Genetic Diversity and Ecological Success of *Staphylococcus aureus* Strains Colonizing Humans^{▽†}

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Received 9 July 2008/Accepted 24 October 2008

The genetic determinants and phenotypic traits which make a *Staphylococcus aureus* strain a successful colonizer are largely unknown. The genetic diversity and population structure of 133 *S. aureus* isolates from healthy, generally risk-free adult carriers were investigated using four different typing methods: multilocus sequence typing (MLST), amplified fragment length polymorphism analysis (AFLP), double-locus sequence typing (DLST), and *spa* typing were compared. Carriage isolates displayed great genetic diversity which could only be revealed fully by DLST. Results of AFLP and MLST were highly concordant in the delineation of genotypic clusters of closely related isolates, roughly equivalent to clonal complexes. *spa* typing and DLST provided considerably less phylogenetic information. The resolution of *spa* typing was similar to that of AFLP and inferior to that of DLST. AFLP proved to be the most universal method, combining a phylogeny-building capacity similar to that of MLST with a much higher resolution. However, it had a lower reproducibility than sequencing-based MLST, DLST, and *spa* typing. We found two cases of methicillin-resistant *S. aureus* colonization, both of which were most likely associated with employment at a health service. Of 21 genotypic clusters detected, 2 were most prevalent: cluster 45 and cluster 30 each colonized 24% of the carrier population. The number of bacteria found in nasal samples varied significantly among the clusters, but the most prevalent clusters were not particularly numerous in the nasal samples. We did not find much evidence that genotypic clusters were associated with different carrier characteristics, such as age, sex, medical conditions, or antibiotic use. This may provide empirical support for the idea that genetic clusters in bacteria are maintained in the absence of adaptation to different niches. Alternatively, carrier characteristics other than those evaluated here or factors other than human hosts may exert selective pressure maintaining genotypic clusters.

Staphylococcus aureus is an important human pathogen, but it also colonizes a large fraction (20% to 60%) of healthy humans without causing any harm to the great majority of carriers. *S. aureus* is a particularly problematic organism because of the apparent ease of acquiring antibiotic resistance, as exemplified by methicillin-resistant *S. aureus* (MRSA). The frequency of MRSA among hospital-associated strains has been increasing steadily over the last 40 years, but the speed of this process varies greatly among different locations.

Despite the prevalence of hospital-associated MRSA, infections caused by carriage strains also constitute a significant problem. A majority (over 80%) of *S. aureus* carriers who developed blood infection after admission to a hospital became infected by their own colonizing strains (36, 38). Until recently, colonizing strains were typically methicillin sensitive despite the spread of MRSA in the hospital setting. Now this situation has changed, but the reasons that MRSA, after 40 years of fortuitous inability to colonize people outside the

hospital setting, apparently quite suddenly acquired this crucial ability remain obscure (14). However, this lack of understanding is not surprising. The genetic determinants and phenotypic traits which make an *S. aureus* strain a successful colonizer are largely unknown. Until recently, accurate and accessible typing methods necessary to tackle these issues were not available. Moreover, colonization with *S. aureus* was not considered a serious public health issue until the appearance of community-acquired MRSA (CA-MRSA).

Although a lot is known about factors, both phenotypic (24, 26) and, more recently, genetic (33, 34), predisposing hosts to *S. aureus* colonization, whether specific bacterial genotypes are adapted to specific hosts is unknown. Because human nares are by far the most common niche of *S. aureus*, if different genotypes indeed show differential adaptations they should be evident as adaptations to colonization of phenotypically and genetically variable hosts. Understanding the patterns or, better still, mechanisms of association or adaptation of different bacterial genotypes with carriers may help to shed light on a recent CA-MRSA epidemic.

The phenomenon of nasal carriage of *S. aureus* has been the object of a great number of studies (reviewed in references 26 and 35), but the diversity and population structure of *S. aureus* isolates from a sufficiently large sample of healthy people have rarely been investigated (10, 22). Unbiased sampling from a

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† Supplemental material for this article may be found at <http://aem.asm.org/>.

[▽] Published ahead of print on 31 October 2008.

healthy population is crucial for understanding the basic biology of *S. aureus* as well as providing an essential reference point for studies concerned with specific subject groups, such as hospital patients or human immunodeficiency virus carriers.

We set out to examine the diversity and population structure of colonizing *S. aureus* strains from a healthy adult population to investigate the differential success of bacterial strains and the potential reasons for these differences. Moreover, we set out to determine whether different bacterial genotypes appear to be associated with different types of carriers. To achieve these goals, we sampled a defined population from a specific location. At the same time, we investigated which of several typing methods are most useful for these goals, employing the well-established method of multilocus sequence typing (MLST) (9) and the increasingly popular *spa* typing method (17, 19) and comparing them to the more recently developed amplified fragment length polymorphism analysis (AFLP) (22, 37) and double-locus sequence typing (DLST) (21) methods.

MATERIALS AND METHODS

Sample collection. Nasal swabs were collected from 405 newly employed hospital personnel during their first medical checkup at a tertiary care hospital in Lausanne, Switzerland. From each subject, one nasal swab was obtained (Amies agar transport swabs; Copan). At the same time, demographic data and medical and employment history were obtained. The strains were collected over a period of 9 months. Ethical clearance was sought and obtained from the ethical committee of the University of Lausanne.

The swabs were stored at 4°C and processed within 3 weeks. Each swab was vigorously rubbed in 1 ml Tris-EDTA buffer and then quantitatively plated on an SAID (bioMérieux) specific chromogenic plate (*S. aureus* colonies appear green, and other staphylococci appear white). After 24 to 48 h of incubation at 37°C, green colonies were counted, inoculated into 800 µl of tryptic soy broth, incubated overnight at 37°C, and frozen at -80°C. To detect colonization with even a very small number of bacteria, the swab was transferred to 5 ml of Bacto staphylococcus broth (Difco) and incubated overnight at 37°C. The enrichment broth was then plated on an SAID plate and incubated overnight at 37°C. If any green colonies were found, they were processed in the same manner as those from primary plates. Eight isolates per subject were collected from directly inoculated plates, as well as another isolate from the plate inoculated with enrichment broth. To screen for MRSA, the enrichment broth was placed on MRSAselect (Bio-Rad) selective chromogenic agar, which gives MRSA colonies a pink appearance. Phenotypic screening for resistance to methicillin on MRSAselect plates was followed by PCR screening for an internal fragment of the *mecA* gene with the following primer pair: *mecA*_{Sa_fw_865}, 5'-AAA AAG CTC CAA CAT GAA GA-3'; and *mecA*_{Sa_rv_1211}, 5'-GTT GAA CCT GGT GAA GTT GT-3'.

Genotyping. DNAs were extracted from 600-µl to 1-ml overnight cultures grown in brain heart infusion broth as previously described (8). AFLP was performed as described previously (22, 37), with some modifications. At least two isolates per person were genotyped, one from a direct inoculation plate and one from an enrichment plate. Approximately 100 to 150 ng of chromosomal DNA was digested with the restriction enzymes MboI and Csp6I (Fermentas). Oligonucleotide linkers for Csp6I (5'-TAG TCA GGA CTC AT-3' and 5'-GAC GAT GAG TCC TGA C-3') and MboI (5'-CTC GTA GAC TGC GTA CC-3' and 5'-GAT CGG TAC GCA GTC TAC-3') were ligated to the digestion mixture. This was followed by a nonselective round of amplification using the primer pair 5'-GAC GAT GAG TCC TGA CTA C-3' and 5'-GTA GAC TGC GTA CCG ATC-3'. For selective amplification, 6-carboxyfluorescein-labeled primers were used. The extensions of selective primer Csp6I contained either G or AA, and the extensions of selective primer MboI contained either G or C. Markers originating from C-AA and G-G amplifications were analyzed. The amplified material was analyzed with an ABI3100 automated sequencer (Applied Biosystems).

MLST analysis was performed as previously described (9) on 133 *S. aureus* isolates, typically one per person. Whenever a previously unknown allele was found, the typing procedure was repeated starting from new DNA extraction material.

DLST was performed as previously described (21). Highly polymorphic frag-

TABLE 1. Characteristics of the participants

Characteristic	No. of participants with characteristic/no. without (%) ^a	
	Carriers (n = 130)	Noncarriers (n = 275)
Gender	49/81	86/189
Recent hospitalization (<6 mo)	7/123 (5.4)	15/260 (5.5)
Recent antibiotic use (<6 mo)	17/111 (13.3)	51/221 (18.8)
Recent employment in health care (<3 mo)	60/70 (46.2)	124/150 (45.3)
Chronic skin condition (eczema or psoriasis)	20/110 (15.4)	17/257 (6.2)
Diabetes	0/129	3/271 (1.1)
Kidney conditions	1/128 (0.8)	2/273 (0.7)
Current use of immunosuppressants	1/127 (0.8)	4/269 (1.5)
Liver conditions	0/129	0/273

^a Except for gender, for which data are given as number of males/number of females.

ments of the *spa* and *clfB* genes were amplified and sequenced. Similar to the MLST scheme, in DLST each unique sequence is given a consecutive number, and a combination of the two numbers uniquely identifies the DLST genotype. Unlike the case for MLST, two isolates with exactly the same alleles can be assumed to be very closely related.

The repeat region of the *spa* gene was amplified as previously described (17, 30). The *spa* types were assigned with an online *spa* database (<http://www.spaserver.ridom.de/>). BURP clustering was performed with default settings.

Data analysis. Electropherograms of AFLP patterns were analyzed with the help of GeneMapper software (Applied Biosystems). Fragments of 75 to 500 bp were included. In this analysis, the markers corresponded to genomic fragments of unique size. The marker sets (bin sets) created by GeneMapper were manually corrected so that unreliable markers were identified by comparison of several isolates from the same subject. Unreliable markers were removed from the analysis. Each marker was scored as either 0 or 1, with 157 and 170 markers per G-G combination and C-AA selective primer combination, respectively. Most analyses were done on a concatenated data set (327 markers).

Bayesian phylogeny was examined with MrBayes (28). The runs had 10,000,000 generations, 100,000 trees were sampled, and the first 1,000 trees were discarded as "burn-in." In addition, a neighbor-joining (NJ) tree based on Nei and Li distances was constructed with 1,000 bootstrap replicates, using Phylip (12).

Multilocus sequence types (STs) were identified by comparison to the *S. aureus* MLST database (<http://www.mlst.net/>). Each unique sequence defines an allele, and a unique combination of alleles defines an ST. STs were assigned to groups by use of the criterion of five common alleles (10), with the help of eBURST (vs3) (11). Several methods of phylogeny building were also applied to the concatenated data set. Bayesian phylogeny was examined as outlined above. An NJ tree (bootstrapped 1,000 times) was based on F84 and JK distances. In addition, a maximum likelihood tree using PhyML (15, 16) with 100 bootstrap replicates was constructed. DLST data were also analyzed with eBURST. All statistical analyses were done with the R package (Foundation for Statistical Computing [<http://www.R-project.org/>]).

RESULTS

Carriage. A total of 130 of 405 (32%) volunteers were *Staphylococcus aureus* carriers. In 101 cases, *S. aureus* colonies were found on primary plates; the remaining carriers were detected only after overnight incubation of cultures in enrichment broth, implying that the number of bacteria in vivo was very low. The basic epidemiological data are summarized in Table 1. The studied population consisted of generally healthy adults (18 to 65 years of age) with a low incidence of risk factors, with one exception. Nearly one-half of the volunteers (46%) had recently worked in a health care setting.

As reported previously, the percentage of carriers decreased with age (logit regression; $F_{1,402} = 6.6$ and $P = 0.015$); the

FIG. 1. Pie chart representing the diversity in 133 carriage isolates of *S. aureus*. Each section represents an isolate. Thicker black lines separate different DLST types. Sections of the same color represent isolates of the same ST; similar colors represent clusters of STs as defined by eBURST analysis. Red stars denote MRSA isolates.

mean ages of carriers and noncarriers were 30.7 years and 33.0 years, respectively. We also found that volunteers suffering from chronic skin problems, such as eczema or psoriasis, were more likely to be carriers of *S. aureus* (Fisher's exact test; $P < 0.005$). We did not find any other effect of medical history on *S. aureus* carriage. Concerning in vivo abundance, more *S. aureus* CFU ($\log_{10}$ transformed) were found in the carriers who had recently been hospitalized (previous 6 months) (mean = 2.16; standard deviation = 0.80) than in the carriers who had not (mean = 1.44; standard deviation = 0.98) (analysis of variance; $F_{1,102} = 4.00$ and $P = 0.048$).

Diversity. According to MLST, there were 37 unique STs among 133 isolates (Fig. 1). Seven new alleles and 10 STs were not previously recorded. New STs found in this study were submitted to the MLST database and newly designated (ST1158 to ST1167). The much higher resolution of DLST allowed us to distinguish 111 unique genotypes, and with the two methods combined, 115 unique genotypes among 133 strains were found. Thus, nearly all of the subjects had a unique strain. Only three subjects were found to be colonized with more than one strain of *S. aureus* (and one with two very similar strains).

Despite very diverse colonization, there was one particularly successful DLST genotype, 26_110 (ST30, *spa* type t012), which colonized eight carriers. We found a (nonsignificant) trend that this dominant genotype occurred more often in carriers who had a recent history of employment in health care (75% versus 46% among all carriers), who were treated with antibiotics (25% versus 15% among all carriers), or who were recently hospitalized (12% versus 6% among all carriers).

spa typing identified 79 different types, which is slightly less than the number of different *spa*_500 loci used in DLST. This is not surprising, as the two loci largely overlap, with *spa* repeats typically completely enclosed within the *spa*_500 locus.

Thirteen new types were identified and newly designated (t4023 to t4035).

All but one pair of isolates produced patterns that were different in at least 1 of 327 AFLP markers (see Fig. S1 in the supplemental material). Eighty-eight percent of the 327 markers were polymorphic. The maximum number of different markers between a pair of isolates was 89, with an average of 29. When the cutoff level for distinguishing different strains was taken to be fewer than five bands, 95 distinct genotypes could be distinguished. One strain was nontypeable by AFLP, with abnormal chromatograms and a very high run-to-run variability.

Two isolates were MRSA. They belonged to two different clusters, ST105 (DLST 2_36, *spa* type t067) and ST45 (DLST 1_1, *spa* type t038). DLST revealed that both types were previously known to colonize and infect patients in the same region (1). Both MRSA carriers had recently worked in health care settings.

Population structure. MLST data analyzed with eBURST delineated nine clusters and 11 singleton types among 37 different STs. Clusters contained sequence types which differed by two loci at the most. All new STs were single- or double-locus variants of known STs. In most cases, STs within each group differed by one or two (ST7 and ST1159) point mutations. There were two exceptions. ST942 was grouped together with ST707 as its double locus variant. However, one allele (*arcC*) of ST942 had five substitutions and another (*yqiL*) had three substitutions in comparison to ST707. This is more consistent with recombination than with point mutation and is evident as a rather large genetic distance between the two isolates (Fig. 2A). Similarly, ST291 and ST398 were also double locus variants. They differed in the *aroE* and *yqiL* alleles by two and four substitutions, respectively, pointing to a possibility of recombination.

FIG. 2. Population structure of 133 carriage isolates of *S. aureus* according to various typing methods. Clusters identified by eBURST analysis of MLST data are indicated by colors. Red stars denote MRSA isolates. Fifty percent consensus Bayesian trees of concatenated MLST (A) and AFLP (B) data are shown. The posterior probability is the proportion of trees supporting a given branch. (C) eBURST analysis of DLST data. The size of the circles corresponds to the number of isolates. Lines join isolates which differ in one of the two loci. (D) BURP clustering based on *spa* types. The size of circles is proportional to the number of isolates. Blue circles are inferred founders. The thickness of the lines represents the cost or distance between *spa* types. *spa* types which have been struck through had to be excluded from analysis because of a small number of repeats (<5).

FIG. 3. Association between number of isolates in each genetic cluster and mean in vivo abundance. Means and standard deviations of *S. aureus* CFU estimated for the entire sample are shown.

Bayesian analysis of the concatenated MLST data set gave results which were highly concordant with the eBURST analysis (Fig. 2A). All clusters delineated by eBURST had 100% posterior probability support, with the exception of cluster 5, which had 95% support. Thus, groups containing only a few STs appeared as well defined as larger groups which corresponded to previously identified clonal complexes. Moreover, judging from branch lengths, “singleton” STs were also equally distinct. No other branch had >90% support, with the following exception: a long branch with 100% support appeared to divide all the isolates in two parts.

Concatenated AFLP data were used to construct a Bayesian tree (Fig. 2B). The tree was highly concordant with MLST data in the delineation of genotype clusters. All clusters displayed 100% support based on AFLP data, with the exception of ST707 and ST942 (54%). The groups which contained isolates of only one sequence type showed good AFLP support (minimum level, 93%). The major divide found by MLST was 98% bootstrap supported by AFLP data. A bootstrapped NJ tree also highly supported the existence of clonal complexes, but the support for the major divide was 75% (not shown). The majority of deeper branches had <90% bootstrap support. The main inconsistency between the MLST-based and AFLP-based results was the existence of a 100% supported branch in the AFLP tree. This was absent in the MLST results.

eBURST analysis of DLST data identified 15 groups and 39 singletons (Fig. 2C). All of the groups identified by eBURST were contained within clusters identified by analysis of MLST data, with the exception of *spa*₅₀₀ allele 109, which occurred in isolates belonging to both ST34 and ST10.

BURP analysis of *spa* types identified 15 groups and 17

singletons. Six types were excluded because of an insufficient number of repeats (<5) (Fig. 2D). Similar to DLST analysis, *spa* type t166 was represented by two STs, ST10 and ST34. Likewise, one of the *spa* clusters spanned two divergent STs, ST101 and ST25, which share only one MLST locus (*aroE*).

Effects of bacterial genotype. To test for the effects of bacterial genotype on some aspects of colonization phenotype, we had to group genetically related isolates. We used clusters identified by eBURST analysis of MLST data because their relevance was also highly supported by AFLP. We decided, however, to split ST707 and ST942 because their close relatedness was strongly questioned by both AFLP and sequence-based MLST analyses. We decided to keep ST291 and ST398 in one group because AFLP analysis unambiguously supported this branch. Thus, there were 21 clusters, which we designated with the number of the most numerous ST. Some clusters (six) contained only one isolate, and no statistical inference was possible for them.

The genotype of *S. aureus* found in the carriers appeared to be affected by a history of previous hospitalization (Fisher's exact test; P value = 0.04915). This result was entirely due to an unusual result for cluster 291 (ST398 and ST291). Two of three carriers (66%) colonized by cluster 291 were recently hospitalized (i.e., in the last 6 months), while the incidence of recent hospitalization among all the carriers was only 6%. Accordingly, when cluster 291 was removed from the analysis, this effect disappeared.

There was no association of *S. aureus* genotype with the carrier's age, sex, recent antibiotic treatment, or previous employment in health service. However, the in vivo abundance of bacteria depended on the genotype (analysis of variance; $F_{14,84} = 1.92$ and $P < 0.04$) (Fig. 3).

DISCUSSION

Genetic diversity. Genetic diversity among carriage strains of *S. aureus* was extremely high, with the majority of carriers being colonized with a distinct strain. The most successful isolate colonized only 7/130 (5.4%) carriers. This is in sharp contrast to hospital-acquired MRSA isolates from the same region, which were collected at the same time. In that case, 50% of the patients were infected or colonized by an identical DLST type, and only 92 DLST types among 438 isolates were found (1). To date, pulsed-field gel electrophoresis (PFGE) has been a method of choice for finest-scale epidemiology. PFGE resolution appears similar to DLST resolution (21), and in earlier studies which utilized PFGE, substantial diversity in carriage isolates was found. In probably the largest study, 146 types could be distinguished among 296 isolates (20). However, as with any method which relies on analysis of DNA fragment patterns, quantification and comparison between studies are not always straightforward, and diversity is a function of an arbitrary cutoff level used to identify distinct genotypes. Since DLST uses sequence data, our data unambiguously showed the extreme diversity in carriage strains. As could be expected, *spa* typing resolution was inferior to DLST resolution. Using the *clfB-500* locus greatly increased the typing resolution, further confirming its importance for microepidemiological studies.

MLST, although having a resolution inferior to that of DLST, provides an important possibility for global comparisons. The "Oxford study" (6, 10) used MLST to investigate population structure in a large unbiased collection of *S. aureus* isolates from healthy adult carriers. There were 49 STs among 179 carriage isolates, while in the present study we found 37 STs among 133 isolates. Thus, diversity on the level of MLST appeared very similar in both studies. Moreover, the same dominant clonal complexes (CC5, CC8, CC15, CC22, CC25, CC30, and CC45) were found in both studies. More recent studies from diverse locations (18, 23, 29) also confirmed the global presence of these groups.

The resolution of AFLP was intermediate between those of MLST and DLST. For this analysis, we adopted a safe cutoff level of at least a five-band difference to designate a distinct genotype, but as mentioned above, the definition of a distinct genotype is arbitrary.

Population structure and phylogeny. MLST data, although lacking in resolution, provide a very good tool to survey global epidemiology and the evolutionary history of bacterial species. Our data support the patterns observed in the well-defined population of the Oxford study (10). Some genotypes which are very abundant in terms of number of isolates also have large numbers of clonal descendants (single-locus variants). Such groups are thought to have arisen by recent clonal expansion, and their existence is interpreted as recent ecological and evolutionary success (4). All new STs in our data set likely originated by point mutations. In the whole data set, there were possibly two STs which were most likely products of recombination detected by MLST. DLST analysis confirmed another case of recombination, namely, a previously described large chromosomal replacement between ST10 and ST30 (27). This gave rise to ST34, in which the region containing *spa* originated from ST10. Accordingly, *spa*₅₀₀ allele 109 occurred in isolates belonging to both ST34 (cluster 30) and ST10

(cluster 10) (Fig. 2C). On the whole, our findings confirm the predominantly clonal mode of evolution in *S. aureus*.

Analysis of AFLP data largely agreed with inferences based on MLST. In particular, the AFLP-based assignment of isolates to clusters identified by analysis of MLST data was very robust. Both analyses identified the same clusters of isolates, with Bayesian analysis support in most cases being 100%. Given the fact that AFLP screens the whole genome for polymorphism in a largely unbiased manner, the clusters identified by MLST are thus relevant to the whole genome of *S. aureus*. We also concluded that carefully performed AFLP can be used with high confidence to assign *S. aureus* isolates to clonal complexes for a fraction of sequencing costs. Melles et al. (22) found a similar concordance between AFLP and MLST results, as well as the particular distinctness of ST30 and ST45.

DLST loci encompass highly polymorphic and quickly evolving repeat domains of two genes, i.e., *clfB* and *spa* (21). However, the analyzed sequences consist of incomplete repeats. Therefore, the clustering is based solely on allelic information and works only for types which are identical in one of the two loci. Despite the fact that the *spa* typing-based BURP algorithm does use sequence data directly to infer relatedness among types, it did not appear to perform better than eBURST analysis of DLST. BURP analysis correctly clustered slightly more isolates belonging to the same MLST cluster, but on the other hand, a sizable proportion of isolates had to be excluded from analysis due to an insufficient number of repeats. In addition, STs 101 and 25 were clustered together by BURP, despite being rather divergent, suggesting either another case of recombination or homoplasy. This implies that using a second locus is important for inferring relatedness, even for highly clonal *S. aureus* isolates. Nevertheless, neither method can be considered suitable for more serious phylogenetic or evolutionary studies because only the closest relatedness among types can be detected. Both, however, are highly appropriate for epidemiological applications.

Neither AFLP nor MLST gave a very clear picture of the relationships among the clusters, despite very good agreement in delineating groups of closely related isolates representing recent clonal expansions. Cooper and Feil (5) proposed a phylogeny of *S. aureus* with a major division into two definitive groups, based on concatenated fragments of 30 genes encompassing 18 kb. The most obvious difference in our results was unambiguous placement of ST22 far from ST45 and ST30, while in the study of Cooper and Feil (5), ST22 clustered together with ST45 and ST30. While MLST-based analysis might be biased by a small number of loci, AFLP-based phylogeny also unambiguously rejected a close association of ST22 with ST30 and ST45. While more markers certainly permitted the construction of a more robust phylogeny, the choice of strains might be as important. Different studies are likely to include different rare recombinants, as a large proportion of recombination events results in the creation of unsuccessful and thus rare genotypes. If present, the recombinants can seriously distort the overall phylogenetic picture, and we conclude that it will be rather difficult to construct an "ultimate" phylogeny of this species.

MRSA. Only two carriers were colonized with MRSA (>2%), similar to the results of other studies of risk-free populations (20, 31). Moreover, using DLST, we were able to

identify recent employment at local health services as the most likely source of MRSA colonization in both cases. The *spa* types of both isolates (t038 and t067) have also been detected in many European countries. We conclude that in this region, true community-acquired MRSA is rare.

Ecological success and bacterial genotype. It is clear that dominant clones of *S. aureus* change over time, both in hospital settings (2, 7) and in colonization, as exemplified by the recent CA-MRSA epidemic in the United States (3). We found that some bacterial genotypes, such as DLST type 26_110, as well as some genotype clusters, such as C45 and C30, were clearly more successful, as they were able to colonize more carriers than the others. However, the basis of this success is unclear. The most predominant genotypes did not show a particularly high abundance in vivo, showing that this trait is not necessarily the key to success. A bacterial genotype's success should be evident as spread in the carrier population over time, either by displacement of other genotypes or by colonization of previously uncolonized carriers. These possibilities are best tested in a longitudinal study where the same cohort of carriers is sampled over time. Such a study is currently under way in our labs.

The population structure of *S. aureus* was similar to that in previous studies (10), namely, isolates formed distinct clusters. Adaptation to different ecological niches has been proposed as a driving selective force responsible for the appearance and maintenance of the clusters (4); this would make clonal complexes meaningful biological entities. It has even been suggested that for *S. aureus* clonal complexes should be elevated to the species status (32). We found only a little evidence of an association of bacterial genotype with different host attributes, such as sex, age, or medical history. The association of cluster 291 (ST291 and ST398) with recent hospitalization was based on a very small sample size and needs to be confirmed. Similarly, there was a trend for a dominant DLST genotype 26_110 (ST30) association with health service, but due to the small sample size, it is not possible to verify its significance.

Thus, our results question the idea that genotype clusters or clonal complexes in *S. aureus* are adapted to different niches. The issue remains of how clearly the identity of genotype clusters in *S. aureus* is maintained in the absence of adaptation. Our data may lend support to the idea that genotype clusters, at least for this species, may be maintained in the absence of positive selection (13). Alternatively, it is possible that adaptation to other factors, such as phages or other bacteria, exerts more selective pressure on *S. aureus* than does adaptation to a human host. Moreover, the possibility of association with carrier characteristics not evaluated here, such as genotypic factors, remains to be tested by sampling colonizing *S. aureus* isolates from carriers of diverse genotypes originating from diverse locations. More generally, understanding evolutionary history and population structure requires sampling from a wide geographical range, and such studies yield interesting insights (18, 29).

ACKNOWLEDGMENTS

This work was supported by a Marie Heim Vögtlin grant (PMPDA-106195) from the Swiss National Science Foundation to O.S.

We thank all of the volunteers for their participation and the staff of Medecine du Personnel of CHUV, in particular Ursina Schild Abegglen, for performing the sampling.

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