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Absence of Patient-to-Patient Intrahospital Transmission of *Staphylococcus aureus* as Determined by Whole-Genome Sequencing

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ABSTRACT Nosocomial transmission of pathogens is a major health care challenge. The increasing spread of antibiotic-resistant strains represents an ongoing threat to public health. Previous *Staphylococcus aureus* transmission studies have focused on transmission of *S. aureus* between asymptomatic carriers or used low-resolution typing methods such as multilocus sequence typing (MLST) or *spa* typing. To identify patient-to-patient intrahospital transmission using high-resolution genetic analysis, we sequenced the genomes of a consecutive set of 398 *S. aureus* isolates from sterile-site infections. The *S. aureus* strains were collected from four hospitals in the Houston Methodist Hospital System over a 6-month period. Importantly, we discovered no evidence of transmission of *S. aureus* between patients with sterile-site infections. The lack of intrahospital transmission may reflect a fundamental difference between day-to-day transmission events in the hospital setting and the more frequently studied outbreak scenarios.

IMPORTANCE Previous studies have suggested that nosocomial transmission of *S. aureus* is common. Our data revealed an unexpected lack of evidence for intrahospital transmission of *S. aureus* between patients with invasive infections. This finding has important implications for hospital infection control and public health efforts. In addition, our data demonstrate that highly related pools of *S. aureus* strains exist in the community which may complicate outbreak investigations.

Staphylococcus aureus is a leading cause of morbidity and mortality worldwide. It causes the majority of bloodstream and soft tissue infections in the developed world (1–5) and is responsible for more annual deaths in the United States than HIV (6). It is also a major contributor to hospital-acquired infections (7–9). Practices implemented to reduce patient-to-patient transmission include an emphasis on hand hygiene and environmental cleaning, as well as screening and decolonization protocols, with various levels of evidence supporting the success of these efforts (7, 8, 10–16). Current methods employed to track intrahospital transmission include traditional epidemiology (patient location, shared caregiver) and molecular typing of the infecting strain. However, recent evidence suggests that current typing methods are not sufficiently sensitive to reveal small differences in strains that are related but not necessarily directly transmitted (17).

Although *S. aureus* is a heterogeneous species, the majority of human disease (both hospital and community acquired) is caused by a relatively small subset of clones (18–21). Current typing methods, including multilocus sequence typing (MLST or ST type) and *spa* typing, rely on sequencing short segments of a few genes and lack the resolution to genetically differentiate between related but distinct isolates (22–27). Several recent studies using next-generation DNA sequencing technology have underscored its ability to distinguish between isolates deemed identical by traditional typing methods (17, 23–25, 27–31).

Despite its well-established role in nosocomial infections, recent evidence has questioned the extent to which patient-to-patient transmission contributes to acquisition of *S. aureus* within an intensive care unit (ICU) environment (31). Other studies examining person-to-person transmission have focused on asymptomatic carriers, outbreak scenarios, or *S. aureus* clones not prevalent in the United States (23–25, 28, 31). The primary goal of this study was to determine the extent of intrahospital strain transmission events involving invasive *S. aureus* infections. To this end, we sequenced the genomes of consecutive sterile-site *S. aureus* isolates (*n* = 398 strains) collected from 305 patients over a 6-month period. The associated patient demographic clinical information allowed mapping of potential links between patients infected with genetically related strains. Our data set also permitted us to address the within-host microevolutionary rate of two of the most prevalent sequence types causing the majority of infections in the United States: ST8 and ST5.

RESULTS

Whole-genome sequencing of consecutive *S. aureus* isolates. We sequenced the genomes of 398 consecutive sterile-site *S. aureus* isolates recovered from 305 patients hospitalized in the Hous-
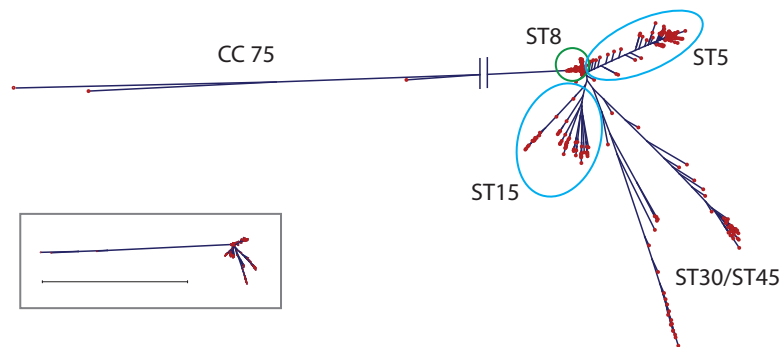
FIG 1 Radial phylogenetic tree mapping the 398 isolates of the HMHS-SA collection based on SNP distance to reference strain FPR3757. Isolates are represented by red filled circles, and numbers correspond to 4 general groupings identified through whole-genome sequencing. Group 1 (green circle) is primarily ST8 organisms that group very tightly with the ST8 reference strain. Group 2 (blue circles) encompasses two groups, both approximately equidistant from the reference strain. One is primarily ST5 isolates, and the other is ST15 strains and others. Group 3 is predominantly ST30 and ST45, and group 4 is rare members of CC75, not previously isolated in the United States. The tree is truncated to enhance detail. (Inset) To-scale version of tree; bar, 0.800.

ton Methodist Hospital System (HMHS-SA) (see Fig. S1A and B in the supplemental material). There were 192 methicillin-resistant *S. aureus* (MRSA) isolates and 206 methicillin-susceptible *S. aureus* (MSSA) isolates. Of the 398 isolates, 69 (17%) were collected from patients in an intensive care unit. The organisms studied come from patients admitted at four different hospitals in the Houston metropolitan area. Houston is the fourth largest and most ethnically diverse city in the United States, and as such, the system hospitals serve a large and multinational population (32). Sixty-seven patients (22%) had multiple isolates, including 47 patients with 2 isolates, 11 patients with 3 isolates, 5 patients with 4 isolates, and 4 patients with 5 isolates. Twenty-four of these 67 patients had multiple isolates collected on the same day from different anatomic sites. The remaining 43 patients had at least two isolates collected on different days. The longest time span separating 2 isolates taken from a single patient was 144 days.

The bacterial genomes were compared to a corrected reference genome of the USA300 *S. aureus* strain FPR3757 to identify single nucleotide polymorphisms (SNPs) (33). This strain was chosen because it represents a prototypical ST8 MRSA reference genome and has been characterized extensively (19, 33–38). After exclusion of mobile genetic elements (MGEs), the 398 isolates differed from the FPR3757 reference genome by an average of 15,464 SNPs (range, 4 to 177,869; standard deviation [SD], 19,629.8), highlighting the considerable genetic diversity of the strain sample.

Our analysis identified 4 primary genetic clusters present in the HMHS-SA sample that broadly corresponded to MLST designations (Fig. 1). The first cluster consisted of 147 isolates, predominantly ST8 *S. aureus*, which differed from the reference strain by an average of 120 SNPs (range, 4 to 885; SD, 162.3). The second cluster included 194 isolates that differed by an average of 16,690 SNPs (range, 1,113 to 24,286; SD, 4075.0) and represented predominantly ST5 *S. aureus*. The third cluster diverged from the reference genome by an average of 46,659 SNPs (range 28,037 to 57,038; SD, 7,109.2) and consisted primarily of ST30 and ST45 organisms. Lastly, the fourth cluster contained 3 extreme genetic outliers, with the isolates differing from the FPR3757 reference genome by an average of 157,834 SNPs (range, 139,413 to 177,869; SD, 19,278.7). These organisms were most closely related to MSHR1132, a highly divergent *S. aureus* clone initially isolated in Australia (39). Previous analysis has revealed that MSHR1132 be-

longs to the clonal complex 75 (CC75), a group of organisms so genetically divergent from the majority of *S. aureus* strains characterized to date that it is often difficult to identify them by traditional typing methods (40). Interestingly, one of the three CC75 patient strains belongs to ST1223, a member of CC75 that has been isolated previously only in Cambodia and South America (39, 41, 42).

Diversity of MLST types present in the 398 *S. aureus* isolates. To correlate the genome data with traditional typing methods, the MLST types of the isolates were computationally determined and mapped onto the phylogenetic tree (Fig. 2). Predictably, strains with the same MLST type clustered into the same distinct branch. This analysis confirmed that there was a considerable fine-structure genomic diversity among strains assigned to the same MLST. Moreover, it reaffirmed a conclusion by Harris et al. (25) that, compared to lower-resolution typing methods, whole-genome sequencing establishes a greater degree of genetic divergence that can enhance identification of intrahospital transmission events.

In addition to validating considerable inter-MLST-type variability, our data reveal substantial *S. aureus* diversity present within the hospitalized population. Although the highest proportion of strains that we examined belonged to the ST8 or ST5 clonal type typical of the majority of MRSA infections occurring in the United States (18–21, 43, 44), we also identified a significant number of unique isolates that belonged to other ST types (Table 1). Our data set contains the first reported isolate of ST1223 (CC75) *S. aureus* in North America, livestock-associated ST97 clones, and multiple novel MLST clonal types that represent single locus variants of ST5, ST97, and ST1159 organisms (Tables 1 and 2).

Lack of patient-to-patient transmission of sterile-site isolates. To begin to distinguish between genetically related and unrelated strains on the basis of whole-genome sequence data, we first established a baseline for related isolates. We sequenced the genomes of 95 distinct colonies cultured from a single renal abscess specimen obtained from a patient infected by an ST8 *S. aureus* strain. These isolates were collected from a patient at an unaffiliated hospital and are not included in the 398-isolate HMHS-SA set. The average pairwise distance between these 95 genomes was 7.4 SNPs (range, 4 to 17; SD, 2.5). By comparison, among the 147 ST8 isolates present in our 398-specimen data set,
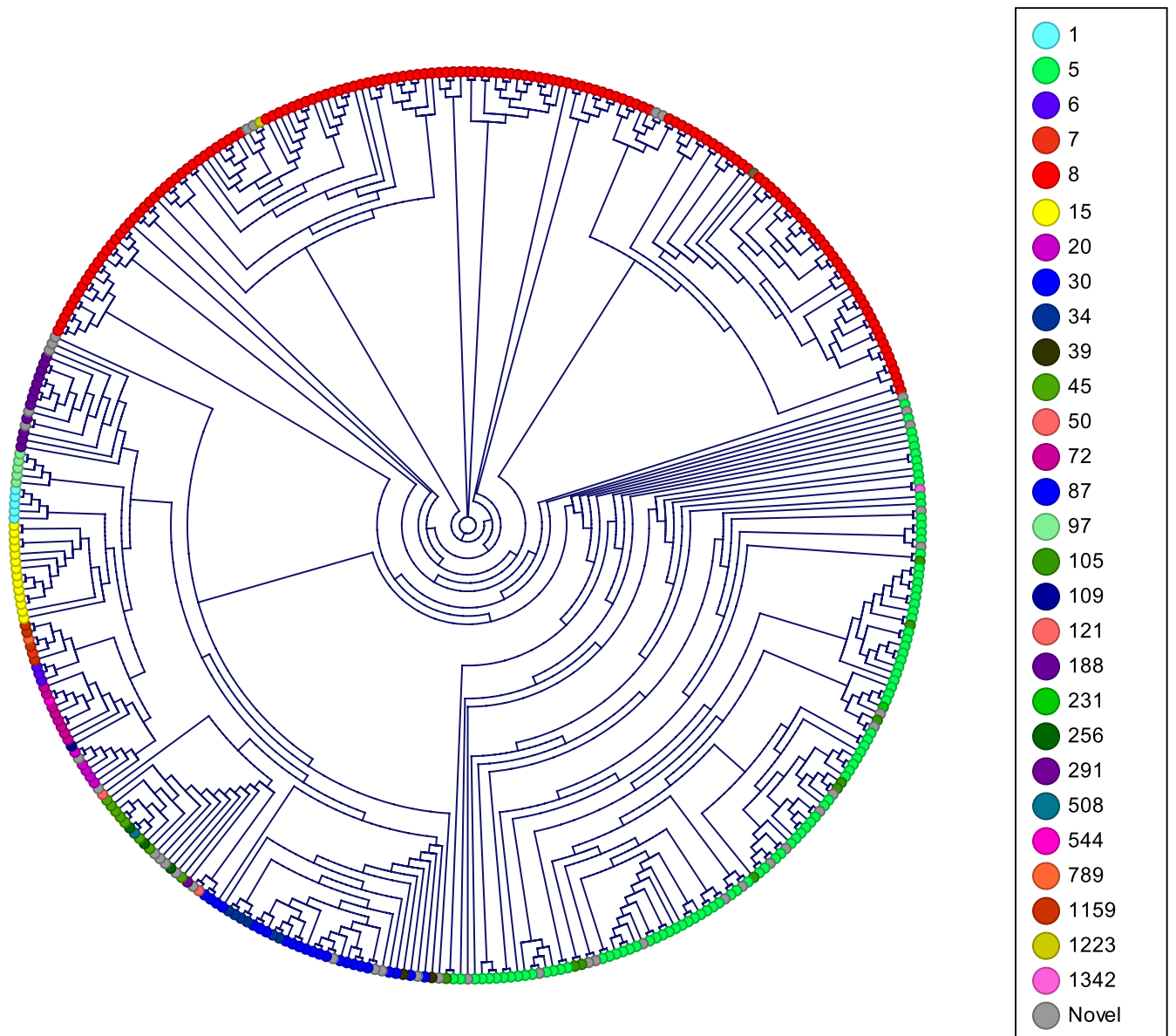
FIG 2 Circular cladogram colored by MLST. All MLST STs of a given clonal complex are colored shades of the same color. MLST was determined using a combination of SRST2 software and manual sequence review. Strains with a novel MLST allele type are labeled in gray ("Novel").

organisms collected from the same patient on the same day but from different anatomic sites had an average of 27.5 SNPs (range, 1 to 96; SD, 39.6). This average is skewed by a single isolate pair collected from bone and associated abscess tissue. With the outlier removed, the intrahost ST8 isolate pairs had an average of 4.7 SNPs (range, 1 to 9; SD, 3.3). This intrahost genetic diversity is similar to that previously reported for CC22 and CC30 *S. aureus* clones (31, 45).

To identify potential intrahospital transmission events among closely related organisms, we chose the two most abundant ST types (ST8 and ST5) for further analysis. We compared ST8 isolates to the ST8 reference genome (strain FPR3757) and ST5 isolates to a representative ST5 genome (strain N315) to determine the most relevant SNPs for the individual isolates (33, 46). The pairwise distance between all isolates in each set (ST5 and ST8)

was then determined. The average pairwise SNP distance among all ST8 strains in our data set was 58.3 SNPs (range, 0 to 858; SD, 118.7) and was 94.0 SNPs (range, 0 to 322; SD, 83.7) for the set of ST5 strains.

To begin to determine which strains were potentially acquired in the hospital setting, we considered the level of within-host SNP diversity and the average pairwise distance of isolates within the HMHS-SA collection. Thus, we chose to examine all isolate pairs with a pairwise distance of 40 SNPs or less. This SNP threshold is in line with thresholds used for studies of within-host variation of nasal carriage isolates and an intensive care unit outbreak investigation (31, 45). Consequently, in the 398-strain data set, there were 86 ST8 and 40 ST5 isolate pairs that exhibited a pairwise distance of 40 SNPs or less. For ST8 strains, 34 of the 86 isolate pairs were isolates collected from the same patient. For the re-

TABLE 1 MLST profiles of HMS-SA isolates[a]

MLST	No. of isolates
1	5
5	96
6	3
7	1
8	140
15	13
20	5
30	19
34	6
39	2
45	8
50	1
72	8
87	2
97	5
105	9
109	1
121	1
188	11
231	1
256	3
291	1
508	1
544	1
789	1
1159	4
1223	1
1342	1

[a] MLST data for 350 of the 398 isolates which belong to a previously defined MLST type. The remaining 48 isolates were identified to have unique allele profiles unrecognized in the current MLST database. The novel MLST allele profiles have been summarized in Table 2.

TABLE 2 Novel MLST allele profiles[a]

Novel MLST	SLV	No. of isolates	Allele in gene:						
			arcC	aroE	glpF	gmk	pta	tpiA	yqiL
A	ST5	6	1	4	1	4	12	1	100
B	ST5	2	1	4	90	4	12	1	10
C	ST97	1	3	1	1	1	1	28	3
D	ST1159	1	3	4	1	4	4	44	141
E	ST5	1	3	4	1	4	12	1	10
F	ST5	1	1	4	1	4	12	137	10
G	ST188	1	3	1	1	8	1	137	1
H	ST8	6	3	3	1	1	4	4*	3
I	ST8	1	3	3	1	1*	4	4	3
J	ST12	1	1	3	1	8	11*	5	11
K	ST188	1	3	1	1	8	1	1	1*
L	ST188	1	3	1	1	8	1	1*	1
M	ST20	1	4	9	1	8	1	10	8*
N	ST2689	1	1	4	1	4	293*	1	10
O	ST30	2	2	2	2	2	6	3	2*
P	ST30	1	2	2*	2	2	6	3	2
Q	ST45	1	10	14	8	6	10*	3	2
R	ST45	2	10	14	8	6*	10	3	2
S	ST50	1	16	16	12	2	13	13	15*
T	ST630	1	12	3	1	1	4	4	3*
U	ST8	2	3	3	1	1	4*	4	3
V	ST5	1	1	4	1	4	64*	1	10
W	ST188	1	3	1	290*	8	1	1	1
X	ST30	1	2	2	2	2	6	3	271*
Y	ST5	1	1*	4	1	4	12	1	10
Z	ST2793	2	151	406	321*	34	256	261	323
AA	ST45	1	10*	14	8	6	10	3	2
AB	ST5	2	1	4*	1	4	12	1	10

[a] Forty-eight isolates identified by SRST2 software had unique allele profiles not present in the MLST database. Each novel allele combination represents a single locus variant (SLV) of a defined MLST type, followed by the number of isolates present with that MLST type. The MLST alleles are listed, with novel allele sequences represented with an asterisk appended to the closest known allele sequence where indicated. Novel types A to G represent novel combinations of known alleles, while the remaining novel MLST types (H to AB) represent combinations containing one novel allele sequence, resulting in a new MLST type. The novel ST type combinations and allele sequences have been submitted to the *S. aureus* MLST database (http://saureus.mlst.net/). Novel allele sequences may also be found in the supplemental material.

maining 52 isolate pairs, 36 isolate pairs had the *S. aureus* infection present upon admission, 27 isolate pairs had significant differences in antibiotic susceptibility, and 29 were isolated from patients in different hospitals without a shared caregiver (see Table S1 in the supplemental material). Thus, in these ST8 isolates, our analysis identified no pairs of related isolates (average pairwise distance, <40) from different individual patients with a plausible transmission chain or other epidemiological linkage. For ST5 strains, 17 of the 40 isolate pairs were isolates collected from the same patient. Of the remaining 23 isolate pairs, 22 patients had *S. aureus* infection present upon admission. The one remaining isolate pair had significant differences in antibiotic susceptibility. In addition, 15 of the 22 ST5 isolate pairs present on admission involved patients in different hospitals without a shared caregiver (Table S1). In the ST5 isolates, our analysis identified no pairs of related isolates (average pairwise distance, <40) from different individual patients with a plausible transmission chain or other epidemiological linkage. This process is summarized in Fig. 3 (flow chart).

Intrahost bacterial microevolution. Within-host evolution of asymptomatic *S. aureus* carriage strains has been recently studied using whole-genome sequencing (45), but there are currently no data that define within-host microevolution over time in the setting of sterile-site infections. The distinction is important as there are potentially very different selective pressures exerted on sterile-site isolates versus strains isolated from asymptomatic carriers. It has also been observed that ST8 isolates tend to be more clonal and

less variable than other ST types (35). We examined genomic variation among strains collected over time from the same patient. In this set of isolates ($n = 30$), the average time between the two isolates for ST8 strains ($n = 15$) was 24.6 days (range, 1 to 144 days; SD, 38.9 days) and 20.2 days for ST5 strains ($n = 15$) (range, 1 to 83 days; SD, 23.9 days). By considering SNP accumulation between the two isolates, and the time interval, we determined that ST8 strains accrued an average of 2.2 SNPs per day (range, 0 to 12 SNPs; SD, 3.0 SNPs) compared to ST5 strains that accrued an average of 17.7 SNPs per day (range, 0.6 to 80.5 SNPs; SD, 24.6 SNPs). This difference was statistically significant ($P = 0.031$, unpaired t test with Welch's correction) and is shown in Fig. 4. Although two outlier strains exist in the ST5 population that inflate the mean rate, when these outliers are removed from the analysis, the increased ST5 SNP accumulation rate remains statistically significant compared to that of ST8 organisms ($P = 0.0005$, unpaired t test with Welch's correction). Taken together, our findings are consistent with the clonal nature of the ST8 population (35).

Identification of CC75 organisms. *S. aureus* CC75 is a highly genetically divergent lineage that has been reported only in Aus-
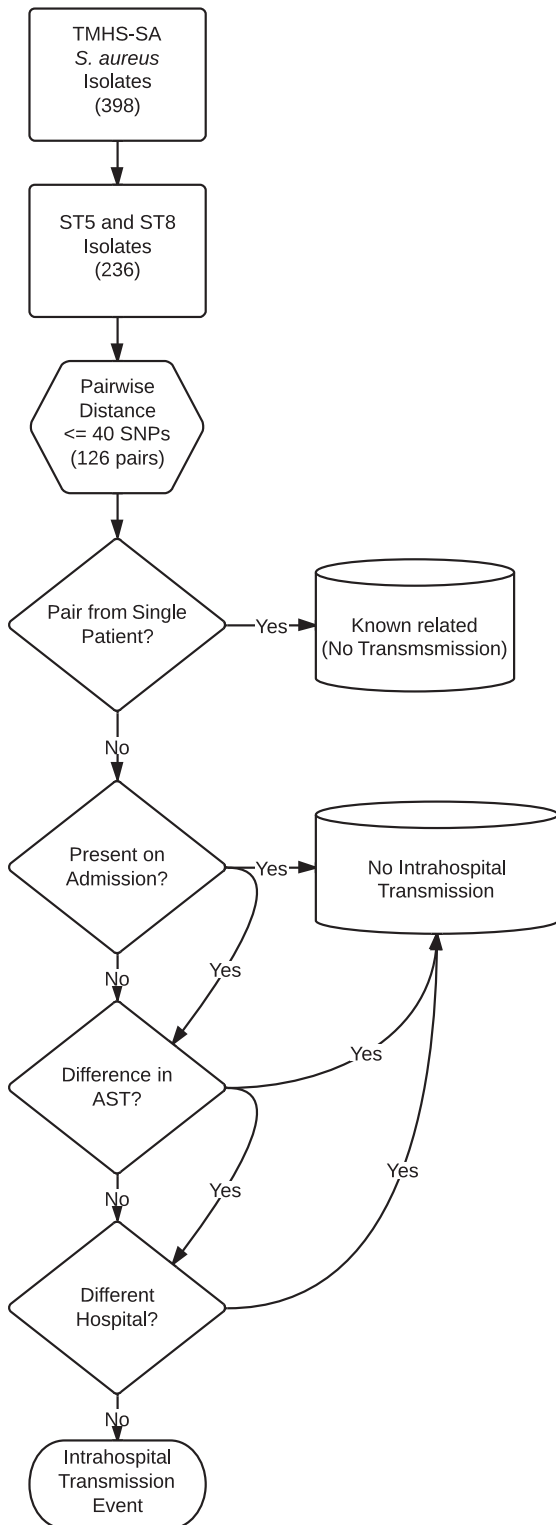
FIG 3 Flow diagram demonstrating the process of screening the 398 *S. aureus* isolates for potential intrahospital transmission. First, the ST8 and ST5 isolates were selected, and any isolate pair with a pairwise distance of 40 SNPs or less was selected for review. If the pair of isolates were from the same patient, no further investigation was performed. If the isolates were from two individual patients, the medical record was reviewed individually to identify if the infections were present on admission, had differences in antimicrobial susceptibility, or were from different hospitals without a shared caregiver. No intrahospital transmission events were identified in these isolates.
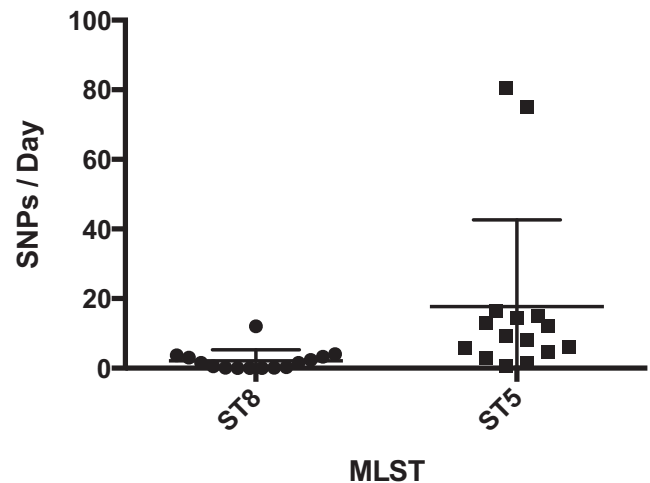

FIG 4 Comparison of the evolutionary rates of ST8 and ST5 clones in the HMHS-SA collection. For patients with multiple ST8 or ST5 *S. aureus* isolates collected over time, the evolutionary rate was calculated. The mean and quartile ranges are indicated for each MLST type. The increased evolutionary rate in ST5 isolates collected over time from the same patient can be observed. The evolutionary rates between the two groups are significantly different with a *P* value of 0.031 using an unpaired *t* test with Welch's correction.

tralia, Cambodia, and South America (39–42). Unexpectedly, our Houston data set included three isolates that clustered with CC75, thus representing the first report of these organisms in North America. Interestingly, our three CC75 isolates are phylogenetically divergent (17,002 to 20,915 SNPs) from the only available CC75 reference genome, MSHR1132 (Fig. 5) (39). Consequently, these newly identified clones may represent the emergence of a novel clonal complex in North America.

DISCUSSION

In our study of invasive *S. aureus* disease across a multihospital system, we could identify no closely related isolates with an obvious intrahospital transmission path. In addition, there was no significant clustering of isolates based on the hospital of origin (Fig. 6). We discovered some isolate pairs that were closely related genetically yet lacked an obvious transmission chain. Many of these isolate pairs consisted of patients with infections already present on admission to the hospital. Subsequently, our results suggest that there are clonal pools of *S. aureus* present within the community that result in patients being infected with closely related organisms prior to their admission to the hospital. This result concurs with observations made recently by Prosperi et al. (29). Thus, it may not be unusual for two patients to be admitted to the same hospital with highly related strains, but conventional typing methods would not differentiate between this and a scenario in which patient-to-patient transmission had occurred. This situation has been previously suggested (31) and underscores the need not only to document infections present at admission but to use tools that provide the necessary resolution to distinguish the two. One potential limitation is that we chose to study only sterile-site infections. Any transmission involving nonsterile sites such as superficial skin or wounds would have been missed in our study.

Our data also provide a framework to assess the average relat-
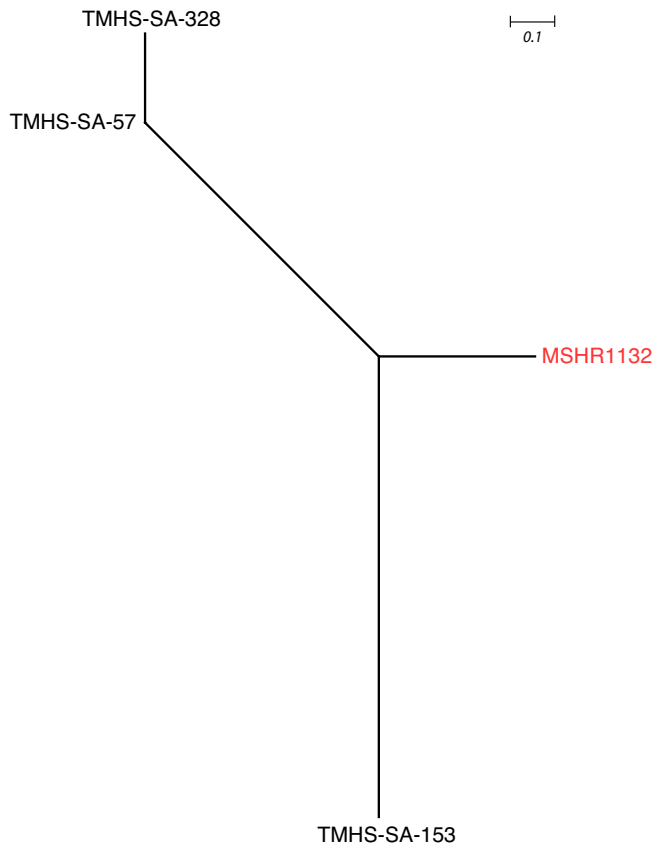
FIG 5 Radial phylogenetic tree of the 3 CC75 HMHS-SA isolates. A clear relationship is evident between HMHS-SA-57 and HMHS-SA-328, which were isolated from the same patient several months apart, compared to HMHS-SA-153. Isolate HMHS-SA-153 was typed as ST1223 by SRST2. HMHS-SA-57 and HMHS-SA-328 share a novel MLST type, which is a single locus variant of ST2793.

edness between isolates collected from the same patient from different sites and over time, compared to isolates collected from unrelated individuals. Similar studies in *Clostridium difficile*, *Klebsiella pneumoniae*, and *S. aureus* have yielded unique insights into both outbreaks and the intrahospital spread of pathogens (17, 23, 29, 47–49). Information obtained from such studies is useful for hospital infection control and public health investigation. Moreover, it is particularly important to be able to differentiate infections acquired prior to hospitalization from those linked to the patient's hospital stay, as insurance reimbursements may soon be denied for hospital-acquired infections (31, 50).

The utility of studying multiple isolates from the same patient collected over time has been previously demonstrated (45, 51). In our study, we analyzed organisms that were collected from the same patient over an extended time interval of up to 144 days. Surprisingly, the microevolutionary SNP accumulation rate observed in these isolates was higher than that reported previously for *S. aureus* (52–55). Several possibilities may explain this difference. Previous estimates of evolutionary rates in *S. aureus* have been derived from analysis of asymptomatic carriage isolates, whereas our analysis was based on organisms cultured from patients with long-term invasive infections. The selective pressures on an invasive isolate are likely to differ significantly from those on

a carriage strain. Furthermore, many prior studies have been conducted on isolates that predominate in Europe, such as ST30 clones, while most of our isolates represent the ST8 and ST5 clonal types prevalent in the United States. We have only one ST30 multiple-isolate patient in our data set; however, the evolutionary rate of this isolate is 0.06 SNPs/day, similar to previously reported rates in this clonal type. Lastly, although the significance of an apparent difference in the microevolutionary rate between ST5 and ST8 *S. aureus* strains is not clear, a lower ST8 microevolutionary rate has been previously described (49). However, this lower evolutionary rate was found in a study of a pediatric population where children with chronic illnesses were excluded. Our study population consists primarily of adults, many of whom suffer from one or more chronic illnesses. This difference in study population may explain some of the observed difference in microevolutionary rate in our study compared to prior published reports.

We had 24 patients (8%) with multiple cultures from different anatomic sites positive for *S. aureus* collected on the same day. These sites were often anatomically contiguous, such as joint fluids and associated bone or tissue.

One unexpected finding was the presence of three isolates (HMHS-SA-57, -153, and -328) belonging to clonal complex 75 and related to ST1223, an ST type associated with infections originating in Australia and Southeast Asia (39, 40). This ST type has not been described previously in North America. Isolates from CC75 are known to be difficult to classify using MLST due to their highly divergent allele sequences, which may lead to difficulty in identifying these strains by classical MLST methods (39, 40). In our data set, these isolates were derived from two unique patients. One patient was of Southeast Asian descent with multiple chronic medical problems that predispose to infection. He had bacteremia with two isolates collected approximately 4 months apart (HMHS-SA-57 and -328). The second patient (HMHS-SA-153) was a Hispanic male who presented to a community emergency department with a joint infection. Of note, these two patients lived in the same community, which is home to a large Southeast Asian population (32).

Our most significant finding, the lack of identifiable intrahospital, patient-to-patient *S. aureus* transmission, was discernible only through whole-genome sequencing. Using conventional, low-resolution typing strategies would not have led to the same conclusions. Moreover, high-resolution whole-genome sequencing must be used in conjunction with clinical and epidemiological information to understand the complex population dynamics potentially at play in an outbreak situation. This is particularly true of an organism as ubiquitous as *S. aureus*, where asymptomatic carriage of the pathogen is prevalent and numerous potential transmission pathways exist. Inasmuch as highly related isolates may be imported into the hospital in multiple independent events, studies of hospital epidemiology using low-resolution typing methods may result in erroneous conclusions.

MATERIALS AND METHODS

Specimen collection and processing. All sterile-site *S. aureus* isolates identified and processed in the Houston Methodist Hospital Clinical Microbiology Laboratory were collected from 15 May 2011 to 16 November 2011. The Houston Methodist Hospital System in Houston, Texas, is comprised of 7 hospitals (1 central and 6 outlying hospitals throughout the greater Houston metropolitan area); 4 of these participated in the current study. Corresponding patient demographic and infection source data were also collected. Patient ages ranged from <1 month to 103 years
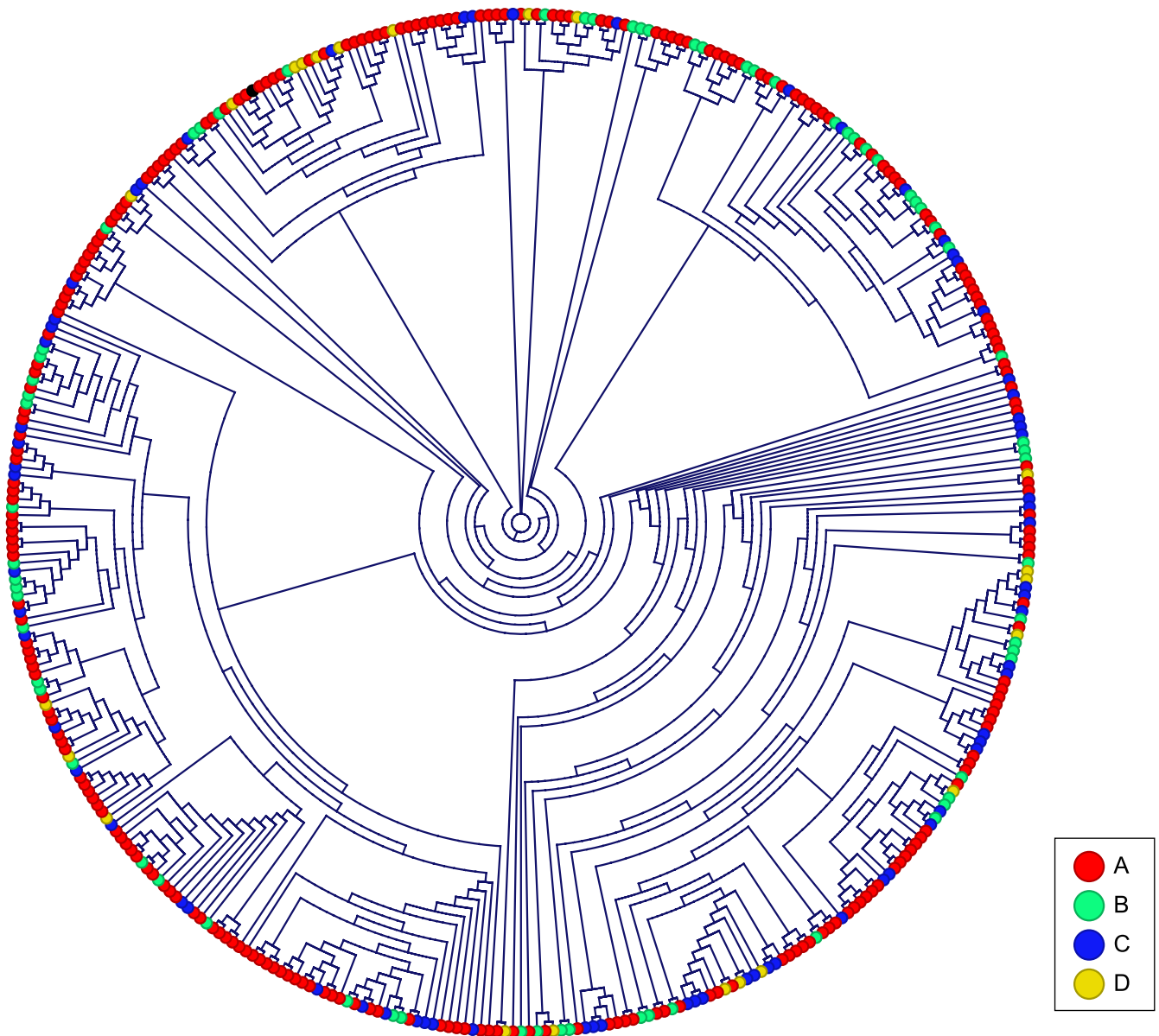
FIG 6 Cladogram showing no significant clustering based on hospital of origin. Small clusters of isolates from hospital B, C, or D represent multiple isolates collected from the same patient at that hospital.

(average age, 58.3 years; SD, 19.0 years), and 59% were male. The sample set was essentially adults, with only 8 of 305 patients younger than 21 years (median, 60 years). The study protocol was approved by the Houston Methodist Research Institute Institutional Review Board (protocol IRB1010-0199). The 95 perinephric abscess isolates studied were derived from a single specimen collected from a pediatric patient. Isolates were stored at −80°C in tryptic soy broth (TSB) containing 20% (vol/vol) glycerol and then grown overnight in 2 ml TSB at 37°C. Overnight culture (1 ml) was collected and placed into a 2-ml tube containing lysing matrix B (MPBio, Santa Ana, CA). The samples were lysed with a FastPrep96 system (MPBio) for two 60-s cycles at 1,600 rpm. Lysates were centrifuged for 1 min at 13,000 × *g*, and the supernatant was transferred to a Qiagen MDX robot for automated DNA extraction with the Qiagen One-For-All kit (Qiagen, Valencia, CA). The extracted nucleic acid was treated with RNase A for 15 min at room temperature. The quality of the extracted DNA was assessed with the NanoDrop spectrophotometer (Thermo,

Fisher, Waltham, MA) and quantified for library preparation with the high-sensitivity double-stranded DNA (dsDNA) assay on the Qubit 2.0 (Life Technologies, Carlsbad, CA).

Whole-genome sequencing and analysis. Libraries were prepared from the purified DNA using either a custom in-house protocol (56) or Illumina Nextera XT chemistry (Illumina, San Diego, CA). DNA libraries were sequenced with either an Illumina GAIIx, MiSeq, or HiSeq 2000 instrument. SNPs were identified by VAAL (57). VAAL data were processed into aligned FASTA files using the scripts prephix and phrecon that were developed in-house (available from http://www.github.com/codinghedgehog/). Phylogenetic trees were generated with FastTreeMP using the generalized time-reversible model and visualized with SplitsTree, Dendroscope, and CLC Genomics Workbench (CLC Biosystems, Cambridge, MA) (58–61). Pairwise distance matrices were made with Geneious R6 (Biomatters, Auckland, New Zealand). MLST type was determined from whole-genome sequence data using SRST2 (http://

katholt.github.io/srst2/) and the *S. aureus* MLST database (http://saureus.mlst.net/).

Strain whole-genome sequence FASTQ files have been deposited with NCBI under BioProject PRJNA252378.

Examination of related isolates. All ST8 and ST5 isolate pairs with pairwise distances below the threshold of 40 were selected for further review. First, the patient source for the two isolates was identified. If isolates were from the same patient, this was noted and no further review was performed on that pair. Next, the electronic medical records of both patients in the isolate pair were reviewed to determine if *S. aureus* infection was identified on the day of admission either in the chart or with positive cultures collected at the time of admission. In addition, it was noted if the patients in the isolate pair were in different hospitals within the system, if any caregivers present in the medical record were shared between the patients in the isolate pair, or if the antibiotic susceptibility for the isolates in the pair differed significantly (defined as either MRSA versus MSSA or the difference in susceptible or resistant as defined by CLSI criteria for at least one antibiotic). The results of these determinations are described in Table S1 in the supplemental material. The study protocol was approved by the Houston Methodist Research Institute Institutional Review Board (protocol IRB1010-0199).

Statistical analysis and graphs. Graphs were constructed and statistical analyses were performed with Prism version 6.0 (GraphPad, La Jolla, CA).

SUPPLEMENTAL MATERIAL

ACKNOWLEDGMENTS

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