Open Forum Infectious Diseases

MAJOR ARTICLE

Dynamic Networks of Methicillin-Resistant *Staphylococcus aureus* in Communities Drive Hospital Transmission

Scott A. Cohen,[1,2] Massimiliano S. Tagliamonte,[1,3,4] Carla Mavian,[1,3] Nicole M. Iovine,[5] Yuting Zhai,[1,6] Kwangcheol C. Jeong,[1,6] Mattia Prosperi,[1,2] J. Adrian Tyndall,[7] Marco Salemi,[1,3] and J. Glenn Morris Jr.[1]

[1]Emerging Pathogens Institute, University of Florida, Gainesville, Florida, USA, [2]Department of Epidemiology, College of Public Health and Health Professions and College of Medicine, University of Florida, Gainesville, Florida, USA, [3]Department of Pathology, Immunology, and Laboratory Medicine, College of Medicine, University of Florida, Gainesville, Florida, USA, [4]Bioinformatics Core, Interdisciplinary Center for Biotechnology Research, University of Florida, Gainesville, Florida, USA, [5]Division of Infectious Diseases, Department of Medicine, College of Medicine, University of Florida, Gainesville, Florida, USA, [6]Department of Animal Sciences, College of Agriculture and Life Sciences, University of Florida, Gainesville, Florida, USA, and [7]Morehouse School of Medicine, Atlanta, Georgia, USA

Background. Although methicillin-resistant *Staphylococcus aureus* (MRSA) transmission has traditionally been viewed separately in hospital and community settings, this distinction is increasingly blurred. We used whole-genome sequencing and epidemiologic analyses to characterize the movement of MRSA across these interfaces in a rural–urban population.

Methods. Serial cross-sectional sampling of MRSA isolates occurred at a tertiary care hospital between 2010 and 2019. Community-onset MRSA was prospectively isolated from patients presenting to the emergency department with acute skin and soft tissue infections (SSTIs), while hospital-onset MRSA was sampled before (2010), during (2015–2017), and after (2019) this community collection period. MRSA transmission was assessed using a joint application of epidemiological approaches and phylodynamic analysis of whole-genome sequences.

Results. After whole-genome sequencing on community and hospital MRSA isolates, phylogenetic analysis revealed 2 major clades distinguished by clonal complex (CC) CC8/t008 and CC5/t002 *spa* types. Multiple independent introductions of MRSA lineages from the community to the hospital were observed. Geographic clustering of community-onset MRSA was uniquely present outside of the urban center. Subjects with rural residence or livestock exposure were more likely to have community-onset MRSA SSTI compared with those with non-MRSA SSTI.

Conclusions. MRSA transmission in hospital settings was introduced from strains with ancestral origins in community settings. Although community-onset MRSA transmission appears sustained with limited influence from hospital strains, more comprehensive surveillance is required to quantify this relationship. Nosocomial MRSA outbreak prevention strategies should target unique aspects of the community in addition to the hospital, particularly hot spots, risk behaviors, and strain reservoirs.

Keywords. methicillin-resistant *Staphylococcus aureus*; hospital-acquired infection; rural; phylogenetics; whole-genome sequencing.

Methicillin-resistant *Staphylococcus aureus* (MRSA) infections are the leading cause of antibiotic-resistant infections in hospitals and are an important cause of outpatient and acute care visits [1, 2]. Historically recognized as a predominantly health care–associated pathogen (HA-MRSA), MRSA epidemiology has evolved over the past several decades with the emergence of community-associated strains (CA-MRSA), particularly due to the USA300 strain in the United States [3]. These CA-MRSA strains are now frequently implicated in skin and soft tissue infections (SSTIs) seen in outpatient and acute care settings. While overall SSTI incidence may be stabilizing or declining [4, 5], the shifting landscape of MRSA transmission continues to challenge public health interventions and clinical management [6].

The traditional distinctions between HA-MRSA and CA-MRSA are becoming increasingly blurred. CA-MRSA strains, once confined to healthy individuals without recent health care exposure, are now frequently implicated in health care–associated infections [7]. International reports and case studies have documented instances of community-derived MRSA strains emerging as the cause of hospital epidemics and becoming the dominant strains in nosocomial infections [7]. In contrast, livestock-associated MRSA (LA-MRSA) remains a well-defined entity in international contexts where specific clonal complexes are linked to agricultural exposure. However, in the United States, LA-MRSA is less understood,

Received 25 February 2025; editorial decision 23 April 2025; accepted 25 April 2025; published online 29 April 2025

Correspondence: Scott A. Cohen, MPH, Emerging Pathogens Institute, University of Florida, PO Box 100009, Gainesville, FL 32610 (scohen211@ufl.edu).

Open Forum Infectious Diseases®

https://doi.org/10.1093/ofid/ofaf264

though livestock exposure has been identified as a potential risk factor for MRSA infection. These overlapping transmission routes underscore the complexity of MRSA epidemiology and highlight the need for more research in mixed rural and urban settings, where traditional CA-MRSA risk factors, such as close contact, may not fully explain transmission patterns.

Molecular *S. aureus* typing techniques have been used to characterize evolution and transmission during local and national epidemics. Traditional methodologies, such as pulse-field gel electrophoresis, lack the discriminatory power to detect related strains and subtypes of clonal bacteria. Advancements in whole-genome sequencing (WGS) and single nucleotide polymorphism analysis permit establishment of a temporal network of transmission across diverse settings and elucidation of transmission events that would be otherwise undetected with traditional techniques [7]. More specifically, use of WGS techniques to classify MRSA has improved our knowledge of the genetic changes associated with different MRSA types, along with information about resistance, virulence, and host adaptation [8]. Further, integrating these genomic data with epidemiological information offers a comprehensive approach to better understand MRSA transmission pathways within and between community and hospital settings. Previous studies in Chicago successfully integrated genomic and epidemiological data, demonstrating that community factors are primary drivers of hospital-onset MRSA infections [9]. However, transmission data of this type are more limited for nonurban communities.

In this longitudinal analysis, we combined epidemiological and phylodynamic analyses using WGS to elucidate MRSA transmission networks within and between community and hospital settings in a mixed rural and urban population. Further, we sought to identify the geographic, behavioral, environmental, and social factors that influence community MRSA transmission.

METHODS

Study Design

Serial cross-sectional community and hospital MRSA sampling was performed at a tertiary care hospital between 2010 and 2019 (Figure 1). For sampling the community-onset study population, pediatric and adult patients presenting to the emergency department at a large tertiary care teaching hospital (UF Health Shands, Gainesville, FL, USA) with evidence of a skin or soft tissue infection (SSTI) were prospectively enrolled between August 2015 and January 2017. SSTI was defined as acute (<4 weeks), nonpostoperative infection such as abscess, cellulitis, furuncle, carbuncle, folliculitis, and insect bites. Patients who actively work in a health care setting or provide routine patient care in outpatient or inpatient settings were excluded. Information about potential subjects' medical history, as well as demographic and contact information, was used to confirm subject eligibility. After voluntary written consent, a questionnaire was completed by participant interview to collect patient demographics, medical history, social history, hygiene habits, and exposure history. Medical records were reviewed to verify and reconcile demographic information and social information, as well as to elicit documentation of past medical history and prior treatment for SSTI. At the completion of the interview, a culture of the SSTI (ie, wound culture) and a culture of the anterior portion of the nasal cavities were collected by study personnel. MRSA was confirmed in accordance with the Clinical & Laboratory Standards Institute guidelines.

For sampling in the hospital setting, specimens from hospital-acquired MRSA-positive infections collected as part of routine standard-of-care procedures were sampled concurrently with the community study population (2015–2017) and 2 years after the community study period (2019). Hospital-acquired infections were defined as infections that were confirmed by positive cultures from specimens collected at least 48 hours after admission and that were not present at time of admission. Whole-genome sequencing data of hospital-onset MRSA isolates collected in 2010 from the same tertiary care center were also extracted for analysis [8].

Data Analysis

Associations between patient-specific demographic, social, and medical determinants with microbiological results were derived by 1-way analysis of variance for normal, continuous variables and by the Fisher exact test (2-tailed) for categorical variables. All associations were tested at an alpha level of .05

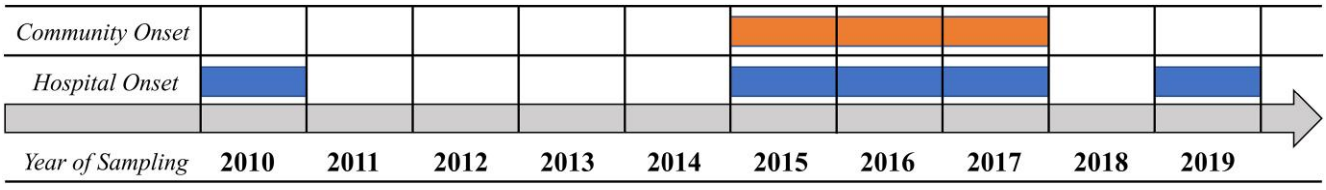

Figure 1. Study timeline of community and hospital MRSA isolate sampling. Color-filled boxes with the corresponding year indicate the time periods when sampling occurred for each location. Boxes are colored by location of sampling. Abbreviation: MRSA, methicillin-resistant *Staphylococcus aureus*.

to determine statistical significance. Statistical analysis was conducted using SAS 9.4 (SAS Institute, Cary, NC, USA).

Geographic analysis was implemented using self-reported home residences collected at the time of community sampling. Residences were geocoded and spatially joined to corresponding census tracts using ArcMap 10.3 (ESRI, Redlands, CA, USA). Rural or urban status was assigned to eligible participants by assigning categories derived from the USDA Economic Research Service 2010 Rural-Urban Commuting Area (RUCA) coding classification. "Urban" census tracts were defined as RUCA codes 1.0–3.0, and "Rural" census tracts were defined as RUCA codes 4.0–10.6 [10]. Heatmaps using kernel density estimation, weighted using the overall emergency department utilization rates for subjects' respective census tracts, were created to assess the geographic distribution of study participants and microbiological results in ArcMap.

Whole-Genome Sequencing

Whole-genome sequencing was performed as previously mentioned [11], with slight modifications. Briefly, the *S. aureus* strains were grown in Tryptic Soy Broth (BD, Franklin Lakes, NJ, USA) at 37°C. Genomic DNA was extracted using the DNeasy Blood & Tissue Kit (Qiagen, Germantown, MD, USA), following the manufacturer's protocol for gram-positive bacteria. Sequencing libraries were constructed using the Nextera XT sample preparation kit (Illumina, San Diego, CA, USA) and loaded in the Illumina Miseq platform. Raw read quality was assessed with FastQC, version 0.11.7 [12]; Illumina adapters and quality trimming were performed with Trimmomatic, version 0.36 [13]. SPAdes, version 3.13.0 [14], was used to produce whole-genome sequences; identification of isolates as *S. aureus* was verified with prokka, version 1.14.6 [15]. Identification of multilocus sequence types (MLSTs) was performed using MLST 2.0 [15], virulence genes were detected using Abricate [16] with the Virulence Factor Database [17], and SCC*mec* typing was performed using SCCmec Finder 1.2 [18]. Pulsed-field gel electrophoresis (PFGE) types of clonal complex (CC) 8 and CC5 isolates were inferred based on SCC*mec* types and toxin profiles [1, 19].

Phylogenetic Analysis

To assess the transmission networks between hospital and community settings, the time-scaled Bayesian phylodynamic inference framework was implemented. Whole genomes were aligned using progressiveMauve, version 2.3. [20], and alignment was tested for evidence of recombination with Gubbins, version 2.4.1 [21]. Parsimony-informative SNPs were retained from nonrecombinant segments, and phylogenetic signal was verified by likelihood mapping [22] as implemented in IQTREE, version 2.0.6 [23]. IQTREE was also used to assess the best nucleotide substitution model with ascertainment bias correction for variable position [24], and subsequently to produce a maximum likelihood tree with 1000 bootstrap replicates. Clock signal of the alignment was assessed by calculating the correlation between root-to-tip genetic divergence and sampling dates using TempEst (Supplementary Figure 1) [25]. Time-scaled trees were estimated using the Bayesian phylodynamic inference framework in BEAST, version 10. Multiple independent runs were combined for a total of 500 and 750 million Markov chain Monte Carlo generations for the 20 taxa tree and the 61 taxa tree, respectively. The HKY nucleotide substitution model was used with empirical base frequencies and gamma distributions of site-specific rate heterogeneity [26]; the SNPs' evolutionary rate was set as 7.57×10^{-5} nucleotide substitutions per year [8, 27]. Constant size and Bayesian Skyline Plot demographic priors were compared by stepping stone and path sampling, and the latter was selected based on the model log marginal likelihood [28]. The maximum clade credibility trees were edited using ggtree [29–31]. To evaluate the presence of distinct genetic relatedness within identified significant risk factors due to potential selective pressures, estimates of genetic compartmentalization, including distance-based methods (nearest-neighbor statistic and Wright's measure of population subdivision [32]) and tree-based methods (Slatkin-Madison statistic [33] and Simmonds association index [34]), were obtained using HypHy [35].

RESULTS

For community-onset MRSA sampling, we prospectively enrolled 200 patients presenting to the emergency department at a tertiary acute care hospital in North-Central Florida with SSTI from August 2015 to January 2017, of whom 182 (91%) were eligible for analysis. Of the 182 subjects, 85 (46.7%) were female and 31 (17.0%) were pediatric patients (<18 years of age). Eighty-three (45.6%) subjects had *S. aureus* isolated from wound culture, of whom 30 were methicillin-susceptible and 53 were methicillin-resistant. Forty (22.0%) subjects had *S. aureus* isolated from nasal culture, of whom 15 were methicillin-susceptible and 25 were methicillin-resistant. Of the 78 MRSA-confirmed isolates, 41 (7 nasal and 34 SSTI) underwent WGS processing. For hospital-onset MRSA sampling, a convenience sample of 36 inpatient MRSA isolates was obtained and sequenced, including 6 isolates from before community sampling (2010), 17 concurrent with community sampling (2015–2017), and 13 from after community sampling (2019). Overall, WGS data were obtained for 77 MRSA isolates and 4 MSSA isolates (Supplementary Table 1).

Among community (n = 45) and hospital-onset (n = 36) MRSA isolates selected for whole-genome sequencing, genetic marker typing revealed 2 clonal complexes, CC5 (n = 20) and CC8 (n = 61). All CC8 isolates were ST8, and n = 59 (98.3%) were confirmed to be USA300 based on genetic marker inference. One CC8/ST8 isolate (KCJ3K737) was determined to be SCC*mec* IV, *spa* type t064, PVL-negative, and *sea*- and *seb*-positive,

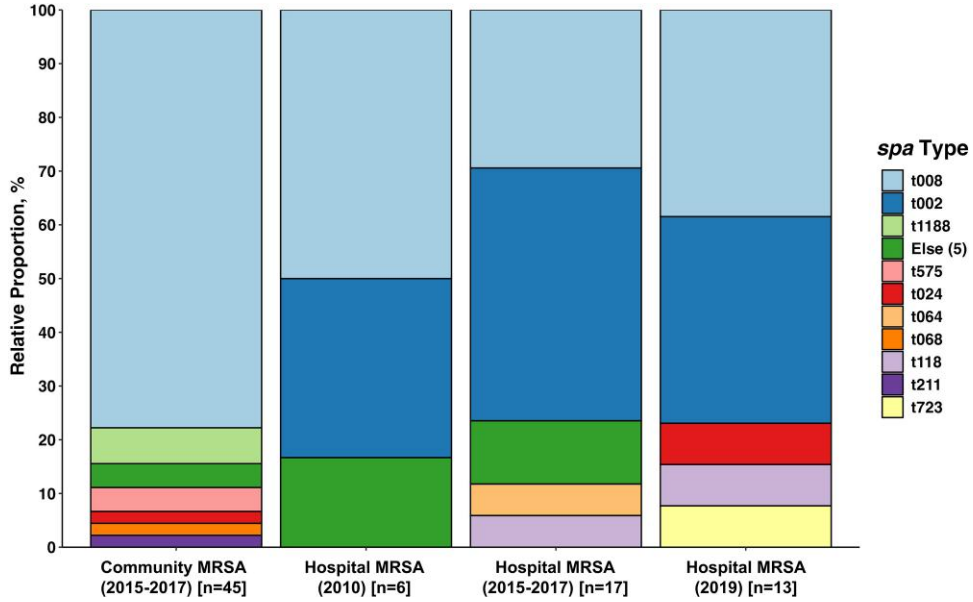

Figure 2. Relative proportions of *spa* types of MRSA isolates by sampling source. Relative proportions are defined as the percentage of isolates with a given *spa* type for each sampling source (ie, community [2015–2017], hospital [2010], hospital [2015–2017], hospital [2019]). Whole-genome sequences were used to infer *spa* types. Proportions are colored by *spa* type as shown in the figure legend. Eleven *spa* types assigned to 1 isolate were categorized as "Else," as shown in the legend. Abbreviation: MRSA, methicillin-resistant *Staphylococcus aureus*.

indicating USA500/Iberian classification. Among CC5 isolates, 5 (25%) and 15 (75%) were determined to be USA800 and USA100 classifications, respectively. Full genetic marker typing for WGS isolates is compiled in Supplementary Table 1.

There were 15 unique *spa* types identified, where 59.3% (n = 48) were t008 and 18.5% (n = 15) were t002. Community-onset MRSA isolates had a significantly greater prevalence of t008 *spa* types compared with hospital MRSA isolates (77.8% vs 36.1%; $P < .001$). Conversely, hospital-onset MRSA isolates had a significantly greater prevalence of t002 *spa* types compared with community MRSA isolates (0% vs 41.2%; $P < .001$). The prevalence of *spa* type across hospital time periods was not significantly different for t008 or t002. Figure 2 summarizes the frequencies and distributions of *spa* types across sampling settings.

To assess the transmission dynamics between community and hospital MRSA isolates, a time-scaled Bayesian phylodynamic framework was implemented. After testing for phylogenetic signal, a final phylogenetic analysis was performed independently on 2 distinct phylogenetic clades (Figure 3). Clades were distinguished by clonal complex, *spa* types, and sampling sources. The larger clade (n = 61) (Figure 3*A*) consisted of only CC8 and predominantly t008 *spa* types (n = 48, 84%), as well as isolates with *spa* types t024, t064, t068, t118, t1188, t211, t2558, t622, t723. The smaller clade (n = 20) (Figure 3*B*) consisted of only CC5 and predominantly t002 (n = 15, 75%) and isolates with *spa* types t088, t105, t214, t575. Median estimates of the time to most recent ancestor (TMRCA) were 2004 (95% HPD: 1992–2009) and 1992 (95% HPD: 1948–2008) for CC8 and CC5 clades, respectively. The estimated genome-wide (2.8 Mb) substitution rate was 3.0×10^{-6} (95% HPD: 1.4×10^{-6}–4.5×10^{-6}) for CC8 clade and 3.0×10^{-6} (95% HPD: 9.4×10^{-7}–5.1×10^{-6}). A maximum likelihood tree revealed similar intermixing of *spa* types (Supplementary Figure 2).

Among hospital MRSA isolates, 18 were included in the CC8/larger clade and 18 were included in the CC5/smaller clade. Two community MRSA isolates were included in the smaller clade. The CC8-dominant clade phylogeny identifies multiple independent introductions of MRSA lineages from the community to the hospital setting, while the CC5-dominant clade phylogeny reveals the opposite. Hospital MRSA isolates from 2019 (KCJ3K391) and 2016 (KCJ3K756) were identified in the same lineage as isolates from the 2010 sampling period (indicated by notation "[A]" in Figure 3*A*). The 2 community isolates with *spa* types t002, MSP138, and MSP222 were the only community MRSA isolates with ancestral origins to the hospital strains (indicated by notation "[B]" in Figure 3*B*).

To understand the factors leading community MRSA transmission, patient-level demographic, social, behavioral, and geographic characteristics among the community-onset study participants were assessed and displayed in Table 1. Demographic characteristics, including age, sex, racial group, and ethnicity, were not associated with nasal or SSTI microbiology results. Subjects with a positive MRSA nasal culture were 3.30 (95% CI, 1.37–7.95) times more likely report current smoking history and 2.65 (95% CI, 1.12–6.24) times more likely to report current alcohol history than those with

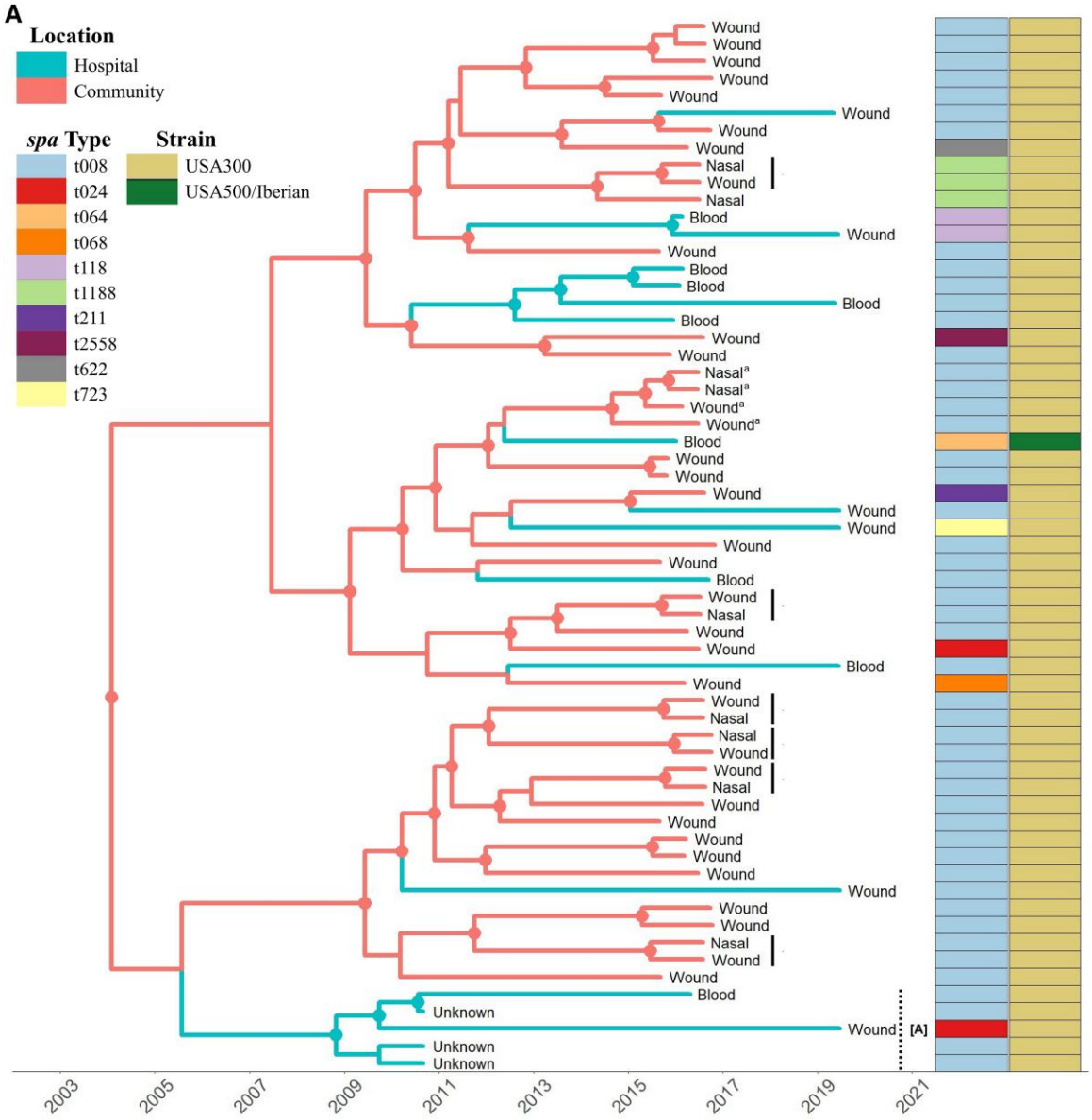

Figure 3. Bayesian maximum clade credibility tree of MRSA isolates. *A,* CC8/large clade. *B,* CC5/small clade. The MCC tree was inferred from whole-genome MRSA isolates scaled in time and colored by location of sampling (location). Circles in the internal node indicate posterior probability support of >0.9. Tree tips indicate the sampling source of isolates. The tree-associated heatmap indicates the *spa* type and US strain of isolates, as shown in the legend. Black bars next to the tips indicate isolates obtained from the same participant. [a]Methicillin-susceptible isolates. Abbreviations: MCC, maximum clade credibility; MRSA, methicillin-resistant *Staphylococcus aureus.*

negative MRSA nasal culture (Supplementary Table 2). Subjects with a positive MRSA SSTI culture were 2.95 (95% CI, 1.29–6.73) times more likely to report having been exposed to livestock animals than those with a negative MRSA SSTI culture. There were no associations observed between MSSA SSTI culture results and social or medical patient-level determinants (Supplementary Table 3). Those residing in rural census tracts had 3.45 (95% CI, 1.36–8.77) greater odds of having a positive MRSA nasal culture and 2.37 (95% CI, 1.07–5.24) greater odds of having a positive MRSA SSTI culture compared with those living in an urban census tract. Heatmaps illustrating the spatial distribution of all community SSTI subjects, those with a positive MRSA SSTI culture, and those with a positive MSSA SSTI culture are presented in Figure 4.

Colonization of the nasal cavity with MRSA and MSSA was significantly associated with concurrent isolation of MRSA ($P < .001$) and MSSA ($P = .021$) from SSTI, respectively. Classification performance metrics of using nasal colonization status to predict SSTI-causative organisms are displayed in Supplementary Table 4. Phylogenetic analysis supported instances of intra-host transmission where MRSA strains isolated from the nasal cavity and from infections sites, collected at the same time, were most closely related and shared the same lineage (Figure 3*A*).

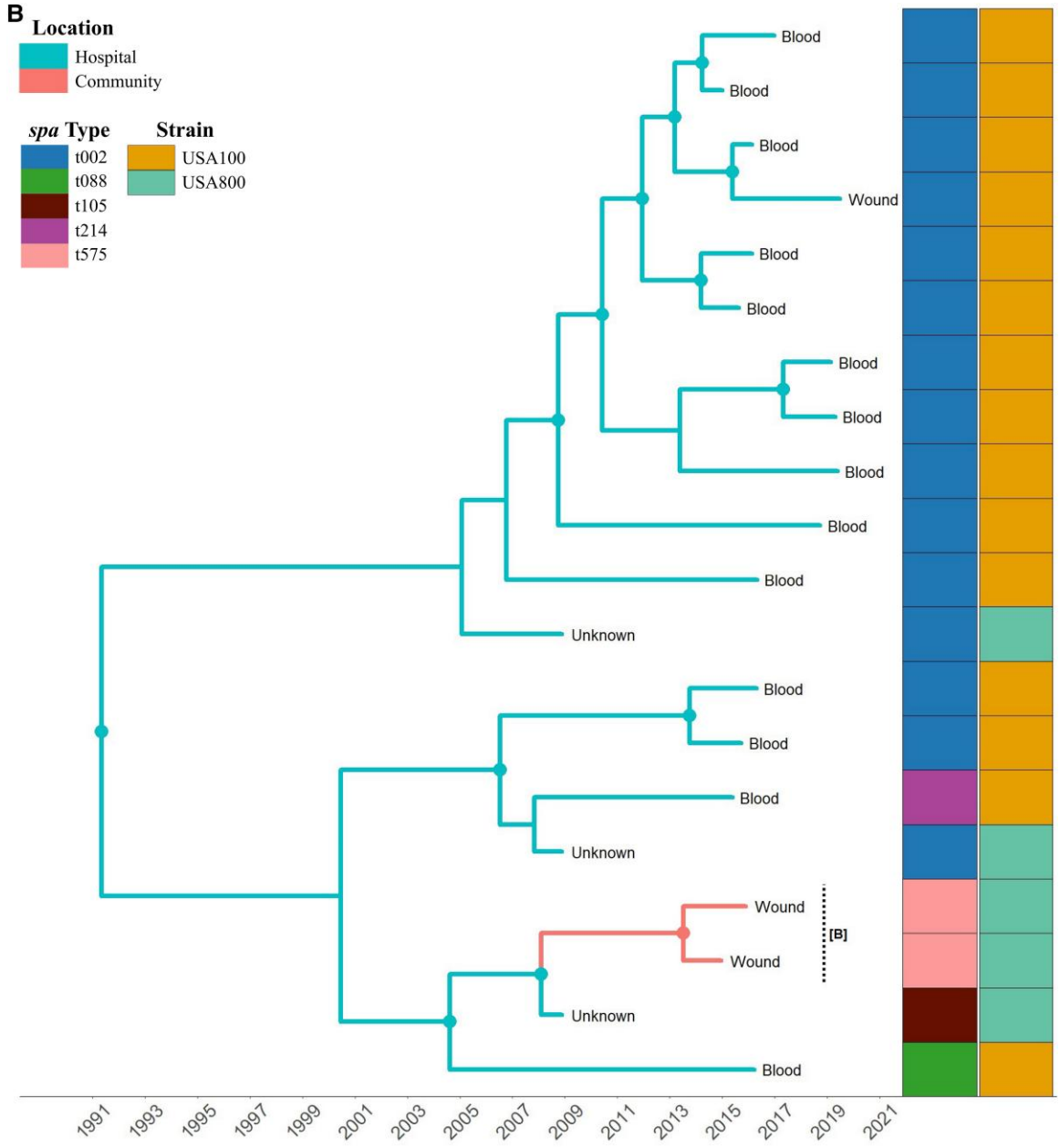

Figure 3. Continued.

Genetic compartmentalization was evaluated to assess whether isolates from subjects with identified risk factors (ie, rurality and livestock exposure) evolved independently. No compartmentalization was observed using distance-based or tree-based methods for either risk factor (Supplementary Table 5).

DISCUSSION

In this longitudinal community assessment, we examined the dynamics of MRSA transmission within and between hospital and community settings and highlighted epidemiological factors contributing to community MRSA transmission. By leveraging WGS data from MRSA isolates obtained from community and hospital settings over a 10-year period, we identified several independent reintroductions of MRSA lineages into the hospitals that were derived from ancestral community MRSA strains, as well as individual lineages persisting in hospital settings. Further, transmission of MRSA from the hospital to the community setting was limited, particularly among USA300/t008 *spa* type, although more comprehensive surveillance is required to quantify the relative contribution of hospital and community strains.

Table 1. Demographics and Social and Medical Demographics Among Community SSTIs by MRSA Culture

Characteristic	No. (%) of Subjects With Wound Culture Result		*P* Value
	MRSA (n = 53)	Non-MRSA (n = 129)	
Age			
Mean age ± SD, y	36.4 ± 21.0	37.8 ± 20.3	.676
Pediatric (age <18 y)	10 (18.9)	21 (16.3)	.669
Sex			
Male	31 (58.5)	66 (51.2)	.415
Female	22 (41.5)	63 (48.8)	…
Race			
Black	10 (18.9)	31 (24.0)	.530
White	39 (73.6)	83 (64.3)	…
Other	4 (7.5)	15 (11.7)	…
Ethnicity			
Hispanic	6 (11.8)	14 (11.0)	.920
Non-Hispanic	47 (88.2)	115 (89.0)	…
Social history			
Current alcohol consumption	21 (39.6)	44 (34.11)	.500
Current smoking history	24 (45.3)	47 (36.4)	.316
Illicit drug use (<30 d)	2 (3.8)	5 (4.1)	.884
Currently attending school	6 (11.3)	23 (18.0)	.373
Currently employed	23 (43.4)	44 (34.7)	.311
Recently incarcerated (<6 mo)	5 (9.6)	8 (6.4)	.668
Recent piercings or tattoos (<6 mo)	5 (9.4)	11 (8.6)	.999
Current member of any gym	3 (5.7)	12 (9.4)	.559
Current member of any team sports	2 (3.8)	6 (4.7)	.999
Medical history			
Have health insurance	22 (41.5)	37 (28.7)	.195
Recent surgery (<30 d)	3 (5.6)	5 (3.9)	.693
Have a primary care physician	23 (43.4)	50 (38.8)	.619
Previously treated for an SSTI (<12 mo)	21 (39.6)	60 (46.5)	.417
Family/household history of SSTI	9 (17.0)	25 (19.4)	.931
Recent antibiotics use (<6 mo)	31 (58.5)	77 (60.2)	.849
Animal exposure			
Exposure to animals (domestic or livestock)	36 (67.9)	87 (68.0)	.999
Exposure to livestock (specifically)	14 (26.4)	14 (10.9)	**.010**
Exposure to domestic (specifically)	35 (66.0)	83 (64.3)	.866
Population density (census tract)			
<100 per sq. mile	30 (56.6)	48 (37.2)	**.021**
≥100 per sq. mile	23 (43.4)	81 (62.8)	…
Rural–urban status (census tract)			
Rural census tract	14 (26.4)	17 (13.1)	**.049**
Urban census tract	39 (73.6)	112 (86.9)	…

Bold type indicates $P < .05$.

Abbreviations: MRSA, methicillin-resistant *Staphylococcus aureus*; SSTI, skin and soft tissue infection.

In the last 2 decades, the initial "location-based" distinction between HA-MRSA and CA-MRSA has become more and more blurred as infections due to CA-MRSA lineage strains have become more common in the health care setting [36]. Historically, these CA-MRSA infections have distinct genetic differences from HA-MRSA: They tend to be resistant to fewer non-β-lactam antibiotics and to carry a smaller version of the genetic region responsible for methicillin resistance (ie, SCC*mec*), often producing Panton-Valentine leukocidin and causing infections in healthy persons [3]. This pattern aligns with our observation of limited introductions of hospital-onset MRSA strains into community settings. In fact, nearly all community-onset MRSA isolates in our study were USA300, the majority of which were PVL-positive. However, our detection of multiple USA300 introductions into the hospital suggests sustained intrahospital transmission and genetic mixing. Similar findings have been reported in other studies, which suggests that the historical categorizations of MRSA as strictly "hospital-" or "community-associated" can be oversimplified [8, 9]. Notably, we also identified a

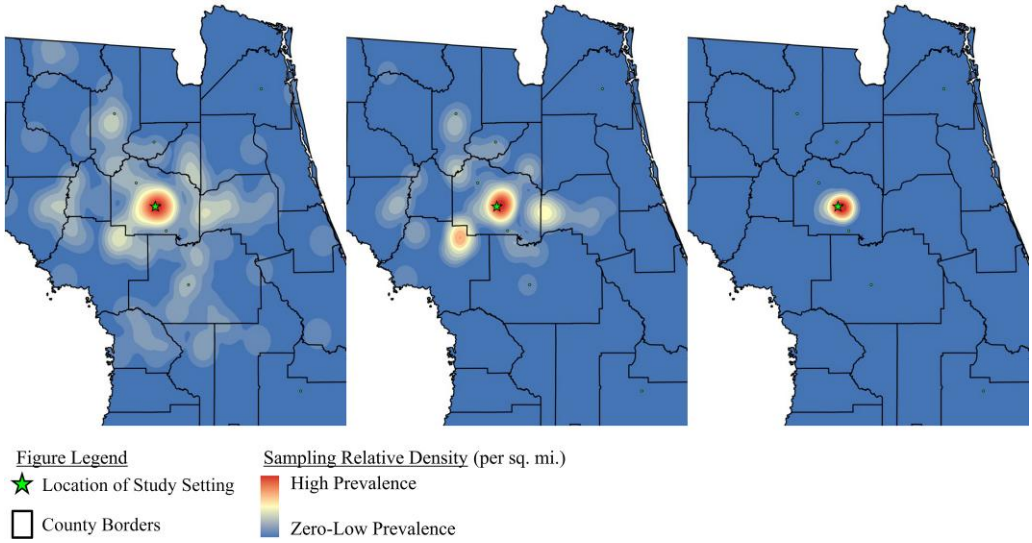

Figure 4. Kernel density heatmaps illustrating the spatial distribution of community *S. aureus* isolates by methicillin sensitivity. A, All community samples. B, MRSA community isolates. C, MSSA community isolates. The heatmap was defined to the study site catchment area in North-Central Florida. Abbreviation: MRSA, methicillin-resistant *Staphylococcus aureus*.

community-onset USA500/Iberian strain through genetic marker inference. Although historically distinguished from USA300, some USA500/Iberian strains can be closely related phylogenetically [19]. Further, an outbreak of the USA500/Iberian strain in Georgia from 2012 to 2015 [37], a region geographically close to our North-Central Florida study site, highlights the potential for wider circulation of this lineage.

With the health care setting not being a significant source of community transmission, the origins of non-hospital-acquired MRSA strains are unclear, especially in rural environments. Our data illustrate that the geographic distribution of community MRSA infections is nonuniform, where there is a higher probability of MRSA infection and colonization in the more rural areas and those with livestock exposure. Further, the lack of compartmentalization of genetic lineages between rural status or livestock exposure supports nonindependent evolutionary histories in isolates among those with these characteristics. While we did not observe the MRSA isolates typically associated with LA-MRSA (ie, CC398/ST398), there is greater diversity in strain types in North America, including CC8/ST8 and CC5/ST5 being found in North American livestock [38]. Previous studies conducted outside of the United States have demonstrated the genetic similarity of community MRSA isolates and MRSA sampled in livestock settings [39]. It has been suggested that 1 possible mechanism for this genetic homology is that there is bidirectional dissemination between livestock and community where those who are in close contact with livestock are at increased risk of MRSA colonization [40]. While non-US studies have demonstrated that livestock-associated MRSA transmission in the health care setting is low risk [41], the epidemiologic experience in the United States has been less clear [42]. With the blurring of traditional epidemiological definitions of MRSA in hospital and community settings, zoonotic reservoirs, and transmission of MRSA into health care settings by means of community spread should be considered as an emerging threat to controlling nosocomial antimicrobial resistance outbreaks.

Our study confirms important known patterns of MRSA transmission. Nasal colonization with MRSA has been known to be a risk factor for invasive MRSA infection for several decades, and intervention strategies, such as decolonization treatments and active screening surveillance, have been designed to target and reduce MRSA transmission [43]. Despite the widespread use of surveillance, the pragmatic evidence for clinical effectiveness and cost-effectiveness is debated [44]. In a recent meta-analysis, MRSA colonization had high specificity but low to moderate sensitivity in predicting MRSA as the causative pathogen for the respective SSTI [45]. In our study, we observed the same trend in the classification performance of nasal colonization to predict SSTI-causative MRSA where we estimated a specificity of 97.7% and sensitivity of 41.5%. While less established [46], this same trend was observed for MSSA colonization in predicting MSSA SSTI and supports the potential utility of MSSA screening. Our whole-genome sequencing approach further establishes the genetic linkage between strains isolated from the nasal cavity and SSTIs from the same patients and the presence of intrahost transmission; however, the directionality of this pattern cannot be determined with our analysis.

There are several limitations to this study. First, the study relies on data from a single site, which may provide insufficient

sampling to explore generalizable transmission patterns between community and hospital settings. Second, hospital sampling was acquired on convenience, which limited the ability to identify factors that could influence MRSA transmission during outbreaks in the nosocomial setting. However, the primary aim of the study was to assess patterns between the community and hospital rather than intrahospital transmission networks. Third, hospital MRSA sampling was acquired from a diverse source of infection (eg, blood, SSTI) while community sampling included only SSTI, although no association was observed between sampling source and genetic lineage. Fourth, not all MRSA community isolates that were sampled underwent whole-genome sequencing; however, evaluation of the mechanism of missingness using patient characteristics and missing sequences supports that these data are "missing at random" and unlikely to influence our main conclusions of the phylogeny (Supplementary Table 6). Additionally, the estimated TMRCA is consistent with those previously reported for the USA300 [47] and CC5 [48] clades, as well as the estimated genome-wide substitution rates [27]. Fifth, while our study suggests geographic differences in MRSA-related SSTIs, it was not designed to directly compare relative rural–urban MRSA prevalence, and the observed rural associations may reflect disproportionate rates of non-MRSA SSTIs in urban areas, while the nasal swabs are historically the standard and most well-studied method for identifying MRSA colonization [45, 49]; we recognize that our analysis cannot be extended to other anatomic sites of colonization.

In conclusion, in this longitudinal community assessment, we observed that MRSA transmission in hospital settings was largely introduced from MRSA species with ancestral origins in community settings. Although community-onset MRSA transmission appears sustained with limited influence from hospital strains, more comprehensive surveillance is required to quantify this relationship. Nosocomial MRSA outbreak prevention strategies should target unique aspects of the community rather than focusing solely on the hospital. Particular attention would be needed in the identification of community hot spots, the role of strain differences, risk behaviors, and possible strain reservoirs in the community.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Acknowledgments

Financial support. This project is supported by the University of Florida's (UF's) "Creating the Healthiest Generation" Moonshot initiative, which is supported by the UF Office of the Provost, UF Office of Research, UF Health, UF College of Medicine, and UF Clinical and Translational Science Institute. Research reported in this publication was also supported by NIH/NIAID R01AI141810 and F30AI183562. Support for the publication of this article was provided by the University of Florida Emerging Pathogens Institute.

Potential conflicts of interest. None to declare.

References

1. Albrecht VS, Limbago BM, Moran GJ, et al. *Staphylococcus aureus* colonization and strain type at various body sites among patients with a closed abscess and uninfected controls at U.S. emergency departments. J Clin Microbiol **2015;** 53: 3478–84.
2. DeLeo FR, Otto M, Kreiswirth BN, Chambers HF. Community-associated meticillin-resistant *Staphylococcus aureus*. Lancet **2010;** 375:1557–68.
3. David MZ, Daum RS. Community-associated methicillin-resistant *Staphylococcus aureus*: epidemiology and clinical consequences of an emerging epidemic. Clin Microbiol Rev **2010;** 23:616–87.
4. Miller LG, Eisenberg DF, Liu H, et al. Incidence of skin and soft tissue infections in ambulatory and inpatient settings, 2005–2010. BMC Infect Dis **2015;** 15:362.
5. Morgan E, Hohmann S, Ridgway JP, Daum RS, David MZ. Decreasing incidence of skin and soft-tissue infections in 86 US emergency departments, 2009–2014. Clini Infect Dis **2019;** 68:453–9.
6. Fritz SA, Shapiro DJ, Hersh AL. National trends in incidence of purulent skin and soft tissue infections in patients presenting to ambulatory and emergency department settings, 2000–2015. Clin Infect Dis **2020;** 70:2715–8.
7. Lakhundi S, Zhang K. Methicillin-resistant *Staphylococcus aureus*: molecular characterization, evolution, and epidemiology. Clin Microbiol Rev **2018;** 31: e00020-18.
8. Prosperi M, Veras N, Azarian T, et al. Molecular epidemiology of community-associated methicillin-resistant *Staphylococcus aureus* in the genomic era: a cross-sectional study. Sci Rep **2013;** 3:1902.
9. Popovich KJ, Snitkin ES, Hota B, et al. Genomic and epidemiological evidence for community origins of hospital-onset methicillin-resistant *Staphylococcus aureus* bloodstream infections. J Infect Dis **2017;** 215:1640–7.
10. Hall SA, Kaufman JS, Ricketts TC. Defining urban and rural areas in U.S. epidemiologic studies. J Urban Health **2006**; 83:162–75.
11. Zhai Y, DeSear KE, Cherabuddi K, Morris JG Jr, Jeong KC. Draft genome sequence of a *Burkholderia cepacia* complex strain isolated from a human intra-abdominal abscess. Microbiol Resour Announc **2021;** 10:e00091-21.
12. Andrews S. A quality control tool for high throughput sequence data. **2010**. Available at: http://www.bioinformatics.babraham.ac.uk/projects/fastqc/. Accessed May 8, 2025.
13. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics **2014;** 30:2114–20.
14. Prjibelski A, Antipov D, Meleshko D, Lapidus A, Korobeynikov A. Using SPAdes De Novo assembler. Curr Protoc Bioinformatics **2020;** 70:e102.
15. Seemann T. Prokka: rapid prokaryotic genome annotation. Bioinformatics **2014;** 30:2068–9.
16. Seemann T. tseemann/abricate. **2025**. Available at: https://github.com/tseemann/abricate. Accessed February 11, 2025.
17. Chen L, Zheng D, Liu B, Yang J, Jin Q. VFDB 2016: hierarchical and refined dataset for big data analysis–10 years on. Nucleic Acids Res **2016;** 44:D694–7.
18. Kaya H, Hasman H, Larsen J, et al. SCCmecFinder, a web-based tool for typing of staphylococcal cassette chromosome mec in *Staphylococcus aureus* using whole-genome sequence data. mSphere **2018;** 3:e00612-17.
19. Bowers JR, Driebe EM, Albrecht V, et al. Improved subtyping of *Staphylococcus aureus* clonal complex 8 strains based on whole-genome phylogenetic analysis. mSphere **2018;** 3:e00464-17.
20. Darling AE, Mau B, Perna NT. progressiveMauve: multiple genome alignment with gene gain, loss and rearrangement. PLoS One **2010;** 5:e11147.
21. Croucher NJ, Page AJ, Connor TR, et al. Rapid phylogenetic analysis of large samples of recombinant bacterial whole genome sequences using Gubbins. Nucleic Acids Res **2015;** 43:e15.
22. Strimmer K, von Haeseler A. Likelihood-mapping: a simple method to visualize phylogenetic content of a sequence alignment. Proc Natl Acad Sci U S A **1997;** 94:6815–9.
23. Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol Biol Evol **2015;** 32:268–74.
24. Lewis PO. A likelihood approach to estimating phylogeny from discrete morphological character data. Syst Biol **2001;** 50:913–25.
25. Rambaut A, Lam TT, Max Carvalho L, Pybus OG. Exploring the temporal structure of heterochronous sequences using TempEst (formerly Path-O-Gen). Virus Evol **2016;** 2:vew007.
26. Hasegawa M, Kishino H, Yano T. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. J Mol Evol **1985;** 22:160–74.

27. Gray RR, Tatem AJ, Johnson JA, et al. Testing spatiotemporal hypothesis of bacterial evolution using methicillin-resistant *Staphylococcus aureus* ST239 genome-wide data within a Bayesian framework. Mol Biol Evol **2011;** 28:1593–603.
28. Hall MD, Woolhouse ME, Rambaut A. The effects of sampling strategy on the quality of reconstruction of viral population dynamics using Bayesian skyline family coalescent methods: a simulation study. Virus Evol **2016;** 2:vew003.
29. Yu G. Using ggtree to visualize data on tree-like structures. Curr Protoc Bioinformatics **2020;** 69:e96.
30. Yu G, Lam TT, Zhu H, Guan Y. Two methods for mapping and visualizing associated data on phylogeny using Ggtree. Mol Biol Evol **2018;** 35:3041–3.
31. Yu G, Smith DK, Zhu H, Guan Y, Lam TT. Ggtree: an R package for visualization and annotation of phylogenetic trees with their covariates and other associated data. Methods Ecol Evol **2017;** 8:28–36.
32. Hudson RR, Boos DD, Kaplan NL. A statistical test for detecting geographic subdivision. Mol Biol Evol **1992;** 9:138–51.
33. Slatkin M, Maddison WP. A cladistic measure of gene flow inferred from the phylogenies of alleles. Genetics **1989;** 123:603–13.
34. Wang TH, Donaldson YK, Brettle RP, Bell JE, Simmonds P. Identification of shared populations of human immunodeficiency virus type 1 infecting microglia and tissue macrophages outside the central nervous system. J Virol **2001;** 75: 11686–99.
35. Kosakovsky Pond SL, Poon AFY, Velazquez R, et al. Hyphy 2.5—a customizable platform for evolutionary hypothesis testing using phylogenies. Mol Biol Evol **2020;** 37:295–9.
36. Deurenberg RH, Stobberingh EE. The evolution of *Staphylococcus aureus*. Infect Genet Evol **2008;** 8:747–63.
37. Frisch MB, Castillo-Ramírez S, Petit RA, et al. Invasive methicillin-resistant *Staphylococcus aureus* USA500 strains from the U.S. Emerging Infections Program constitute three geographically distinct lineages. mSphere **2018;** 3: e00571-17.
38. Butaye P, Argudín MA, Smith TC. Livestock-associated MRSA and its current evolution. Curr Clin Micro Rep **2016;** 3:19–31.
39. Abd El-Hamid MI, Bendary MM, Merwad AMA, Elsohaby I, Mohammad Ghaith D, Alshareef WA. What is behind phylogenetic analysis of hospital-, community- and livestock-associated methicillin-resistant *Staphylococcus aureus*? Transbound Emerg Dis **2019;** 66:1506–17.
40. van Cleef BA, Monnet DL, Voss A, et al. Livestock-associated methicillin-resistant *Staphylococcus aureus* in humans, Europe. Emerg Infect Dis **2011;** 17:502–5.
41. Würtz ET, Bønløkke JH, Urth TR, et al. No apparent transmission of livestock-associated methicillin-resistant *Staphylococcus aureus* CC398 in a survey of staff at a regional Danish hospital. Antimicrob Resist Infect Control **2017;** 6:126.
42. Smith TC. Livestock-associated *Staphylococcus aureus*: the United States experience. PLoS Pathog **2015;** 11:e1004564.
43. Boyce JM. MRSA patients: proven methods to treat colonization and infection. J Hosp Infect **2001;** 48:S9–14.
44. Fätkenheuer G, Hirschel B, Harbarth S. Screening and isolation to control meticillin-resistant *Staphylococcus aureus*: sense, nonsense, and evidence. Lancet **2015;** 385:1146–9.
45. Butler-Laporte G, De L'Étoile-Morel S, Cheng MP, McDonald EG, Lee TC. MRSA colonization status as a predictor of clinical infection: a systematic review and meta-analysis. J Infect **2018;** 77:489–95.
46. Noorani HZ, Adams E, Glick S, Weber S, Belinson S, Aronson N. Screening for Methicillin-Resistant Staphylococcus aureus (MRSA): Future Research Needs: Identification of Future Research Needs From Comparative Effectiveness Review No. 102. Agency for Healthcare Research and Quality; **2013**.
47. Bianco CM, Moustafa AM, O'Brien K, et al. Pre-epidemic evolution of the MRSA USA300 clade and a molecular key for classification. Front Cell Infect Microbiol**2023**; 13:1081070.
48. Smith JT, Eckhardt EM, Hansel NB, Rahmani Eliato T, Martin IW, Andam CP. Genome evolution of invasive methicillin-resistant *Staphylococcus aureus* in the Americas. Microbiol Spectr **2022;** 10:e0020122.
49. Wertheim HF, Melles DC, Vos MC, et al. The role of nasal carriage in *Staphylococcus aureus* infections. Lancet Infect Dis **2005;** 5:751–62.