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Global Epidemiology and Evolutionary History of *Staphylococcus aureus* ST45

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ABSTRACT *Staphylococcus aureus* ST45 is a major global MRSA lineage with huge strain diversity and a high clinical impact. It is one of the most prevalent carrier lineages but also frequently causes severe invasive disease, such as bacteremia. Little is known about its evolutionary history. In this study, we used whole-genome sequencing to analyze a large collection of 451 diverse ST45 isolates from 6 continents and 26 countries. *De novo*-assembled genomes were used to understand genomic plasticity and to perform coalescent analyses. The ST45 population contained two distinct sublineages, which correlated with the isolates' geographical origins. One sublineage primarily consisted of European/North American isolates, while the second sublineage primarily consisted of African and Australian isolates. Bayesian analysis predicted ST45 originated in northwestern Europe about 500 years ago. Isolation time, host, and clinical symptoms did not correlate with phylogenetic groups. Our phylogenetic analyses suggest multiple acquisitions of the SCC*mec* element and key virulence factors throughout the evolution of the ST45 lineage.

KEYWORDS *Staphylococcus aureus*, ST45, phylogeny, whole-genome sequencing, MRSA, evolution, MRSA evolution

Staphylococcus aureus, an opportunistic pathogen colonizing up to 30% of the human population (1), causes a variety of mild to severe diseases among humans and animals (2). Clonal complex (CC) 45, a cluster of *S. aureus* isolates defined by multilocus sequence typing (MLST) sequence type (ST) 45 and closely related STs, has been shown to branch off near the root of the *S. aureus* population (3). CC45 is characterized by its diversity, consisting of methicillin-susceptible *S. aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA), community-acquired (CA) and hospital-associated (HA), and clinical and commensal clones. Furthermore, CC45 is isolated worldwide, and although less frequently reported in Asia, Africa, and South America (4–6), strains are predominant in North America, Australia and Europe (7) (Fig. 1).

CC45 contains several clinically important MRSA clones. ST45-MRSA-II, also known as MRSA-USA600, is widely spread in North America, and had been reported in other areas such as Hong Kong and Australia (7). In North America, USA600 is a major cause of endocarditis and bloodstream infections. For the latter, high ST45-MRSA-II mortality

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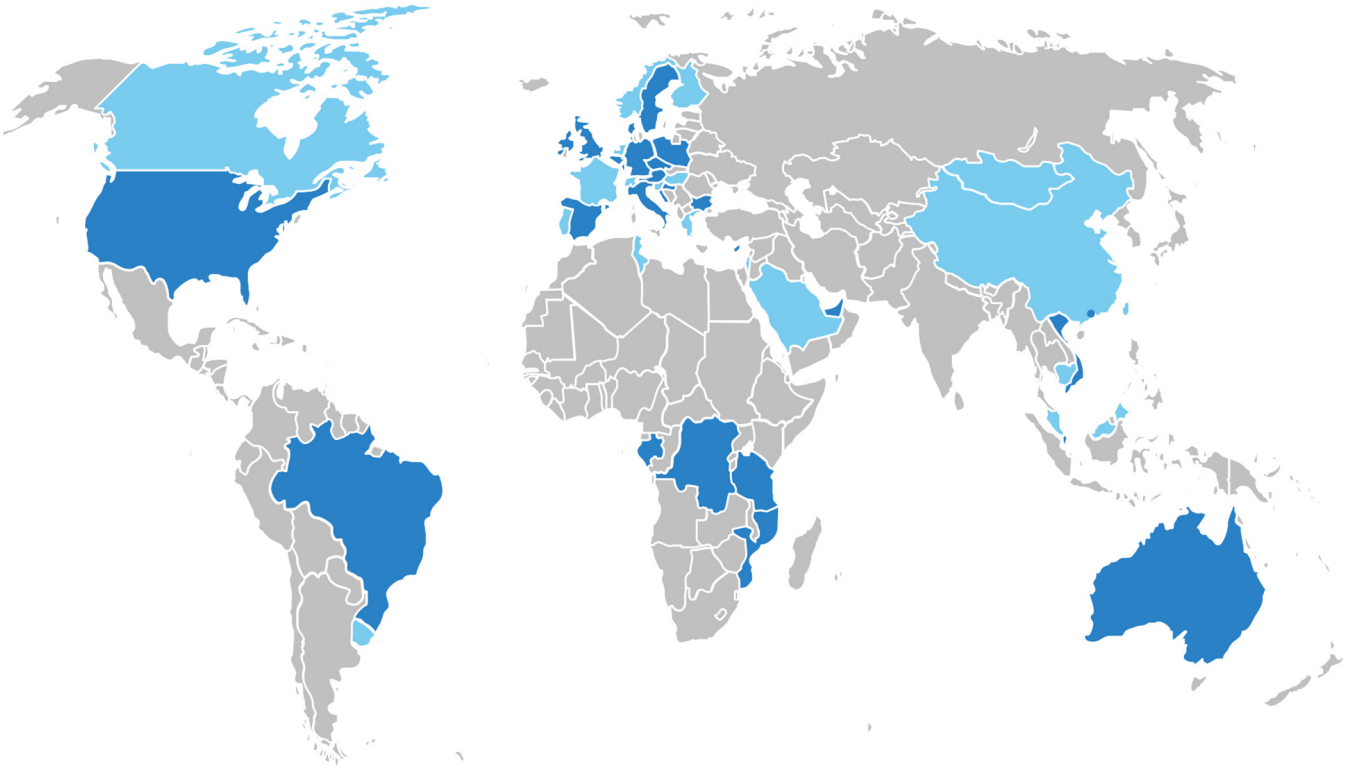

FIG 1 Global distribution of *S. aureus* ST45. Countries with CC45 isolates reported in the literature (as of February 2019) are colored in blue. The darker shade marks countries with ST45 isolates that were available for this study. (The world map template was obtained from https://www.powerpointslides.net/powerpointgraphics/powerpointmaps.html.)

rates have been reported (8, 9), which is consistent with the clone's high survival rates in blood (10). ST45-MRSA-IV also known as Berlin-IV or the Berlin epidemic strain, was initially isolated in Berlin hospitals in 1993, and has been a major clone over large areas of Germany (11). ST45-MRSA-IV and -V, which were the earliest CA-MRSA clones reported among the aboriginal communities in Western Australia (12), are found in most Australian regions. Recently, a multicenter outbreak of ST45-MRSA-V containing deletions in the *spa* gene was reported on the east coast of Australia (13).

ST45 is primarily known as a human-associated clone. However, isolates from aquatic environments (14, 15), animals (16–19), food items (20–24), and livestock producers (25) have been reported. Transmission of potential pathogens between animals and humans, either via direct contact or the food chain, is a rising public health threat (26). Therefore, the presence and diversity of ST45 should be carefully monitored.

Although *S. aureus* ST45 is a global pathogen with a variety of hosts, its population structure and genomic properties seem to differ between or even within regions. In this study, we aimed to identify the temporal and spatial origin, major evolutionary events, and global transmission routes of *S. aureus* ST45. For this purpose, we conducted in-depth, whole-genome sequence (WGS) analysis on 451 temporally and spatially diverse *S. aureus* ST45 isolates.

MATERIALS AND METHODS

Collection of isolates. Our data set consisted of 451 *S. aureus* ST45 WGS sequences (see Table S1 in the supplemental material). It included all ST45 sequences available at public databases (NCBI, pubMLST; until February 2019) which had sufficient metadata, i.e., at least year and country of isolation. Moreover, the sequences had to meet our internal quality criterion, i.e., presence of ≥95% of the previously defined 1,861 *S. aureus* core genome MLST (cgMLST) targets (27). Available read files were downloaded from the European Nucleotide Archive (ENA). To increase temporal and spatial diversity, we screened the MRSA TypeCat (28) and the literature and requested isolates directly from the authors. These additional isolates ($n = 295$) were sequenced on an Illumina MiSeq sequencer by following a 250-bp paired-end protocol (29). After quality trimming (average base quality 30 in a window of 20, downsampling to coverage of

TABLE 1 Metadata of 451 *S. aureus* ST45 isolates included in this study

Region	Country	Yr(s)	No.	Source
Australia	Australia	2000–2016	47	This study
Eastern Africa	Mozambique	2011	1	This study
	Tanzania	2008–2011	6	5[b]
Middle Africa	DR Congo	2013–2015	2	5[b]
	Gabon	2011	25	5[b]
North America	USA	1999–2017	73	25,[b] 68,[a] 69,[b] 70[a]
South America	Brazil	2005–2006	2	6,[b] 71[b]
Eastern Asia	Hong Kong	2000	1	This study
Southeastern Asia	Singapore	2009–2010	5	72[a]
	Vietnam	2017–2018	2	This study
Western Asia	Cyprus	2006	1	53[a]
	United Arab Emirates	2008	1	73[b]
Eastern Europe	Bulgaria	2006	1	53[a]
	Czech Republic	2006	2	53[a]
	Poland	2006	2	53[a]
Northern Europe	Denmark	1970–2009	79	This study
	Ireland	2003	1	Wellcome Sanger Institute[a]
	Sweden	2006	9	53[a]
	United Kingdom	2002–2017	39	This study, 74–77[a]
Southern Europe	Croatia	2006	3	53[a]
	Italy	2013–2016	7	78[a]
	Spain	2006–2015	4	53[a]
Western Europe	Austria	2006	2	53[a]
	Belgium	2006	5	53[a]
	Germany	2003–2018	130	This study, 5,[b] 53,[a] 79[a]
	Luxembourg	2010	1	TGEN-North[a]

[a]Downloaded from public database.
[b]Isolates sequenced in our lab.

180), the files were *de novo* assembled using Velvet v1.1.04 (30) and SeqSphere+ v4.1.90 software (Ridom GmbH, Münster, Germany). Overall, the 451 sequences were from isolates cultured between 1970 and 2018 and originated in Africa ($n = 34$), America ($n = 75$), Asia ($n = 10$), Australia ($n = 47$), and Europe ($n = 285$) (Table 1).

Genome analysis. Assembled genomes were screened for the presence of genes associated with regulation, resistance and virulence (Data Set S1 in the supplemental material) using the BLASTN algorithm (31) and different allele libraries implemented in SeqSphere+, which have been published by Strauß and colleagues (32). Additionally, ABRicate v0.9.8 (https://github.com/tseemann/abricate) was used with different databases, namely, CARD (33), AMRFinderPlus (34), VFDB (35), and PlasmidFinder (36), to search for antimicrobial resistance (AMR) genes, virulence factors, and plasmids, respectively. To determine SCC*mec* types, a custom ABRicate database was built from the SCC*mec*Finder database (https://bitbucket.org/genomicepidemiology/sccmecfinder_db/src/master/). Ambiguous results were reassessed directly from read files using the web-based SCC*mec*Finder (37). The results were manually screened for certain patterns, such as regional or phylogenetic clustering with specific AMR or virulence-associated genes. Furthermore, group-specific single nucleotide polymorphisms (SNPs) were identified using the "Find group-specific SNV" function implemented in SeqSphere+.

Phylogenetic analysis. After extraction of the up to 1,861 cgMLST targets (27), nucleotide sequences of all targets present in the 451 sequences were concatenated. A multiple sequence alignment (MSA) of these sequences was created using online MAFFT v7 (38). Recombinant regions were identified using Gubbins v2.4.1 software (39) and regions affected by recombination were removed from the alignment. A new, recombination-free MSA was created from the remaining targets resulting in a total sequence length of 418,491 bp from 553 cgMLST targets. From both alignments, a maximum likelihood (ML) tree was generated using the RAxML-HPC BlackBox tool v8.2.12 (40) on the CIPRES Science Gateway (41). The resulting trees were visualized and edited using FigTree v1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/) and iTol v4.0.3 (42). Differences between these trees were examined using the treespace package (43) in R v4.0.2 (44).

Ancestral dating. The recombination-free MSA was applied for coalescence-based analysis, including ancestral dating, using the BEAST software package v1.10.4 (45). Using chain lengths of 50,000,000 and logged every 1,000 states, several model combinations, including different substitution models, strict and relaxed clock, as well as different coalescent models, were tested and compared by ratios of marginal likelihoods using the model comparison function, implemented in Tracer v1.7.1 (46), with the AICM criterion and 100 bootstrap replicates. The final analysis was run using the GTR substitution model with site heterogeneity and four gamma categories, a strict clock and logistic growth. The prior for the clock rate was set to uniform (0 to 1, initial = 0.0001). Sampling dates in years were included as tip dates and country of isolation was used as an additional trait. All other parameters were left at default settings. The BEAST analysis was run on the CIPRES Science Gateway (41). Four independent runs were started and checked for convergence. Moreover, a priors-only file was run to ensure the informativeness of the data. The

four runs were combined and downsampled to 10,000 states using LogCombiner v1.10.4 from the BEAST package, discarding 10% burn-in from each run. After analyzing the MCMC trace files of the combined file in Tracer v1.7.1 (46), a maximum clade credibility tree (mcc) was calculated in TreeAnnotator v1.10.4 using median node heights. The resulting phylogeny was inspected using FigTree v1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/) and visualized in iTol v4 (42).

Data availability. Raw sequence data of isolates sequenced for this study were deposited in the European Nucleotide Archive under study accession no. PRJEB40321. Assemblies of two isolates have been deposited at GenBank under BioProject no. PRJNA650390. Single accession numbers for all isolates are provided in Table S1 in the supplemental material.

RESULTS

ST45 phylogeny and spatiotemporal distribution. An ML tree was created based on the concatenated cgMLST target sequences present in all sequences (765 of 1,861 target genes). To ensure our results were not biased by horizontal gene transfer (HGT) events, probable recombination sites were identified and the affected target genes were purged from the alignment. Gubbins identified 57 regions of potential HGT in the alignment, spanning 212 cgMLST genes. To assess the impact of these regions on the overall phylogeny, we compared ML trees of both alignments, including and excluding HGT-affected targets (Fig. S1 in the supplemental material). The related tree distance using the Kendall-Colijn metric (47) and country of origin as category was 7.189, which confirmed that the topology of both trees is comparable. Although the comparison showed that the overall topology and position of isolates within the tree did not change, by removing the HGT-affected targets the branch lengths were altered. Consequently, all further analyses were based on the purged alignment.

To identify potential groups within the ST45 phylogeny, we analyzed the distribution of associated metadata of isolates such as the region and the year of isolation, as well as genomic features across the phylogeny (Fig. 2). The tree topology showed a very basal split into two distinct sublineages separated by long branches with strong geographical signatures. With some exceptions, the two sublineages fitted regional differences. The larger sublineage (European/North American sublineage = EU/NA, $n = 373$) primarily consisted of isolates from Europe ($n = 276$, 74%) and North America ($n = 70$, 19%), with a few isolates from Australia ($n = 18$, 5%) and a few unrelated isolates from Africa, Asia, and South America ($n = 9$, 2%). The second sublineage (African/Australian sublineage = AFR/AUS, $n = 78$) could be further divided into two groups. One group (AFR, $n = 31$, 40%) almost exclusively consisted of African isolates from Gabon and Tanzania, with one exception from Denmark. The other group (AUS, $n = 47$, 60%) primarily included isolates from Australia ($n = 29$, 62%), as well as some isolates originating from Asia ($n = 7$, 15%), Europe ($n = 8$, 17%), and North America ($n = 3$, 6%). With regard to date of isolation, we did not identify any distribution pattern except for closely related isolates coming from individual studies.

To investigate genetic differences between the two sublineages, we screened the HGT-free cgMLST target sequences for sublineage-specific SNPs that were present in the AFR/AUS isolates but not in the EU/NA isolates. In total, 457 AFR/AU-specific SNPs in 188 target genes were detected. Eighty of the SNPs were nonsynonymous substitutions in 62 target genes. The affected genes did not belong to obvious functional groups and, apart from approximately 400 kb around the origin of replication, they were distributed over the entire chromosome (Fig. S2). The absence of the affected genes around the origin of replication was caused by the removal of most of the genes located in this area due to potential HGT.

Genomic features of ST45 isolates. In total, 180 MRSA and 271 MSSA isolates were included in this study. Methicillin resistance occurred in several different clades across the tree. All isolates from the AFR group were MSSA, while the AUS group mainly consisted of MRSA. For the EU/NA group, we did not find an association between methicillin-susceptibility and region, date of isolation, or clade. Thirty MRSA carried SCC*mec* II, 101 SCC*mec* IV, and 49 SCC*mec* V. While SCC*mec* IV isolates occurred in multiple clades, SCC*mec* II was only found in a single monophyletic group with isolates primarily from the United States. The majority of SCC*mec* V isolates belonged to the AUS group and a distinct Australian clade within the EU/NA branch. However, a few

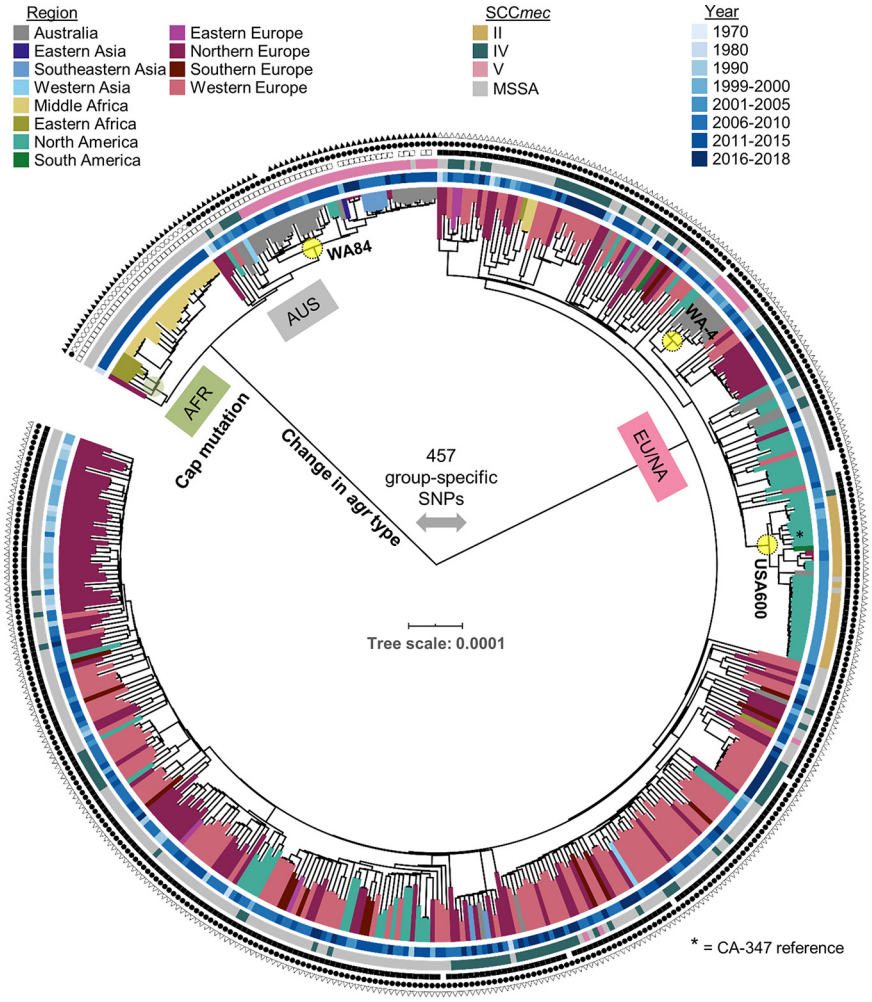

FIG 2 Maximum-likelihood tree of 451 *S. aureus* ST45 isolates, including metadata and genomic features. Branch lengths represent SNPs per site based on a 418,491-bp long alignment of 756 cgMLST target genes. The tree was midpoint rooted. Isolates are colored according to their geographical region of origin. Isolation countries were summarized into geographical regions as defined by the United Nations. Year of isolation is represented on the inner ring and SCC*mec* types on the second ring. On the outer rings, *sasG* presence and *agr* and *cap* type are illustrated with symbols as follows: filled square, *agr*I; empty square, *agr*IV; missing square, ambivalent *agr* results; filled circle, Cap5; empty circle, Cap8; filled triangle, *sasG* present; empty triangle, *sasG* not present; missing triangle, *sasG* ambivalent results. Important lineages are marked with yellow circles and bold text; the CA347 reference genome is highlighted with an asterisk.

SCC*mec* V isolates were found to cluster in different clades (Fig. 2). The different SCC*mec* types identified depict known ST45 lineages. The monophyletic SCC*mec* II clade represents the USA600-like isolates, while ST45-MRSA-IV (Berlin-IV) isolates are scattered over the EU/NA group. The two Australian SCC*mec* V clades in the AUS and EU/NA subgroups depict the WA-84 and WA-4 lineages, respectively.

All isolates were further screened for the presence of different genomic features, such as virulence factors, resistance, and regulatory genes. No correlation between regional group and resistance profile was found. However, *tet*(*K*) was more frequently detected in the AFR ($n = 11$, 35%) and AUS ($n = 35$, 74%) groups than in the EU/NA sublineage ($n = 4$, 1%). Although *blaZ* was present in most AFR ($n = 30$, 97%) and AUS ($n = 38$, 81%) group isolates, it was only detected in 55% of EU/NA ($n = 204$) sublineage isolates. Of the 33 isolates carrying *ermC*, 20 were isolates from the AUS subgroup and *ermA* was primarily found in isolates clustering with the USA600 reference (32 of 42).

Three factors known to influence virulence correlated with the regional groups described above. The regulation factor *agr* genes in the AFR/AUS and the EU/NA

sublineages were *agrIV* and *agr*I, respectively. The African isolates, which formed the AFR group, carried the type 5 capsular polysaccharide *cap5* gene, while all other isolates in the collection carried *cap8*. Apart from two Australian isolates, the virulence factor *sasG*, encoding the *S. aureus* surface protein G, was found in all isolates in the AFR/AUS sublineage but was not detected in the EU/NA sublineage. Only one isolate within the AFR/AUS sublineage carried genes encoding Staphylococcal enterotoxins. A combination of *sec* and *sel* was present in several but not all EU/NA isolates. A few of the EU/NA isolates also were positive for *tsst-1*, which encodes the toxic shock syndrome toxin (TSST). The immune evasion cluster (IEC) was not detected in 13 isolates scattered across the phylogeny. Few isolates in the AUS group lacked *scn*. None of the isolates in the collection carried the *luk-F/luk-S* Panton-Valentine leucocidin (PVL) associated genes. A list of presence/absence data for all genes analyzed can be found in Data Set S1 in the supplemental material.

Phylogeographic analysis and ancestral dating. To gain further insights into the origin and dissemination of *S. aureus* ST45, we reconstructed the isolates' temporal and spatial development with a Bayesian analysis. All runs passed our quality control, i.e., good effective sample size (ESS) values, convergence, and difference to priors-only run. Figure 3 shows the most probable geographical origin of the lineage and all subclades, as well as temporal estimates for the emergence of new clades based on substitution rates and isolates' metadata in an mcc tree. All major nodes and clades described in the following were supported with high (>0.9) posterior probabilities (PP) (Fig. S3). The Bayesian analysis confirmed the major sublineages and regional subgroups described using the ML approach. The estimated mutation rate was 1.29×10^{-6} substitutions per site per year. According to our analysis, the ST45 lineage originated around the year 1500 (median: 1519, 95% highest posterior density [HPD]: 1455 to 1580, PP = 1) in North/West Europe (11% Denmark, 9% Sweden, 8% Germany). A divergence into two distinct sublineages (AFR/AUS and EU/NA) subsequently occurred. The most recent common ancestor (MRCA) of the AFR/AUS sublineage circulated in central Europe (48% Denmark, 45% Germany) around 1945 (95% HPD: 1936 to 1953). A few years later (median: 1950, 95% HPD: 1942 to 1959), transmission of the sublineage's MRCA from Europe (53% Denmark, 38% Germany) to Eastern Africa (PP = 1) occurred, followed by a transmission to Middle Africa (median: 1975, 95% HPD: 1970 to 1983). Around the same time of dispersal in Africa, transmission of the strain to the United States occurred (median: 1950, 95% HPD: 1942 to 1958) and then to Australia (median: 1957, 95% HPD: 1948 to 1966). Finally, this Australian strain (group AUS) was isolated in Asia a few decades later (median: 2000, 95% HPD: 1996 to 2004).

The MRCA of the second sublineage (EU/NA) circulated in the early 20th century (median: 1917, 95% HPD: 1905 to 1928) in Germany (96%). A few decades later (median: 1934, 95% HPD: 1926 to 1945), the strain emerged in the United States before being reintroduced to Europe (median: 1946, 95% HPD: 1939 to 1955) and transmitted to Australia (median: 1955, 95% HPD: 1946 to 1964). Major transmission events are visualized in Fig. 4.

Based on the ancestral dating and distribution of SCC*mec* types, we analyzed when different SCC*mec* elements were acquired in the different groups. According to our analysis, the acquisition of SCC*mec* II, which led to the emergence of USA600, occurred in the 1970s (median: 1972, 95% HPD: 1966 to 1978). Both Australian SCC*mec* V strains acquired the element around 1980 (WA-84, median: 1981, 95% HPD: 1976 to 1987; WA-4, median: 1980, 95% HPD: 1974 to 1986). SCC*mec* IV elements were acquired multiple times within the ST45 population. Some more recent acquisitions include a British lineage that acquired SCC*mec* in the late 1990s (median: 1998, 95% HPD: 1992 to 2003) and a German lineage that can be dated to the beginning of the millennium (median: 2007, 95% HPD: 2003 to 2012).

DISCUSSION

Our results provide an insight into the evolutionary history of the clinically significant *S. aureus* ST45 lineage. We have investigated a global collection of ST45 WGS data

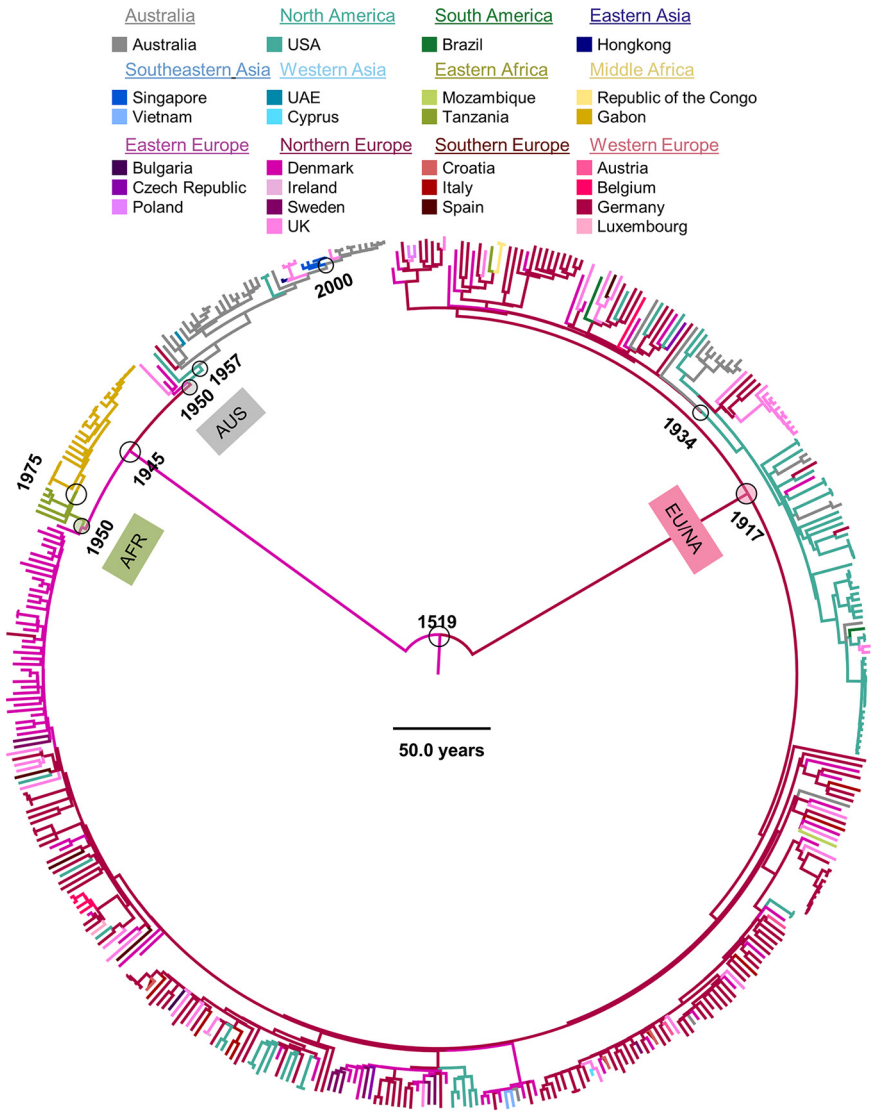

FIG 3 Maximum clade credibility tree resulting from a BEAST analysis of 451 *S. aureus* ST45 genomes. Origins of samples and their most recent common ancestor are represented by branch colors. Median node ages for some most relevant nodes are provided. Names of major sublineages are highlighted: AFR, African; AUS, Australian; EU/NA, European/North American. Refer to Fig. S3 in the supplemental material for 95% HPD intervals and posterior probabilities.

with a vast diversity in terms of temporal and geographical origin, host, and clinical manifestation. We have shown that the ST45 phylogeny is defined by two distinct sublineages with spatial subgroups. Moreover, we have analyzed the origin and global transmission of ST45 and determined the relation of previously described, clinically relevant clones.

ST45 phylogeny depicts geographical subgroups. Albeit with various prevalence, our data set contained ST45 isolates originating from six continents. Although isolates from different regions can be found throughout the tree, certain regional groups are observed within the ST45 phylogeny. There is a very clear separation between isolates from (i) Africa, Australia, and Asia and (ii) Europe and North America. Although ST45 is primarily human-associated, it can occasionally be found in animals and food items. The animal-associated isolates in our data set did not cluster together but were scattered over the phylogeny. Since only two lacked *scn*, the isolates may represent human spillover rather than an animal-associated ST45-sublineage. Similarly, we were not able

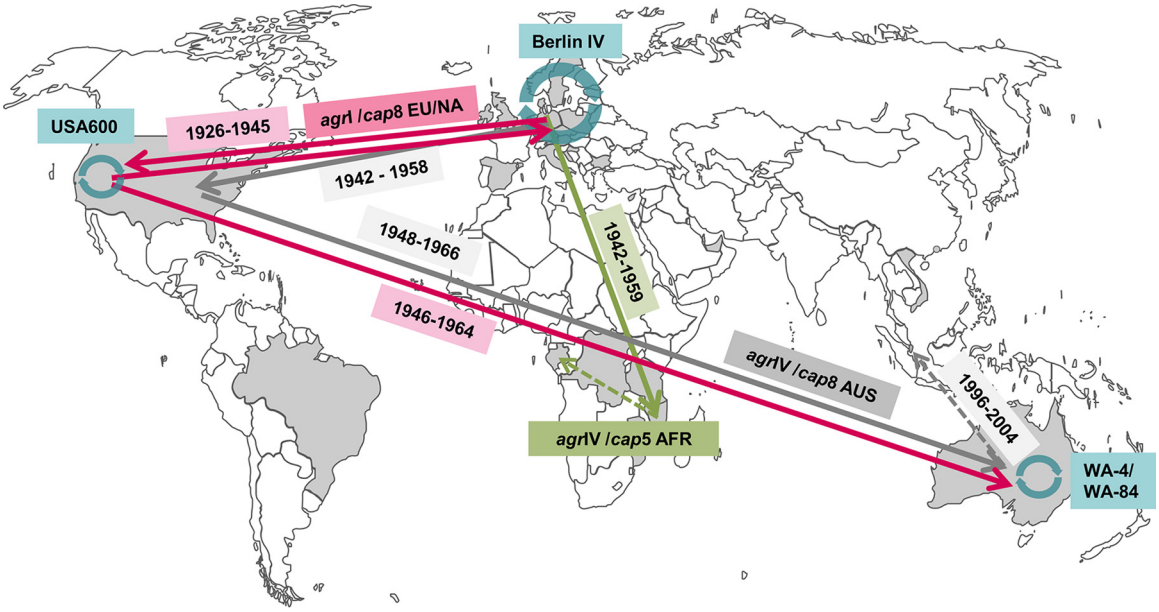

FIG 4 Map of major transmission events. Countries where isolates were obtained from are shaded in light gray. Transmissions are indicated with arrows and colored according to the three major phylogenetic groups that were identified. Acquisition of *agr* and *cap* type is highlighted. The dating of transmission (95% HPD interval) as calculated by BEAST is given. Major lineages circulating to date are marked with blue, circular arrows, and the respective labels. (The world map template was obtained from https://www.powerpointslides.net/powerpointgraphics/powerpointmaps.html.)

to identify monophyletic clusters for the commensal and clinical isolates or the HA and CA isolates.

Multiple acquisitions of SCC*mec*, resistance, and virulence factors within the ST45 population. To further investigate what distinguishes the AUS/AFR sublineage from the EU/NA sublineage, the gene content of the isolates was analyzed. On a core genome level, we identified 457 SNPs that were specific to the AFR/AUS sublineage. The AFR/AUS-specific SNPs were scattered over the entire chromosome in a mosaic-like manner and no certain hot spots were observed. In addition to the core genome, we analyzed the distribution of various genomic features associated with regulation, resistance, and virulence. Presence of genes encoding the virulence factor *sasG*, which was exclusively found in AFR/AUS, and subtypes of the accessory gene regulator *agr* correlated with the divergence between the AFR/AUS and EU/NA sublineages. Capsular polysaccharide type 5 was unique to the African clade. The *agr* locus encodes a signaling pathway, which is essential for the regulation of the expression of virulence factors (48). The surface protein *sasG* promotes adhesion to nasal epithelial cells. Concurrently, if expression levels are high, it can also mask the binding of microbial surface components recognizing adhesive matrix molecules (MSCRAMMs), which also promote cell adhesion, to their ligands. Thus, *sasG* could be advantageous for colonization but also to promote detaching and dissemination via the bloodstream during infection (49). The AFR/AUS sublineage, which carried *agr* IV and *sasG*, comprised the AFR group where all isolates were from nasal carriage and the AUS group where isolates were primarily clinical. Consequently, a direct impact on virulence or adherence could not be inferred. For *sasG*, differences may be explained by expression levels, which would require a detailed study to elucidate. A small number of isolates were missing part of or all of the IEC. Such absences in lineages are typically linked to livestock-associated (LA) MRSA, such as CC398 (50). From our samples, however, only two isolates, one isolated from cheese and one from nasal carriage of a livestock producer, had a known association to livestock. We did not identify genetic markers that could be correlated with clinical manifestation. This is similar to the recent findings by Roe and colleagues (51), who also could not find distinguishing genetic characteristics between

CC45 bacteremia and nasal carriage isolates from Denmark, suggesting that all CC45 isolates could have a potential to cause invasive diseases. Although many ST45 isolates would be classified as CA in terms of host epidemiology, i.e., no history of hospitalization or residence in a care facility (52), none of the isolates in this study were PVL positive.

The distribution of MRSA within our data set indicates that multiple introductions of *mecA* have occurred during the evolution of ST45. Our data suggest different scenarios have occurred for different SCC*mec* elements. All SCC*mec* II isolates were located in one clade, indicating a single acquisition. SCC*mec* IV, however, was found distributed across the phylogeny, sharing several different ancestors with MSSA isolates. Moreover, the Australian SCC*mec* V isolates belong to two very distinct clades, also indicating different introduction events. Although some resistance genes were more common in certain subgroups, no clear correlation between resistance profile and regional group was found. Overall, our analysis shows that inferring ancestral relationships between isolates from genotypic typing, i.e., a combination of virulence markers or resistances, can be misleading. Though a few virulence-related genes correlated with phylogenetic groups, other combinations and SCC*mec* types were introduced or lost multiple times throughout the evolutionary history of ST45.

Distribution of clinically relevant ST45-MRSA clones. USA600 or ST45-MRSA-II, associated with a high mortality rate, is the most frequently reported ST45 clone in the literature. All USA600-like isolates in our data set formed a monophyletic clade and were isolated mainly in the United States, supporting the assumption that USA600 is rarely found outside North America (7). The Berlin epidemic strain in Europe and WA-MRSA-75 in Australia both refer to the well-established ST45-MRSA-IV, which is a predominant clone in Europe (53) and one of the four major MRSA lineages identified in Western Australia (54). This genotype was the most common MRSA clone in our study and was located in the EU/NA sublineage. Another Australian lineage found in indigenous people is WA-4 or ST45-MRSA-V (7). Isolates sharing these features form a distinct Australia-only clade, closely related to the USA600 cluster. All three MRSA clones mentioned so far harbor *agr* type I. Within the Australian *agr*IV group, most isolates were *SCCmec* V, representing the frequently identified WA-84 clone.

The observation of a unique African subgroup of ST45 is novel. A reason for this may be that all isolates belonging to the AFR group are nasal carriage MSSA isolates and therefore of less clinical significance for most biomedical research. The isolates were taken from a study by Ruffing and colleagues (5), which focused on the comparison of *S. aureus* prevalence in Africa to that in Germany. According to their study, *agr* types I to IV are present in African *S. aureus* lineages, whereas in Germany mainly type I with only rare occurrences of *agr* IV was found. This is in line with our findings of *agr* IV in the African and *agr* I in the European isolates. CC45 isolates were significantly less prevalent in Africa than in Germany. Similar to our study, the authors found two distinct ST45 clusters related to geographical origin.

European origin and global spread of ST45. Utilizing a Bayesian analysis, including phylogeographic reconstruction and ancestral dating, we have proposed a model for the emergence and global dissemination of *S. aureus* ST45. Our model suggests that the lineage originated in the early 16th century in Northern Europe. Subsequent to its emergence, a divergence into two distinct sublineages occurred approximately 500 years ago. Presumably, there were more variations, but likely bottleneck events resulted in only these two sublineages able to establish successfully. Because of the long time span, we can only speculate on reasons for this diversification. However, population size changes in pathogens are often a consequence of changes among their hosts (55). Since humans are considered the major ST45 host, human movements might have been a major driver for selection on ST45 sublineages. This phenomenon has been described before, e.g., for *Helicobacter pylori* (56). The opening of Atlantic and African trade routes (57), and thus completely new environments, may have had a large impact on dissemination.

The last common ancestor of the AU/AFR lineage circulated in Europe in the mid-20th century, from where it spread to Tanzania. The African lineage is unique and

so far has not been found on any other continent. This phenomenon has been observed previously in ST8 (58) and may be due to different demographic aspects of developing regions. Potential transmission routes from Africa to other continents are limited since there is significantly less tourism and trading from Africa into Western countries (59). However, it is interesting that the clone can be found in Gabon as well, meaning it was transmitted from a country located on the eastern coast of Africa to a country in the west, where these two regions are separated by the central African rainforest. Our collection lacks isolates from other African countries, especially from North Africa, where travel activities to Europe are higher due to immigration (60). Isolates from China, a strong trading partner for African goods (61), were also not included. Isolates from these regions may be helpful to further elucidate the role of Africa in the evolutionary history of ST45.

In the 1950s, the clone was, based on the most likely model, brought to Australia via the United States. This is a reasonable transmission route given the increased travel frequencies between the two countries during World War II and in the postwar era. Later, around 2000, the clone was likely introduced to Asia from Australia, which is not surprising considering their geographic proximity. Our findings suggest that the different ST45 lineages present in Australia were due to several introductions rather than one Australian ancestor. According to our analysis, SCC*mec* V was introduced to Australia around 1980. This fits well with the fact that MRSA became a notifiable organism in Western Australia in 1982 and the identification of WA-4, which is considered one of the earliest CA-MRSA clones in remote regions of Western Australia, occurred in 1995 (54).

The MRCA of the EU/NA lineage circulated in Germany in the early 20th century. From there, a mainly European genotype evolved that remains the major clone found primarily in Europe. It was carried over to the United States in the 1930s, which could be associated with the emigration wave during the rise of National Socialism in Germany at that time (62). Later in the 20th century, it was reintroduced to Europe and transmitted to Australia while it continued to circulate in the United States and evolved into the highly pathogenic USA600.

The proposed scenario outlines a reasonable evolutionary history for the modern *S. aureus* ST45 population. A European origin is probable, considering the lineage's high prevalence in European countries. Studies on other STs, such as ST8 (58) or ST22 (63), also support a European origin. The suggested mutation rate of 1.29×10^{-6} substitutions per site per year is also in line with other studies reporting mutation rates for *S. aureus* lineages between 1.05 and 2.0×10^{-6} substitutions per site per year (64–67).

Although the drivers of the ST45 evolutionary history are only speculative, several aspects support the proposed model. Nonetheless, the limits of the present study relate to the underlying biases of phylogeographic analyses, where temporal and spatial conclusions arise from imperfect sampling or limited availability of samples. Although we tried to overcome the limitation by including as much diversity regarding sampling sites as possible, the study only provides a defined snapshot of the population. It cannot be ruled out that different clones not captured within the collection may lead to different or new results. We therefore need to emphasize the importance of adding more data from observational population screenings and underrepresented countries in order to reduce the bias toward pathogenic, Western world MRSA in the public databases.

Conclusion. In this study, we analyzed a data set of 451 *S. aureus* ST45 isolates from different settings. ST45 is a highly diverse lineage associated with different global regions, hosts, antimicrobial susceptibility, and clinical manifestations. We analyzed this diversity in relation to the strain's population structure. We clarified the relation of clinically important strains such as the USA600 within the overall phylogeny and identified different regional clusters, including a unique and so-far-undescribed African clade. Moreover, we suggested a possible reconstruction of the origin and evolutionary history of one of the most prevalent and clinically significant global *S. aureus* strains, including the dating of important transmission events.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, XLSX file, 0.04 MB.

SUPPLEMENTAL FILE 2, PDF file, 0.2 MB.

SUPPLEMENTAL FILE 3, PDF file, 0.3 MB.

SUPPLEMENTAL FILE 4, XLSX file, 0.2 MB.

SUPPLEMENTAL FILE 5, PDF file, 0.04 MB.

SUPPLEMENTAL FILE 6, PDF file, 0.03 MB.

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