Molecular differentiation of historic phage-type 80/81 and contemporary epidemic *Staphylococcus aureus*

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Staphylococcus *aureus* is a bacterial pathogen known to cause infections in epidemic waves. One such epidemic was caused by a clone known as phage-type 80/81, a penicillin-resistant strain that rose to world prominence in the late 1950s. The molecular underpinnings of the phage-type 80/81 outbreak have remained unknown for decades, nor is it understood why related *S. aureus* clones became epidemic in hospitals in the early 1990s. To better understand the molecular basis of these epidemics, we sequenced the genomes of eight *S. aureus* clinical isolates representative of the phage-type 80/81 clone, the Southwest Pacific clone [a community-associated methicillin-resistant *S. aureus* (MRSA) clone], and contemporary *S. aureus* clones, all of which are genetically related and belong to the same clonal complex (CC30). Genome sequence analysis revealed that there was coincident divergence of these clones from a recent common ancestor, a finding that resolves controversy about the evolutionary history of the lineage. Notably, we identified nonsynonymous SNPs in genes encoding accessory gene regulator C (*agrC*) and α-hemolysin (*hla*)—molecules important for *S. aureus* virulence—that were present in virtually all contemporary CC30 hospital isolates tested. Compared with the phage-type 80/81 and Southwest Pacific clones, contemporary CC30 hospital isolates had reduced virulence in mouse infection models, the result of SNPs in *agrC* and *hla*. We conclude that *agr* and *hla* (along with penicillin resistance) were essential for world dominance of phage-type 80/81 *S. aureus*, whereas key SNPs in contemporary CC30 clones restrict these pathogens to hospital settings in which the host is typically compromised.

A prominent cause of human infections worldwide is *Staphylococcus aureus* (1). In addition to the overall high burden of *S. aureus* colonization and/or infections among humans (1, 2), the organism readily acquires resistance to antibiotics, and there have been multiple epidemics of antibiotic resistant *S. aureus* infections over the past 60 y (3).

One of the most remarkable bacterial epidemics of the 20th century was caused by a penicillin-resistant *S. aureus* clone known as phage-type 80/81 *S. aureus*. This clone emerged rapidly and became prominent in Australia, Great Britain, Canada, and the United States in the 1950s (4–8). Phage-type 80/81 *S. aureus* caused a wide range of syndromes, including skin infections (boils, carbuncles, and pustules) and fatal sepsis or pneumonia (4). These infections were often reported as unusually severe, a problem compounded by resistance to penicillin—a major problem for treatment. Outbreaks occurred initially in hospitals, especially among newborns, young children, and nursing mothers, but ultimately infections were acquired outside of the healthcare setting as the overall burden of phage-type 80/81 *S. aureus* increased (9). The pandemic lasted for ∼10 y (1953–1963), at which point there was a decrease in the number of colonized newborns in hospitals and a reduction in staphylococcal disease in adults (10). The reason for the decline of phage-type 80/81 *S. aureus* is unknown, but its dominance as a human pathogen ended a few years after methicillin was introduced as a treatment for infections caused by penicillin-resistant organisms.

Methicillin-resistant *S. aureus* (MRSA) emerged in the early 1960s (11) and is now widespread in hospitals and the community in virtually all industrialized countries (reviewed in refs. 3, 12). One of the most successful MRSA clones over the past 20 y is known as epidemic MRSA-16 (EMRSA-16), which was first reported in the United Kingdom in 1992 (13). EMRSA-16 spread to hospitals in Europe (13, 14) and the United States (15–17), and there is also relatively high prevalence of hospital infections caused by methicillin-susceptible *S. aureus* (MSSA) strains related to EMRSA-16 by multilocus sequence type (MLST), which is used to determine whether strains belong to the same clonal complex (15, 18). It is notable that phage-type 80/81 *S. aureus* and EMRSA-16 belong to the same clonal complex 30 (CC30) and may have a recent common ancestor (19). Further, Robinson et al. proposed that a contemporary community-associated MRSA strain known as the Southwest Pacific clone is a descendant of phage-type 80/81 *S. aureus* (19). Although these ideas are intriguing, it is not known whether contemporary epidemic CC30 strains are reemerged descendants of phage-type 80/81 *S. aureus*.

To better understand the molecular and evolutionary basis for these epidemic waves, we genetically characterized 284 historical and contemporary CC30 strains, compared the genome sequence of eight *S. aureus* isolates representative of phage-type 80/81, Southwest Pacific, and contemporary CC30 hospital clones, and evaluated virulence of each in mouse infection models.

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Results and Discussion

Genome Sequencing of Phage-Type 80/81 *S. aureus* and Contemporary CC30 Clones. We first selected eight *S. aureus* clinical isolates representative of three prominent CC30 clones (past and present), including three phage-type 80/81 isolates from the 1960s, for subsequent analysis by whole-genome sequencing (Table 1). These isolates were originally recovered from patients with *S. aureus*

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infections in Australia, France, the United Kingdom, and the United States, and were selected from a collection of 284 CC30 isolates obtained over a 73-y period (1935–2008). We performed whole-genome short-read length DNA sequencing to generate data that were then mapped to the genome of the EMRSA-16 reference strain MRSA252 (20). Using this approach, in combination with conventional capillary DNA sequencing, we identified all single nucleotide polymorphisms (SNPs) and insertions/deletions (InDels) in the core genome and mobile genetic elements (MGEs) of the eight query strains relative to the EMRSA-16 reference strain (Figs. 1 and 2 and Datasets S1–S6).

There were 2,299 unique SNPs in the core genome of the eight query isolates compared with EMRSA-16 and these SNPs appear to be distributed randomly across the entire genome (Dataset S1 and Fig. S1). The eight *S. aureus* isolates differed from EMRSA-16 on average by 703 SNPs, and all nine isolates—the eight query isolates plus EMRSA-16—differed from each another by an average of 723 SNPs (range was 135–1,033 SNPs) (Fig. 1*A*). Twenty-nine percent of these SNPs were located in intergenic regions and among the 1,642 nucleotide changes that were distributed among 1,019 unique ORFs, 63% were synonymous and 37% were nonsynonymous.

Phylogenetic Analyses. We next conducted phylogenetic analyses on the basis of a relatively large segment (1.4 Mb, ~50%) of the genome or using concatenated SNP nucleotides of the core genome to elucidate the genetic relationships among the CC30 isolates (eight query isolates + the EMRSA-16 reference strain) (Fig. 1 *B* and *C*). The three phage-type 80/81 isolates (21295, 22030, and 22251) were the most closely related organisms (differing on average by 165 SNPs) (Fig. 1). EMRSA-16 and contemporary MSSA isolates (3636, 21203, 21247, and 21345)—herein called contemporary CC30 hospital isolates—were also similar to each other, differing by an average of 378 SNPs, but these isolates were more divergent from the phage-type 80/81 and Southwest Pacific clones (Fig. 1). Unexpectedly, the data indicate the Southwest Pacific clone is not likely a direct descendant of phage-type 80/81 *S. aureus* as suggested by Robinson et al. (19). Rather, it evolved from an ancestral strain common to each of the three CC30 sublineages identified here.

The ratio of nonsynonymous-to-synonymous SNPs was ~0.6:1 for each of the three groups of isolates. The low ratio of nonsynonymous-to-synonymous SNPs indicates that SNP diversity has accumulated over a relatively long period and all isolates have undergone purifying selection. In addition, the similar ratio of nonsynonymous-to-synonymous SNPs among the phage-type 80/81 isolates, the Southwest Pacific clone, and EMRSA-16 and related isolates provides support to the idea that each diverged from a common ancestor at roughly the same time.

Core Genome SNPs Present in Virulence Molecules or Gene Regulatory Elements. The nonsynonymous SNPs in the core genome of the eight CC30 isolates compared with the EMRSA-16 reference strain were distributed among 439 unique ORFs (Dataset S1). These SNPs could be segregated into those specific to EMRSA-16 and the contemporary MSSA isolates, but only a limited number (approximately six) of these polymorphisms were present in proven or putative virulence determinants or gene regulatory molecules (Dataset S1). Contemporary CC30 hospital isolates contained nonsynonymous SNPs in *vraE*, *sdrC*, and genes encoding staphylocoagulase (*coa*), aconitase (*citB*), and α-hemolysin (*hla*) that were not present in the phage-type 80/81 or Southwest Pacific clones. These genes encode proteins known to be involved in virulence and/or interaction with the mammalian host, and therefore, SNPs in these genes could alter virulence of the contemporary CC30 hospital clones. Notably, the SNP in *hla* (G→A at position 1,181,065 in EMRSA-16) results in a premature stop codon that presumably inhibits production of the protein (tested below).

In addition, nonsynonymous SNPs were present in the genes encoding heme sensor protein (*hssS*), iron-regulated surface determinant protein H (*isdH*), sortase B (*srtB*), and accessory gene regulator C (*agrC*) (Dataset S1). HssS is the sensor kinase of a two-component gene regulatory system (HssRS) that senses environmental heme and sortase B and IsdH are important for heme-iron uptake and metabolism (21, 22). The SNP in *agrC* (G→A) predicts a Gly-to-Arg substitution at residue 55 ($agrC_{G55R}$) in EMRSA-16 relative to the phage-type 80/81 isolates. Inasmuch as the Agr quorum sensing system is a key regulator of *S. aureus* virulence molecules, $agrC_{G55R}$ could alter virulence of the contemporary CC30 hospital isolates.

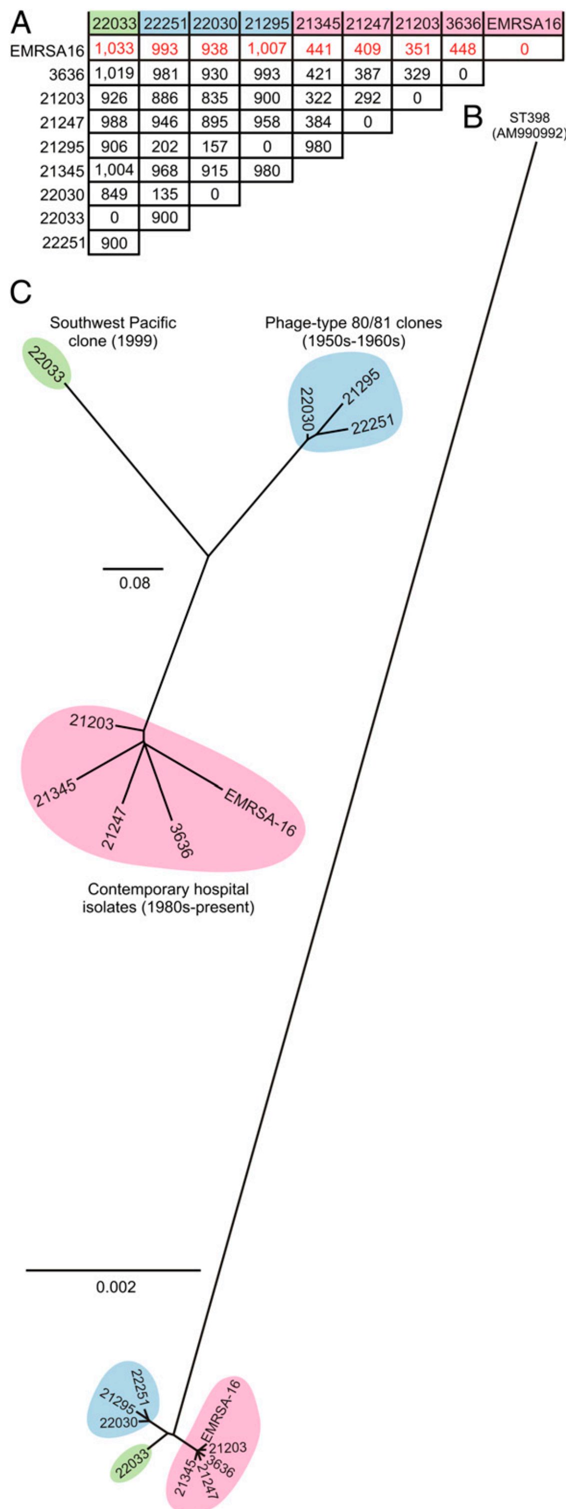
MGEs. *S. aureus* readily acquires new genetic information on MGEs such as prophages, transposons, insertion sequences, and pathogenicity islands. The gene encoding methicillin resistance (*mecA*) is carried by an MGE known as staphylococcal cassette chromosome *mec* (SCC*mec*), of which there are several types. SCC*mec* type II and SCC*mec* type IV were present in EMRSA-16 and the Southwest Pacific clone, respectively; all other strains were MSSA. On the basis of the genome sequence data, 7 of 11 putative MGEs in EMRSA-16 were also present (i.e., not absent or divergent in DNA sequence) in the four contemporary MSSA isolates tested (Fig. 2 and Dataset S3). By comparison, only one of these elements, a genomic island (nucleotides 93,145–101,656 in EMRSA-16), was similar (or present) in the phage-type 80/81 or Southwest Pacific clones (Fig. 2 and Dataset S3). A *S. aureus* genomic island known as νSaα and bacteriophage ϕSa2—MGEs that contain putative or proven virulence factors—were divergent in the phage-type 80/81 and Southwest Pacific clones compared with the EMRSA-16 reference strain. It is possible that divergence in these MGEs alters the phenotype of these isolates.

There were a total of 2,386 SNPs in MGEs across all isolates tested, and most (83.5%) were synonymous SNPs (Dataset S6).

Table 1. CC30 isolates used for genome sequencing

Isolate (other name)	SCC*mec*	Spa type	MLST	Origin	Comment
22030 (M1015)	MSSA	43/t012	ST30	Australia, 1962	Pandemic phage-type 80/81
22251 (65-20)	MSSA	43/t012	ST30	United States, 1965	Pandemic phage-type 80/81
21295 (NRS216)	MSSA	43/t012	ST30	United Kingdom, 1968	Pandemic phage-type 80/81
21203 (NRS112, MN-8)	MSSA	33/t012	ST30	United States, 1980	Contemporary CC30 hospital isolate
EMRSA-16 (MRSA252)	II	16/t018	ST36	United Kingdom, 1997	Epidemic MRSA-16 clone (hospital isolate)
22033 (WBG10049)	IV	19/t019	ST30	Australia, 1999	Southwest Pacific clone, CA-MRSA
3636	MSSA	33/t012	ST30	United States, 1999	Contemporary CC30 hospital isolate
21247 (NRS162)	MSSA	43/t012	ST30	France, 2000	Contemporary CC30 hospital isolate
21345 (NRS267)	MSSA	468/t122	ST30	France, 2002	Contemporary CC30 hospital isolate

Spa type is present as eGenomics BK/Ridom.



A	22033	22251	22030	21295	21345	21247	21203	3636	EMRSA16
EMRSA16	1,033	993	938	1,007	441	409	351	448	0
3636	1,019	981	930	993	421	387	329	0	
21203	926	886	835	900	322	292	0		
21247	988	946	895	958	384	0			
21295	906	202	157	0	980				
21345	1,004	968	915	980					
22030	849	135	0						
22033	0	900							
22251	900								

Fig. 1. Genome- and SNP-based phylogenetic analyses. (*A*) SNP matrix for CC30 strains. Red text indicates the number of SNPs relative to the EMRSA-16 reference strain. Green, blue, and red shading correspond to the Southwest Pacific clone, pandemic phage-type 80/81 isolates, contemporary CC30 hospital isolates, respectively. (*B*) Phylogenetic analysis of eight CC30 isolates and the EMRSA-16 reference strain based upon a contiguous 1.4-Mb region of the genome. An ST398 strain (GenBank accession number AM990992) was used as an outgroup for the analysis because we determined it was more closely related to EMRSA-16 (MRSA252) than other (non-CC30) *S. aureus* strains on the basis of genome sequence analysis. (*C*) Phylogenetic analysis of the same CC30 isolates based upon 2,299 concatenated SNPs in the core genome.

Compared with SNPs in the core genome, the ratio of non-synonymous-to-synonymous SNPs in MGEs among all isolates was 10-fold less (0.06:1), indicating that the evolutionary history of these elements is distinct from that of the core genome.

Non-SNP Mutations. Compared with EMRSA-16, there were several large (>11 bp) InDels distributed throughout the core genome of the other *S. aureus* isolates (Fig. 2 and Dataset S2). InDels specific to phage-type 80/81 isolates and/or the Southwest Pacific clone include in-frame mutations in genes encoding ECM binding protein homolog (*ebh*), *S. aureus* surface proteins A and C (*sasC* and *sasA*), and collagen adhesin (*cna*), all of which are surface proteins.

By comparison with the EMRSA-16 reference strain, there were 192 unique small InDels (insertions ≤4 bp and deletions ≤11 bp) distributed throughout the core genome of the eight query CC30 isolates (Dataset S4). InDels in known virulence molecules or gene regulators, such as those in *hlgA* (encoding γ-hemolysin), *sarS*/*sarH* (staphylococcal accessory regulator S or H), *ebh*, *isdC*, *sdrC*, or *sasA*, were present in only one or two of the isolates. Thus, these mutations do not contribute to segregation of the three sublineages identified here by genome sequencing.

Evolution of CC30 *S. aureus* Includes Accumulation of Key SNPs in *hla* and *agrC*. *S. aureus* produces many secreted virulence molecules, including the cytolytic toxin Hla, that are known to contribute virulence in animal infection models. Production of these molecules is controlled in part by the Agr gene regulatory system. Inasmuch as mutations in *hla* and/or *agr* could potentially alter the ability of *S. aureus* to cause human infections, we determined the extent to which the SNPs identified in *hla* and/or *agrC* are present in a subset ($n = 172$) of the collection of 284 CC30 clinical isolates used here (Fig. 3*A* and Dataset S7). Of these isolates, 122 (70.9%) contained the SNP in *hla* at position 1,181,065 that results in a premature stop codon (hla_{STOP}) and the majority of these isolates were recovered from patients after 1976 (Fig. 3*A*). In addition, 124 of 172 CC30 isolates tested (72.1%) contained the SNP in *agrC*, which resulted in an amino acid change at Gly55 ($agrC_{G55R}$), and most of these isolates were obtained after 1976 (Fig. 3*A*). Similar to the phage-type 80/81 isolates, three CC30 isolates obtained before the phage-type 80/81 pandemic (isolated in 1935, 1940, and 1945) contained intact *hla* and *agrC* genes (Fig. 3*A* and Dataset S7).

Hla Segregates Phage-Type 80/81 and Southwest Pacific Clones from Contemporary CC30 Hospital Clones. To verify that the stop codon in the gene encoding Hla prevented toxin production, we tested culture supernatants of EMRSA-16 and the eight query isolates for presence of Hla (Fig. 3*B*). Hla accumulated in culture media of two phage-type 80/81 clones (22030 and 21295), and the Southwest Pacific clone, but was undetectable in culture supernatants from the other isolates (Fig. 3*B*). We recreated the *hla* stop codon in isolate 22030, a phage-type 80/81 clone, and this mutant strain ($22030hla_{STOP}$) failed to secrete Hla into the culture medium (Fig. 3*B*). Unexpectedly, one of the phage-type 80/81 isolates (22251) failed to produce Hla, and correspondingly, this isolate had a SNP in *agrA* that predicts an amino acid substitution at residue 123 (Cys-to-Phe). In accordance with these data, 22251 produced little or no *RNAIII* (Fig. 3*C*).

A SNP in *agrC* Inhibits Production of *RNAIII*. We found that *RNAIII* expression in EMRSA-16 and three of the other contemporary MSSA isolates were reduced compared with the phage-type 80/81 clones 21295 and 22030, and the Southwest Pacific clone (Fig. 3*C*). To determine whether $agrC_{G55R}$ explains in part the reduced levels of *RNAIII* in these strains, the $agrC_{G55R}$ SNP was engineered in the phage-type 80/81 isolate 22030. The mutant

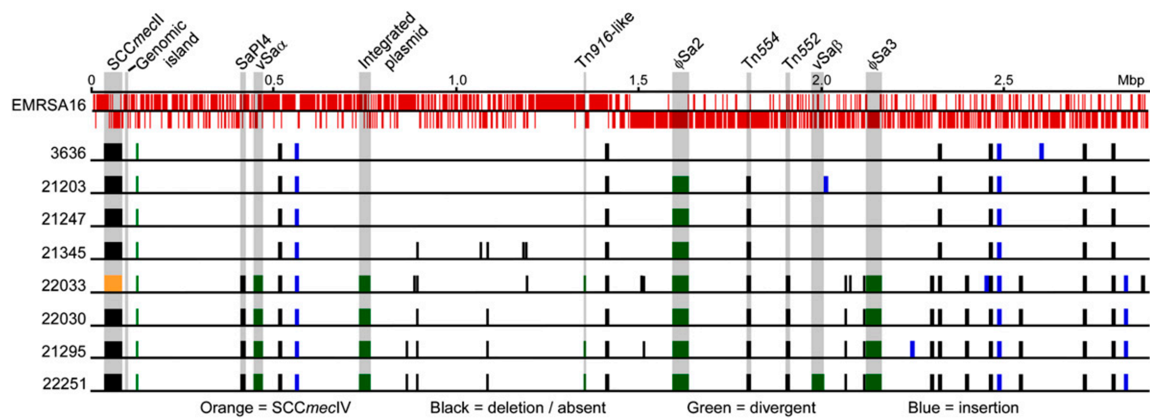


Fig. 2. Large insertions/deletions and mobile genetic elements (MGEs) among the eight sequenced CC30 *S. aureus* strains compared with the EMRSA-16 (MRSA252) reference strain. SCC*mec*, staphylococcal cassette chromosome *mec*; SaPI4, *S. aureus* pathogenicity island 4; Tn, transposon.

strain, 22030$agrC_{\mathrm{G55R}}$, failed to secrete Hla and produced very little *RNAIII*, results consistent with a significant defect in Agr (Fig. 3 *B* and *C*). Strain 21203, a contemporary CC30 isolate, contained the $agrC_{\mathrm{G55R}}$ mutation but had high levels of *RNAIII* (Fig. 3*C*). The molecular basis for this phenotype is not known and difficult to explain, but is perhaps due to a separate compensatory mutation that affects *agr*. Collectively, these data indicate that contemporary CC30 hospital isolates have defects in Agr and fail to produce Hla, changes that likely alter strain virulence and host–pathogen interaction.

Comparative Virulence in Mouse Infection Models. We next compared the ability of the eight query isolates and EMRSA-16 to cause disease in mouse infection models. Mice infected with isolates 21295, 22030, or 22033—phage-type 80/81 and Southwest Pacific clones—had in aggregate significantly decreased survival in a bacteremia model compared with animals infected with the contemporary CC30 hospital isolates (Fig. 4*A* and Fig. S2). Survival in mice infected with the phage-type 80/81 clone containing a SNP in *agrA* (isolate 22251) was similar to that of mice infected with EMRSA-16 and contemporary MSSA isolates, consistent with a prominent role for Agr and/or Agr-regulated molecules such as Hla in *S. aureus* virulence (Fig. 4*A*). Mice infected with 22030$agrC_{\mathrm{G55R}}$ or 22030hla_{STOP} had significantly reduced capacity to cause lethality in the mouse bacteremia model, indicating that these SNPs are largely responsible

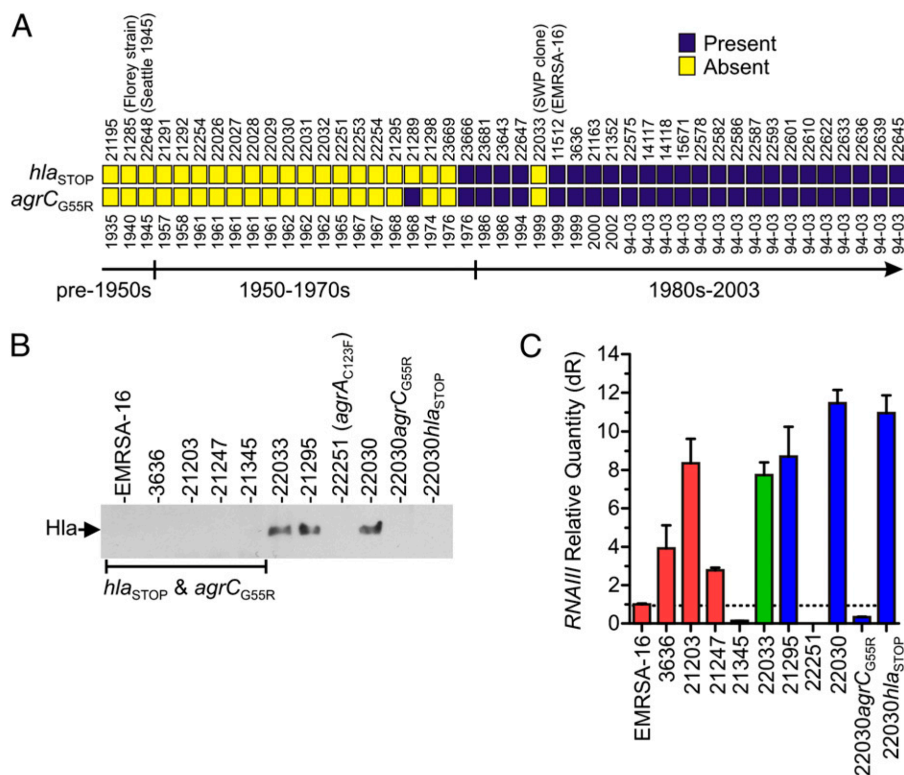


Fig. 3. Evolution of CC30 *S. aureus* includes acquisition of SNPs in *agr* and *hla*. (*A*) Presence or absence of $agrC_{\mathrm{G55R}}$ or hla_{STOP} in a representative subset of CC30 clinical isolates (45 isolates shown). (*B*) Immunoblot analysis of Hla in culture supernatants. (*C*) Relative quantitation of *RNAIII* by TaqMan real-time reverse transcriptase PCR. Dotted line indicates the level of *RNAIII* present in EMRSA-16, set arbitrarily as one. Data for *B* and *C* are representative of three separate experiments and error bars in *C* indicate SEM of triplicate wells from a single experiment. SWP, Southwest Pacific clone.

for the reduced virulence phenotype of the contemporary CC30 hospital isolates (Fig. 4*B*).

Inasmuch as pneumonia was a prominent manifestation of human infections with the phage-type 80/81 clone in the 1950s and 1960s, we compared virulence of the eight query CC30 isolates in a mouse pneumonia model. Survival was 100% for mice infected by intranasal inoculation with the contemporary CC30 hospital isolates and the *agrA* mutant strain 22251 (Fig. 4*C*). By comparison, survival was decreased significantly in the animals infected with phage-type 80/81 isolates 21295 or 22030 (containing wild-type *agrC* and *hla* alleles) (29% or 0%, $P < 0.0001$). Taken together, the data indicate that these representative phage-type 80/81 isolates and the Southwest Pacific clone are in general more virulent than contemporary CC30 hospital isolates, a phenotype attributed largely to an intact Agr gene regulatory system and functional Hla.

Concluding Comment. Here we used state-of-the-art molecular approaches to investigate the molecular and evolutionary basis of the phage-type 80/81 *S. aureus* pandemic, a puzzle that has

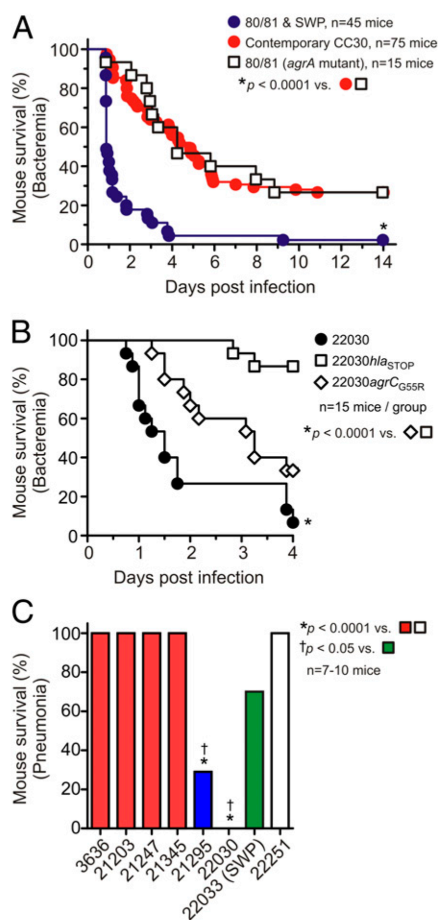


Fig. 4. Virulence of CC30 isolates in mouse infection models. (*A*) Mouse sepsis model. Mice (15 mice per strain) were infected with 5×10^7 cfu of *S. aureus*. Results are grouped as animals infected with pandemic phage-type 80/81 isolates plus the Southwest Pacific clone (45 mice) or contemporary CC30 hospital isolates (75 mice). Results for mice infected with 22251 (containing $agrA_{C123F}$) are plotted separate from the other phage-type 80/81 isolates. (*B*) Impact of $agrC_{G55R}$ and hla_{STOP} mutations on virulence of the phage-type 80/81 clone 22030. The mouse sepsis model was performed as described in *A*. (*C*) Mouse pneumonia model. Mice (7–10 per strain) were infected with $3–4 \times 10^8$ cfu of *S. aureus*. Data are presented as percent survival over a 48-h time period after *S. aureus* lung infection.

remained unresolved for decades. Although the outbreak of phage-type 80/81 *S. aureus* likely involves multiple factors, we found that in contrast to contemporary CC30 hospital isolates, the vast majority of phage-type 80/81 isolates contain a fully functional Agr gene regulatory system and encode full-length Hla. Correspondingly, phage-type 80/81 isolates were highly virulent in animal infection models (Fig. 4). Consistent with these observations, phage-type 80/81 *S. aureus* caused unusually severe human infections, including infections outside of the healthcare setting (6). Despite the success of this clone as a human pathogen, the percent of infections caused by it decreased dramatically by the mid-1960s (10). One possible explanation for the decline of phage-type 80/81 *S. aureus* is use of methicillin for the treatment of penicillin-resistant strains (10), as the worldwide decrease in these infections occurred within a few years after methicillin became widely used as a therapeutic agent. Alternatively, it is possible that phage-type 80/81 *S. aureus* acquired mutations during interaction with the host that in the end rendered it relatively ineffective as a human pathogen. Such a hypothesis is in part supported by our current findings with contemporary CC30 hospital isolates, which by comparison have decreased virulence in mouse infection models (Fig. 4). However, our comparative genome sequence analysis revealed that the contemporary CC30 hospital isolates tested here are not direct descendants of phage-type 80/81 *S. aureus* (Fig. 2). Therefore, these strains are not simply a re-emerged, less virulent version of the epidemic phage-type 80/81 clone.

One of the more interesting features of contemporary CC30 hospital clones is their ability to cause abundant and severe human infections despite the decreased virulence phenotype in animal infection models (Fig. 4). For example, Fowler et al. reported that EMRSA-16 and related isolates (ST36) were significantly associated with increased severity of infection (15). These findings seem at variance with our mouse infection data, but it is important to note that the infections caused by these CC30 strains occurred in the healthcare setting, and as such, infected individuals had underlying risk factors for infection (13, 15). Further, *S. aureus* strains with defective Agr gene regulatory systems (such as the contemporary CC30 hospital isolates studied here) typically have increased levels of surface protein expression, such as protein A and EMRSA-16, and related CC30 MSSA clones are known to be prominent human nasal colonizers (13, 15). Contemporary CC30 *S. aureus* are associated significantly with bloodstream infections and persistent bacteremia (15), which may simply be related to the overall high burden of colonization by these clones. It is possible that the decreased virulence capacity of the contemporary CC30 hospital clones compared with historic phage-type 80/81 *S. aureus* is a characteristic better suited for long-term colonization and persistence in the human host and that under certain predisposing conditions, allows these organisms to cause bacteremia. This idea is also consistent with the known absence of EMRSA-16–like clones as a cause of community-associated MRSA infections, because individuals with such infections are otherwise healthy. It should also be noted that virulence in mouse infection models is not established as being fully correlated with that in humans, a caveat that must be considered in the interpretation of animal infection studies.

The success of epidemic *S. aureus* clones is multifactorial and more information about the host and pathogen is needed to better understand why they emerge and ultimately disappear. The ability to attenuate high-virulence capacity of newly emerged clones is presumably critical to long-term survival of human commensal organisms such as *S. aureus*. Although our studies revealed two possible molecular mechanisms for attenuation of *S. aureus* virulence, more genome sequence information will be required to fully elucidate this process. For

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phage-type 80/81 *S. aureus*, it would also be important to have available the complete genome sequences of several clinical isolates that precede the epidemic of the 1950s. Obtaining the complete genome sequence of hundreds of *S. aureus* clinical isolates coupled with elucidation of specific host genotypes is the next step toward these goals.

Materials and Methods

For full details, see *SI Materials and Methods*.

***S. aureus* Isolates.** Eight *S. aureus* isolates were selected from among a collection of 284 CC30 patient isolates that were recovered over a span of 73 y (1935–2008) and from multiple geographical locations (Table 1).

Generation of Isogenic Mutant Phage-Type 80/81 Strains. The $agrC_{G55R}$ and hla_{STOP} mutations present in EMRSA-16 were created in the phage-type 80/81 clone 22030, according to a published method for allelic replacement (23).

Genome Sequencing and Phylogenetic Analyses. Genome sequencing was performed using a SOLiD 3 System (Applied Biosystems). Data generated by the SOLiD sequencer were analyzed using Corona-Lite (Applied Biosystems) and Zoom (Bioinformatics Solutions).

Phylogenetic analyses were performed using a contiguous 1.4-Mb region of the genome identified by Mauve (University of Wisconsin Genome Center) or with 2,299 concatenated SNP nucleotides in the core genome of all eight query isolates compared with the EMRSA-16 reference strain MRSA252 (NC_002952). DNA sequences were aligned using Clustal W and phylogenetic trees were generated by the neighbor joining method using 1,000 bootstraps in the Geneious software package (New Zealand Genome Center).

Mouse Infection Studies. All animal studies conformed to National Institutes of Health guidelines and were reviewed, approved, and supervised by the institutional animal care and use committees at Rocky Mountain Laboratories (mouse bacteremia model) or the University of Chicago (mouse pneumonia model). The mouse bacteremia and pneumonia models were performed as described previously (24, 25).

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