a
47 consequence of defective transmembrane conductance regulator CFTR protein.
48 Airways of CF patients expose bacteria to selective pressures including host defense,
49 antibiotic therapy and the competition with co-infecting pathogens [7]. This
50 environment favors the emergence of highly adapted variants, which have greater

51 ability to survive and persist compared to their ancestors [8]. Phenotypic variations can
52 result from genetic changes including minor or single nucleotide variations (SNVs) or
53 horizontal gene transfer (HGT), which can be selected if advantageous [9]. Whole-
54 genome sequencing (WGS) allows the identification of underlying molecular
55 mechanisms of phenotypic variants observed during within-host adaptation, including
56 changes in antibiotic resistance, by comparing genomes of adapted isolates to the
57 ones of their isogenic ancestors [10]. In addition, analyzing the variable genome allows
58 clone assignment, typing and assessment of resistance and virulence potential. About
59 25% of *S. aureus* genes can be assigned to the variable genome, which is further
60 divided into core variable genes and mobile genetic elements (MGEs) [11]. The *S.*
61 *aureus* repertoire of virulence genes is diverse and primarily dependent on the clone.
62 Predicting clinical outcome from genotype information remains a major medical issue
63 and large-scale studies reporting *S. aureus* genomic analyses are needed to improve
64 genotype-phenotype relationships.

65 Using a collection of early-late pairs of sequential clonal isolates recovered from 14
66 patients, we recently investigated the consequences of *S. aureus* adaptation on the
67 inflammatory response of respiratory cells [2]. The purpose of the current study was to
68 report the genomic analysis of 14 persistent clones belonging to 12 different sequence
69 types representative of major circulating clones in Europe [12].

70

71 **2. Materials and methods**

72 **2.1. Study populations, bacterial isolates and genome sequencing.**

73 Bacterial isolates and patients' characteristics have been described previously [2].

74 Briefly, at the time of early isolate collection, children ranged in age from 42 days to 13

75 years and from 2 to 21 years at the time of late isolate collection. The CF1 patient was

76 co-colonized with *Pseudomonas aeruginosa* at the time of early and late isolate
77 collection whereas the CF8, CF12, CF13 and CF17 patients were co-colonized with *P.*
78 *aeruginosa* after the time of early isolate collection. *P. aeruginosa* was not found in the
79 other patients.

80 The whole genomes were sequenced on an Illumina MiniSeq instrument (2x150
81 paired-end sequencing) using a Nextera XT library preparation kit (Illumina) following
82 the manufacturer's instructions.

83 **2.2. Genome comparison, molecular typing, resistance and virulence genes**

84 Assemblies were performed with de novo assembler SPAdes 3.9.0 and annotated
85 using Prokka version 1.14.0. Single-nucleotide polymorphisms (SNPs) and small
86 indels were assessed using Snippy v3.1 (<https://github.com/tseemann/snippy>). Briefly,
87 Snippy was used to map the raw short reads of late isolates against the annotated
88 assembly of the cognate early isolates and to classify variants by their predicted
89 functional effect.

90 *S. aureus* isolates were typed by in silico multilocus sequence typing (MLST) using
91 srst2 tool to directly match raw fastq files against the database of allele sequences and
92 of sequence type profiles retrieved from pubmlst.org.

93 The alleles of virulence-associated genes including genes encoding toxins, immune
94 evasion factors, microbial surface components recognizing adhesive matrix molecules
95 (MSCRAMM) as well as typing markers such as capsule and agr group typing markers
96 were retrieved from Strauss *et al* [13]. The resulting database of 1,711 alleles from
97 182 genes were used with srst2 tool to match raw fastq files directly. The online
98 webtool PathogenWatch (<https://pathogen.watch>) and spaTyper 1.0
99 (<https://cge.food.dtu.dk/services/spaTyper/>) were used to predict antibiotic resistance
100 profiles and spa typing respectively. The sequences reported here are available

101 at the National Center for Biotechnology Information's BioProject database under
102 accession PRJNA720901.

103 **2.3. Antimicrobial susceptibility testing**

104 The susceptibility testing was performed and interpreted according to the European
105 Committee for Antimicrobial Susceptibility Testing (The European Committee on
106 Antimicrobial Susceptibility Testing Breakpoint tables for interpretation of MICs and
107 zone diameters Version 11.0 <http://www.eucast.org>). For all isolates, susceptibility to
108 penicillin (1 U), ceftiofur (30 µg), gentamicin (10 µg), tobramycin (10 µg), tetracycline
109 (30 µg), chloramphenicol (30 µg), erythromycin (15 U), clindamycin (2 µg),
110 ciprofloxacin (5 µg), linezolid (10 µg), and trimethoprim- sulfamethoxazole (1.25 +
111 23.75 µg), fusidic acid (10 µg) were determined.

112 **2.4. Determination of mutation frequency**

113 To assess the mutability rate, bacteria were cultivated until the stationary phase in
114 trypticase-soy (TS) broth at 37°C, 175 rpm. At the stationary phase, $\sim 10^8$ bacteria were
115 spread onto TS agar supplemented with 100 µg/mL rifampin or 50 µg/mL streptomycin
116 (in cases of initial resistance to rifampicin) while serial tenfold dilutions of the same
117 cultures were plated onto TS agar without antibiotics for the determination of total
118 colony forming units (CFUs). After 48 h of incubation at 37°C, the number of colonies
119 was counted, and the mutation frequencies to rifampicin were determined relative to
120 the total count of viable organisms plated [14]. The experiments were carried out three
121 independent times.

122 **2.5. Statistical analysis**

123 Data were analyzed using GraphPad Prism 8 software. Results are presented as
124 median \pm 95% CI (confidence interval). The results were compared by nonparametric

125 tests. Median mutation frequencies were compared by the Kruskal–Wallis test. All p
126 values were considered to be significant at $p \leq 0.05$.

127

128 **3. Results**

129 This study investigated 14 pairs of sequential isogenic early and late isolates (14 early
130 and 14 late isolates) separated by 2- to 9-year intervals (Table S1). After de novo
131 assembly of genomes, coding DNA sequences (CDSs) were annotated and mutations
132 were identified by comparing late isolates to early isolates from each patient. The
133 maximum likelihood phylogenetic tree was shown in a previous study [2]. The
134 polymorphisms identified were further categorized by type and predicted impact on
135 coding sequences. The total number of polymorphisms between early and late
136 genomes ranged from 1.5 to 365 mutations per year. The great majority of single-
137 nucleotide polymorphisms (SNPs) fell in coding regions and resulted in missense
138 variants.

139 The largest numbers of polymorphisms between the early and late isolates were
140 observed in patients CF8 and CF10 with 136.7 and 365 mutations per year
141 respectively. Considering that the reported mutation rate is approximately 8 mutations
142 per genome per year for the *S. aureus* species [15], this suggests a particular ability to
143 mutate in the CF8 and CF10 patients' clones. The role of a defect in methyl-mismatch
144 repair (MMR) system, through *mutS* and *mutL* mutations, in hypermutability of *S.*
145 *aureus* has been well described [16]. Therefore, we analyzed the protein sequences of
146 MutS and MutL of CF8 and CF10 isolates. Sequence alignment showed that the
147 amino-acid sequences of MutS and MutL from CF8 and MutL from CF10 were identical
148 between early and late isolates. We found 1 amino-acid change (V516I) in the MutS
149 sequence of the late isolate of CF10 compared to the early isolate.

150 To further test the hypermutability hypothesis, we determined the mutation frequency
151 of isolates from all the patients to rifampin or streptomycin resistance. All the tested
152 strains showed mutation rates below 10^{-7} , which is the mutation rate reported for
153 mutator strains [17] (Fig. 1).

154 Genotyping information regarding ST, CC, spa-type, *agr* group, and capsule type are
155 provided in Table 1. The most frequent *agr* type was type I (9/14), followed by type II
156 (3/14), and type III (2/14). The most frequent capsule type was type 8 (8/14).

157 A summary of the occurrence of non-core (*i.e.*, genetic elements not shared by all
158 isolates) virulence-associated genes as determined by whole genome analysis is
159 presented in Table 1. The immune evasion gene cluster (IEC) is carried by β -
160 hemolysin (*hly*)-converting bacteriophages, which integrate within *hly* gene and
161 therefore prevent β -haemolysin production. It was previously shown to be present in
162 90% of the human clinical *S. aureus* isolates [18]. The IEC was present in the clones
163 of all patients. This cluster harbored the staphylococcal complement inhibitor (*scn*)
164 gene and a different combination of *sea* (encoding staphylococcal enterotoxin A), *sep*
165 (encoding staphylococcal enterotoxin-like P), *sak* (encoding staphylokinase) and *chp*
166 (encoding the chemotaxis inhibitory protein). The enterotoxin gene cluster (*egc*:
167 harboring the *sei*, *seg*, *sem*, *sen*, *seo*, *seu* enterotoxin genes) was present in the clones
168 of 7 out of 14 patients. The toxic-shock syndrome toxin (*tst*) was present in only 1
169 clone.

170 In Table S2, the antibiotic resistance of each isolate and the presence of the related
171 genes are summarized. The predominant antibiotype in early isolates was an exclusive
172 resistance to penicillin through the *blaZ* gene presence (9/14 early isolates). In most
173 of the patients (10/14), no difference was observed in the antibiotype between early
174 and late isolates. In 2 patients, the *blaZ* gene was lost in late isolates, resulting in

175 penicillin susceptibility (late isolates CF10 and CF11); in another 2 patients, point
176 mutations in *rpoB* conferring resistance to rifampicin were observed. In addition, late
177 isolate of CF16 also exhibited mutations in *fusA*.

178 To identify genes potentially involved in the long-term persistence of *S. aureus* in CF
179 lungs in our cohort, we focused on genes mutated in the late isolates of at least 2
180 patients compared to their cognate early isolates (excluding CF8 and CF10 patients).
181 We thus identified 12 genes with SNPs listed in table 2. Mutated genes were involved
182 in carbohydrate metabolism, cell wall metabolism, genetic information processing and
183 adhesion. Concerning CF8 and CF10 patients, most of the more than 900
184 polymorphisms separating early and late isolates were synonymous, missense and
185 intergenic genetic alterations. Notable variations in CF8 and CF10 late isolates include
186 frameshifts and stop codon acquisition in genes encoding staphostatin B (*sspC*),
187 aspartokinase (*lysC*), DNA-3-methyladenine glycosylase 1 (*tag*), Fe²⁺ transporter
188 (*feoB*), and heptaprenyl diphosphate synthase component 2 (*hepT*).

189

190 **4. Discussion**

191 We conducted a genetic analysis of 14 pairs of *S. aureus* sequential isolates from the
192 lungs of patients followed in a French cystic fibrosis center. There is no consensus on
193 the curative or preventive treatment of *S. aureus* in children with CF in France
194 compared to Great Britain [19]. In our center, children received anti-staphylococcal
195 antibiotics only in cases of clinical deterioration. For each patient, only pairs of
196 genetically related isolates were submitted to in-depth analysis. This strategy enables
197 us to easily identify genetic changes distinguishing late from early isolates, to
198 investigate phenotypic consequences of genetic changes (such as antibiotic
199 resistance), and to search for convergent evolution across pairs of isolates. By using

200 comparative genome sequencing of isolates obtained sequentially from respiratory
201 samples of CF patients, several studies have improved our understanding of *S. aureus*
202 adaptation during long-term chronic infections [20]. Despite the coexistence of several
203 lineages in their airways, it has been shown that CF patients harbor a dominant
204 persistent lineage of *S. aureus* [21]. Since, there are no specific ST associated with
205 CF, most frequent ST associated with carriage and infections can be found in CF
206 patients. For instance, animal-related ST398 isolates [22], which cause serious human
207 infections have lately emerged as animal-independent pathogens, particularly in
208 France and are observed among patient of our study. ST398 isolates have already
209 been observed in CF patients [23].

210 Supporting previous reports, the majority of isolates (64.3%) of our study were *agr* I.
211 In addition, *agr* type II (21.4%) and type III (14.3%) were also found, supporting the
212 notion that while the distribution of *agr* types may vary from one geographic region to
213 another, *agr* type I is predominant and *agr* type IV is rare [24, 25].

214 Long *et al.* reported the whole-genome sequencing of 1,382 *S. aureus* isolates
215 collected longitudinally from 246 CF children across the United States, half of whom
216 were infected with MRSA [20]. The authors showed that *S. aureus* lineages undergo
217 convergent evolution in key persistence and antimicrobial-resistance genes. They also
218 showed that *in vivo* mutation rates during CF infection were similar to values estimated
219 in other *S. aureus* populations [20]. In contrast, in the clones of the 14 patients
220 investigated in our study, none were resistant to methicillin as the proportion of MRSA
221 in France is below 10% [4].

222 The importance of mutator strains in CF patients has been initially described in a
223 seminal study carried out on *Pseudomonas aeruginosa* isolates from 30 CF patients
224 revealing that 36% of the patients harbored a hypermutable strain [26]. Indeed, the

225 positive selection of mutators appears to be a common feature of chronic infections
226 [27]. Hypermutable *S. aureus* strains in CF patients has also been reported [28] and
227 attributed to a defective MMR system due to *mutS* or *mutL* mutations [14].
228 Nevertheless, persistence of mutator *S. aureus* strains in CF patients seems far less
229 frequent to what has been observed with *P. aeruginosa* species [29].

230 However, despite a high number of genetic polymorphisms suggesting that patients
231 CF8 and CF10 have mutator strains, no phenotypic observation of high mutation rates
232 was obtained. A likely explanation is that hypermutability has been lost after
233 reacquisition of MMR gene functions thus stabilizing mutations acquired during
234 hypermutability. Another possibility would be the infection of the patient with a strain
235 belonging to the same lineage.

236 Adaptation during the course of infection commonly occurred with selection of
237 advantageous mutations in a set of genes related to persistence such as transcriptional
238 regulators and antimicrobial resistance (e.g., *thyA*, *rpoB*, and *walk*) [8, 20]. In line with
239 previous studies, we identified mutations linked to resistance in *rpoB* and *fusA* genes.
240 We also identified mutations in *ebh* gene encoding a membrane protein involved in
241 intracellular invasion and persistence [20, 30]. Of note, mutations in *agr* locus involved
242 different genes (*agrA*, *agrC* and *agrD*) and have been previously reported [2].

243 To date, the presence of specific virulence genes has not been associated with
244 changes in CF patients' outcome. However, presence of genes does not predict their
245 expression and further explorations relying on proteomics will contribute to improve
246 our understanding of the mechanisms at play in the striking long-term persistence
247 ability of *S. aureus*.

248

249 **Ethics statement**

250 All experiments were performed in accordance with the guidelines and regulations
251 described by the Declaration of Helsinki and the law Huriot-Serusclet on human
252 research ethics and informed consent was obtained for all participating subjects.
253 Clinical isolates of *S. aureus* were obtained from airway secretions from patients with
254 cystic fibrosis at the Necker-Enfants Malades University Hospital, Paris, France.
255 Sputum sampling is part of routine standard care. The research procedure is validated
256 by Ile-de-France 2 IRB (ID-RCB/Eudract: 2016 A00309-42).

257

258 **Declaration of competing interest**

259 All authors declare no competing interests.

260

261 **Acknowledgments**

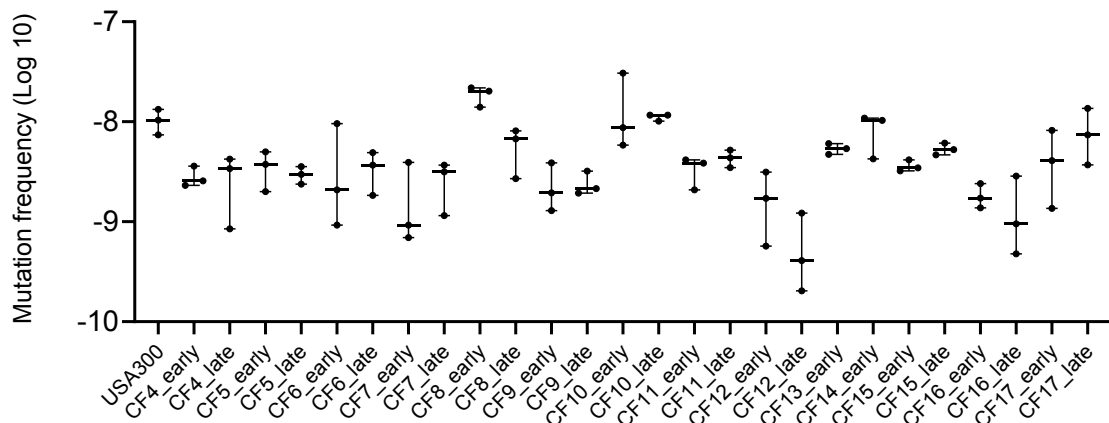
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267

268 **Appendix A. Supplementary data**

269 Supplementary data to this article can be found online.

270 **Figure**



271

272 **Figure 1. Mutation frequencies for clinical isolates and control strain**

273 Mutation frequencies to rifampicin or streptomycin resistance for USA300-LAC strain

274 (USA300) and clinical isolates were determined. USA300 was shown as a control.

275 Spontaneous mutation frequency for each replicate was calculated as the number of

276 antibiotic-resistant colonies on trypticase-soy (TS)- plates containing antibiotics

277 divided by the number of colony-forming units counted following plating on TS-agar

278 plates without antibiotic. CF13_late and CF14_late isolates are not represented due to

279 the lack of obtention of antibiotic-resistant colony. Error bars indicate median with 95%

280 CI (n = 3). Significance was assessed by the Kruskal–Wallis test.

Table 1. Typing and virulence genes in clones of 14 patients

Patient	ST/CC/spa	agr group	capsule genotype	virulence genes
CF4	59/59/t316	1	8	<i>chp, sasG, sak, scn, seb, sek, seq</i>
CF5	45/45/t230	1	8	<i>chp, cna, sak, scn, sec, seg, sei, sel, sem, sen, seo, seu</i>
CF6	6/5/t701	1	8	<i>cna, sasG, lukED, sak, scn, sea,</i>
CF7	398/398/t7880	1	5	<i>chp, cna, scn</i>
CF8	8/8/t8	1	5	<i>chp, sasG, lukED, sak, scn</i>
CF9	398/398/t6587	1	5	<i>chp, cna, scn</i>
CF10	5/5/t002	2	5	<i>chp, sasG, lukED, sak, scn, seg, sei, sem, sen, seo, sep, seu</i>
CF11	2355/1/t4206	2	5	<i>cna, sasG, scn, sec, sei, sel, sem, sen, seo, seu</i>
CF12	1165/45/t4206	1	8	<i>chp, cna, sak, scn, seg, sei, sem, sen, seo, seu</i>
CF13	22/22/t2359	1	5	<i>chp, cna, sasG, sak, scn, seg, sei, sem, sen, seo, seu</i>
CF14	15/15/t084	2	8	<i>chp, sasG, lukED, scn</i>
CF15	SLV-78/t786	3	8	<i>sasG, lukED, sak, scn, sec, sel</i>
CF16	45/45/t31	1	8	<i>chp, sak, scn, sec, seg, sei, sel, sem, sen, seo, seu</i>
CF17	30/30/t018	3	8	<i>chp, cna, sak, scn, sea, seg, sei, sem, sen, seo, seu, tst1</i>

SLV-78: Single locus variant of ST78.

Table 2. Genes with non-synonymous SNPs in several late isolates compared to early isolates (excluding CF8 and CF10)

Ortholog	Gene name	SNP type	Product	Category	Patient
SAUSA300_0020/ SAUSA300_0021	<i>walKR</i>	MS	Two-component system involved in cell wall metabolism and biofilm formation	Cell wall metabolism	CF12, CF17
SAUSA300_0132	<i>epsF</i>	MS	O-antigen biosynthesis glycosyltransferase	Cell wall metabolism	CF16, CF17
SAUSA300_0333	<i>bgfG</i>	MS	Transcriptional antiterminator involved in carbohydrate metabolism	Metabolism (Carbohydrates)	CF14, CF17
SAUSA300_0527	<i>rpoB</i>	MS	DNA-directed RNA polymerase subunit beta	Genetic information processing	CF14, CF16
SAUSA300_0708	<i>hisc</i>	MS, FS	Histidinol-phosphate aminotransferase	Metabolism (Amino acids)	CF4, CF15
SAUSA300_0772	<i>cifA</i>	MS	Cell surface-associated protein clumping factor A	Adhesin	CF9, CF11
SAUSA300_1251	<i>parC</i>	MS, ins	DNA topoisomerase IV, subunit A	Genetic information processing	CF4, CF13, CF17
SAUSA300_1327	<i>ebh</i>	MS, stop	Extracellular matrix-binding protein	Adhesin	CF4, CF15
SAUSA300_1611	<i>valS</i>	MS	Valine--tRNA ligase	Genetic information processing	CF5, CF11
SAUSA300_1851	<i>tagG</i>	MS	hypothetical protein teichoic acid transport system permease protein	Cell wall metabolism	CF12, CF17
SAUSA300_2324	<i>scrA</i>	MS	PTS system, sucrose-specific IIBC component	Metabolism (Carbohydrates)	CF7, CF15
SAUSA300_2541	<i>mgo</i>	MS, FS	putative malate: quinone oxidoreductase 2	Metabolism (Carbohydrates)	CF4, CF16

MS, missense; FS: frameshift; ins: insertion

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Supplementary Materials for

Genomic analysis of *Staphylococcus aureus* sequential isolates from lungs of patients with cystic fibrosis

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This file includes:

Tables S1 to S2

Table S1. General features of detected mutations in *S. aureus* late isolates of 14 patients compared to early isolates

Patient	CF4	CF5	CF6	CF7	CF8	CF9	CF10	CF11	CF12	CF13	CF14	CF15	CF16	CF17
Time since early isolate (years)	4.4	4.2	5.3	2.7	6.7	1.9	3.0	7.1	4.3	2.9	9.4	4.5	9.2	2.0
Single-nucleotide polymorphism	19	29	6	27	903	9	1085	23	18	13	27	63	42	50
Insertion–deletion	1	1	2	2	13	1	10	2	1	1	1	2	9	2
Located in Coding sequence	12	21	5	20	751	8	917	17	16	10	19	45	38	32
Nonsynonymous mutation (%)	9 (75)	14 (66.7)	4 (80)	14 (70)	³⁶³ (48.3)	6 (75)	391 (42.6)	12 (70.6)	11 (68.8)	5 (50)	14 (73.7)	35 (77.8)	31 (81.6)	24 (75)
Frameshift variant	1	1	2	2	13	1	10	2	1	1	1	2	9	2
Missense variant	8	13	2	12	337	4	367	10	9	1	12	34	20	21
Premature stop codon	0	0	0	0	3	0	1	0	1	1	1	1	2	0
Other	0	0	0	0	10	1	13	0	0	2	0	0	0	1
Synonymous mutation	3	7	1	6	388	2	526	5	5	5	5	10	7	8
Intergenic	8	9	3	9	165	2	176	8	3	4	9	18	13	17
Total polymorphisms	20	30	8	29	916	10	1095	25	19	14	28	65	51	52
Mutations/year	4.5	7.1	1.5	10.7	136.7	5.3	365	3.5	4.4	4.8	3.0	14.4	5.5	26

Table S2. Antibiotic resistance in *S. aureus* pairs of early and late isolates of 14 patients

Patient	Resistance phenotype early isolate	Resistance genotype early isolate	Resistance phenotype late isolate	Resistance genotype late isolate
CF4	Pen	<i>blaZ</i>	Pen	<i>blaZ</i>
CF5	Pen	<i>blaZ</i>	Pen	<i>blaZ</i>
CF6	Pen	<i>blaZ</i>	Pen	<i>blaZ</i>
CF7	Ery	<i>ermT</i>	Ery	<i>ermT</i>
CF8	Pen	<i>blaZ</i>	Pen	<i>blaZ</i>
CF9	Ery	<i>ermT</i>	Ery	<i>ermT</i>
CF10	Pen	<i>blaZ</i>	Susceptible	None
CF11	Pen, Tet	<i>blaZ, tetK</i>	Tet	<i>tetK</i>
CF12	Pen	<i>blaZ</i>	Pen	<i>blaZ</i>
CF13	Susceptible	None	Susceptible	None
CF14	Pen, Tmp	<i>blaZ, dfrG</i>	Pen, RA, Tmp	<i>blaZ, dfrG, rpoB_S464P</i>
CF15	Pen	<i>blaZ</i>	Pen	<i>blaZ</i>
CF16	Pen	<i>blaZ</i>	Pen, FD, RA, Ery	<i>blaZ, fusa_P404L, rpoB_R484H</i>
CF17	Pen	<i>blaZ</i>	Pen	<i>blaZ</i>

Pen: Penicillin; Ery: Erythromycin; Tet: Tetracycline; Tmp: Trimethoprim; RA: Rifampin; FD: Fusidic acid