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ORIGINAL RESEARCH

Nasal *Staphylococcus aureus* Carriage and Antimicrobial Resistance Profiles Among Community-Dwelling Adults in Jiangsu, China

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ABSTRACT

***Introduction*:** Persistent nasal carriage has been associated with *Staphylococcus aureus* infection. Previous *S. aureus* studies in Asia have primarily focused on clinical patients, providing limited information on persistent nasal carriage among the general adult population.

***Methods*:** This study examined 143 healthy adults in a community in Jiangsu, China. Nasal swab samples were collected 10 times. The colonization status was identified using SPA typing. We also determined antimicrobial susceptibility, genotype, and genomic characteristics of *S. aureus*.

***Results*:** The prevalence of *S. aureus* nasal carriage among the community individuals was on average 16.78%. The carriage rates of methicillin-resistant *S. aureus* and multidrug-resistant *S. aureus* were 6.29% and 7.69%, respectively. We identified 8.39% persistent carriers, 39.16% intermittent carriers, and 52.45% noncarriers. Furthermore, family members displayed concordance in terms of genotype and genomic characteristics.

***Conclusion*:** Persistent nasal sampling captured intermittent carriers that were missed during short-term sampling, thus highlighting the necessity for regular community testing. SPA typing can serve as a rapid method for determining *S. aureus* colonization. The potential for intrafamilial transmission of *S. aureus* is evident, with persistent carriers being the most probable source of infection.

Keywords: SPA; MLST; Drug resistance; Whole genome sequencing

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Key Summary Points

Why carry out the study?

The SPA typing method indeed serves as a rapid means of identifying carriers of Staphylococcus aureus.

Longitudinal multiple nasal sampling provides a more precise representation of Staphylococcus aureus nasal carriage compared to cross-sectional sampling.

What was learned from the study?

The community population of Jiangsu province demonstrates a higher prevalence of ST59-MRSA strains, characterized by heightened virulence and drug resistance. Consequently, vigilant monitoring of ST59 colonization is warranted.

Linezolid, teicoplanin, and vancomycin may serve as potential last-resort options for MRSA treatment.

INTRODUCTION

Staphylococcus aureus, an opportunistic pathogen, is a major cause of bacteremia, pneumonia, endocarditis, and skin and soft tissue infections (SSTIs) [1]. Methicillin-resistant *S. aureus* (MRSA) is a prevalent multidrug-resistant *S. aureus* (MDRSA) which is resistant to β-lactam and other antibiotic classes [2]. Therefore, treating MRSA infections often proves more challenging and costly than methicillin-susceptible *S. aureus* (MSSA) infections. Community-associated MRSA (CA-MRSA) spreading in hospitals and communities increases MRSA infections, with nasal carriage of *S. aureus* as a major risk factor for both CA and nosocomial infections [3, 4]. Additionally, the majority of studies on *S. aureus* nasal carriage have employed a cross-sectional approach, relying on a single nasal sampling to determine whether the participant is a carrier. However, this leads to misclassifying the patient's carriage state [5, 6]. Therefore, serial sampling of the nasal cavity within the community population can help in ascertaining nasal carriage prevalence and transmission mechanisms of *S. aureus* within these cohorts [7, 8].

Whole-genome sequencing (WGS) offers a high level of precision and can provide additional insights into resistance mechanisms, pathogenicity factors, and population structure [9]. Therefore, this study aimed to (i) report the distinct nasal carriage patterns of *S. aureus* among community-dwelling adults in Jiangsu, China, and (ii) analyze the antimicrobial resistance and genetic characteristics of these isolates to furnish insights into containment and intervention strategies against *S. aureus* infections.

METHODS

Participants

In this study, we retrospectively studied surveillance samples from a community in Jiangsu Province from June 7, 2016 to June 14, 2017. We analyzed 10 nasal swab samples and demographic data (including age, sex, and body mass index (BMI)) from 144 healthy adult residents aged 18–65. The time points for nasal swab sampling were days 0, 3, 7, 10, 14, 17, 21, 42, 90, and 180. Individuals with a history of *S. aureus* infection, hospitalization, admission to a nursing facility, dialysis for renal failure, recent surgery, or the use of permanent indwelling catheters or medical devices within the last year were excluded from this study.

We utilized PASS v15.0 software to calculate the required sample size for the study. Based on domestic and foreign literature research, considering the high population density in China, the carriage rate of *S. aureus* in the community population was set at 35%, with $\alpha = 0.05$, $\beta = 0.10$, and $\delta = 14\%$. Accounting for a potential loss to follow-up rate of approximately 10% during the study process, we determined that at least 127 samples were necessary. Ultimately, the first 144 surveillance samples that met the above exclusion requirements and sample sizes were included in this study.

Nasal Swabs

The participants were tested for *S. aureus* nasal carriage by streaking both anterior nares with sterile moistened cotton swabs to a depth of approximately 1 cm and rotating them for approximately 15 s. Nasal swabs were then placed in tubes containing sterile normal saline and refrigerated at 4 °C before being quickly sent to the microbiology laboratory within 4 h. The workflow of this study is illustrated in Fig. 1.

Culture and Identification

S. aureus colonies were detected according to the National Standard of China GB/T 4789.37–2008. After incubation at 37 °C for 45–48 h, 2–4 typical colonies were selected from each Baird-Parker agar medium (BD, Beijing, China) for subsequent identification. The *S. aureus* strains were identified using plasma coagulase and VITEK-2 biochemical tests.

Definitions for Colonization Status

The *S. aureus* nasal carriage state was assessed on the basis of the results of the nasal swab culture as follows [10, 11]:

- Persistent carriers: Individuals showing ≥ 80% positive results for *S. aureus* in their consecutively tested samples.
- Intermittent carriers: Individuals who tested positive for *S. aureus* in < 80% of their samples.
- Non-carriers: Individuals with no detectable *S. aureus* nasal carriage.

SPA Typing

Polymorphic regions of the *spa* gene were amplified and sequenced for all isolates [12]. The resulting SPA types were used to categorize the carriage state of each individual. For volunteers with intermittent carriage, antimicrobial susceptibility testing and WGS were performed on strains initially isolated from the same SPA type. In cases of persistent carriage, these analyses encompassed the first strain, last strain, and strain marking a change in SPA type. Isolates that could not be classified as having any known SPA type were defined as nontypable (NT).

Drug Sensitivity Identification

The antimicrobial susceptibility of the isolates was determined using the BD Phoenix M50 Automated Microbiology System, and evaluated 23 antibiotics across seven classes (Supplementary Table 1). *Staphylococcus aureus* ATCC29213 was used as a quality control strain. The results were interpreted on the basis of SIR (S: Sensitive; I: Intermediate; R: Resistant) according to the Clinical and Laboratory Standards Institute (CLSI M100-S32) guidelines. MRSA was defined as *S. aureus* expressing the *mecA* gene and/or Phenocillin®, while MDRSA strains were classified as MRSA and any isolates resistant to at least three antimicrobial classes [13].

WGS

The genome was sequenced using the MGISEQ-2000 platform. The raw data were filtered and analyzed using CLC Genomics Workbench 22.0.4 software (Qiagen, Hilden, Germany) and underwent de novo assembly to generate contig sequence files for each strain. The sequence types (STs) were determined by comparing them with corresponding allelic profiles in the Multilocus sequence typing (MLST) database (http://www.pubmlst.org). BioNumerics software was utilized to create minimum spanning trees (MST), while the genetic distances in the single nucleotide polymorphisms (SNPs) between every pair of genome were computed using the CLC software. Phylogenetic trees were constructed using iTOL v6.

COL(NC_002951.2/USA/human/ST25) and 12 *S. aureus* strains sourced from the National Center for Biotechnology Information (NCBI) were used as references (Supplementary Table 2) [14]. All sequence data were submitted to the NCBI. Accession numbers will be provided during the review process.

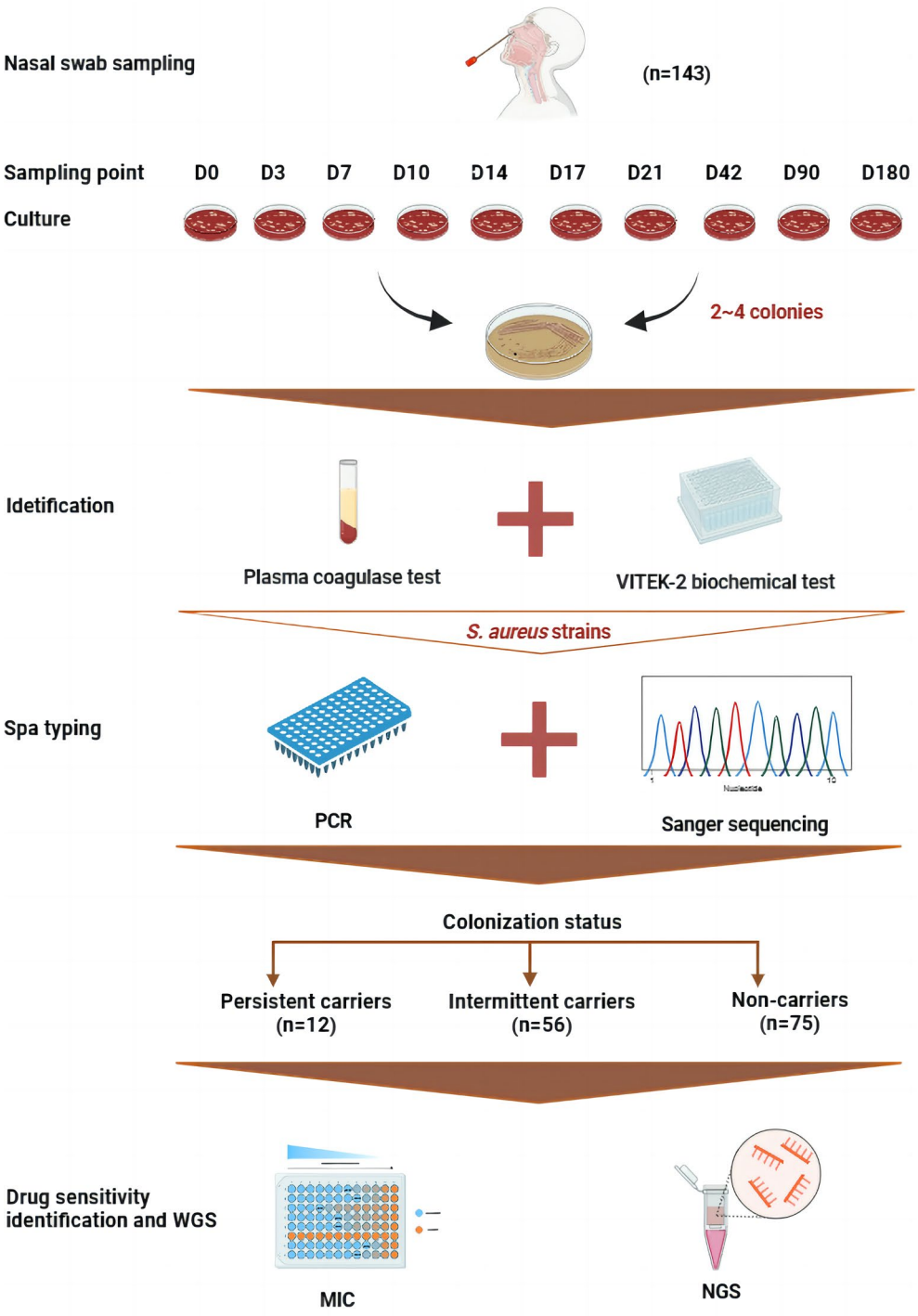

Fig. 1 Workflow of this study. *N* represents the number of volunteers

Genetic Characterization Analysis

The ResFinder database served as a reference for analyzing antibiotic resistance genes (ARs), whereas the Virulence Factor Database (VFDB) was utilized to screen virulence factors (VFs). The results from these databases were directly obtained through the CLC Genomics Workbench using FASTA format data as input. To identify and compare the Integrative Conjugative Elements/Integrative Mobile Elements (ICEs/IMEs), the online tool ICEfinder (sjtu.edu.cn) was employed to support the use of both FASTA and GenBank format files.

Statistical Analysis

The data were analyzed using SPSS 26.0 to assess possible associations between *S. aureus* nasal carriage and age, sex, or BMI using ordinal logistic regression analysis. Statistical significance was set at $p < 0.05$.

Ethical Approval

This study used samples from previously in routine monitoring, and the Ethics Review Committee at the Jiangsu Provincial Center for Disease Control and Prevention decided that it does not need a special ethical review. This study was performed in accordance with the Helsinki Declaration of 1964 and its later amendments. We made sure to protect the privacy of the people involved and followed all the ethical rules when publishing the article.

RESULTS

Participants

One participant dropped out, leaving 143 volunteers who consecutively provided nasal swabs over the 10 time periods. From each nasal swab sample, we selected 2–4 colonies for analysis, and 47.55% (68/143) of the individuals exhibited *S. aureus* nasal carriage. Across 10 cross-sectional samplings, the nasal carriage rates of *S. aureus* were displayed in Fig. 2. The

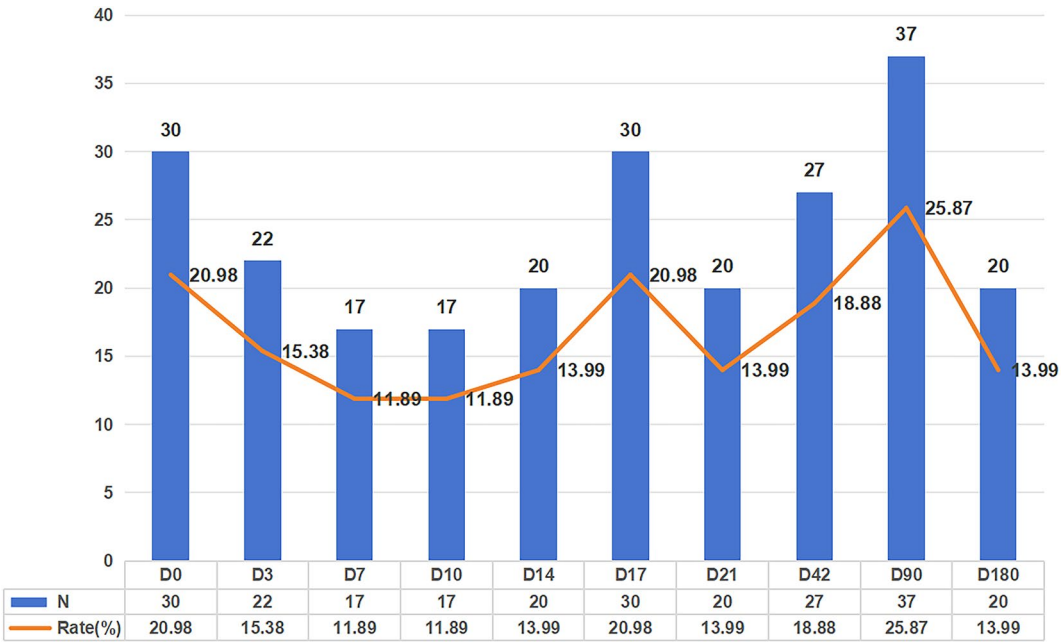

Fig. 2 Nasal *S. aureus* carriage rates in 10 cross-sectional samples. *N* number of nasal carriers of *S. aureus*

average nasal carriage rate of *S. aureus* in the 10 samplings was 16.78%.

The study cohort consisted of 65 men and 78 women. The median age and BMI were 47 and 24, respectively. Women, younger individuals, and those with a higher BMI exhibited a greater propensity for colonization by *S. aureus*. However, none of these differences were statistically significant (Table 1).

SPA and Carrier State Analysis

A total of 240 *S. aureus* strains were isolated and subjected to SPA typing analysis. The SPA typing results revealed 44 distinct SPA types and eight NTs in 225 and 15 strains, respectively. The prominent SPA types included t437, t015, and t164.

On the basis of SPA results, we found that approximately 8.39% (12/143) of the participants could be categorized as persistent carriers, 39.16% (56/143) as intermittent carriers, and 52.45% (75/143) as noncarriers. Two discernible patterns of *S. aureus* carriage emerged: continuous carriage with a single SPA type, and transitions between SPA types (Table 2, Supplementary Table 3). The majority of persistent carriers (66.67%, 8/12) exclusively presented with one SPA type throughout the study period. In total, 107 strains were selected for antimicrobial susceptibility testing and WGS.

Table 1 Analysis of sample population characteristics associated with *S. aureus* carriage state (both persistent and intermittent carriers)

Factors	Level	β	OR	95% Confidence interval	*P* Value
Gender	Female	Reference			
	Male	− 0.219	0.803	0.420, 1.535	0.507
Age		− 0.011	0.989	0.955, 1.024	0.534
BMI		0.037	1.038	0.938, 1.148	0.475

MLST Typing

A total of 25 STs were identified among the 107 strains (Table 2, Fig. 3a and Supplementary Table 3). ST59 was the most prevalent ST, the second and third most common clusters were ST398 and ST1281. Of the 15 STs belonging to six different clonal complex (CC), CC1 emerged as the most prevalent, followed by CC8, CC15, CC5, and CC45. Among the nasal carriage isolates from persistent carriers, three CCs including seven STs were detected: CC1, CC5, and CC45 (Fig. 3b). CC15 were exclusively detected in intermittent carriers, indicating a heightened prevalence of intermittent carriage among individuals colonized by this particular lineage. Among the MRSA strains analyzed in this study, the three predominant types were ST59, ST398, and ST508 (Fig. 3c).

Antimicrobial Susceptibility

Resistance analysis revealed that 107 strains were susceptible to nine antibiotics (Table 3). Evaluation of oxacillin resistance demonstrated that MRSA accounted for approximately 24.30% (26/107) of the isolates. Furthermore, MDRSA constituted 38.32% (41/107), with 68.29% (28/41) originating from persistent carriers (Supplementary Table 1). Drug resistance of MRSA strains are shown in the Supplementary Table 1.

The samples from the initial cross-sectional sampling (day 0) of 143 individuals were subjected to drug sensitivity tests. The prevalence of MRSA and MDRSA carriers was approximately 6.29% (9/143) and 7.69% (11/143), respectively. Among the 10 nasal samplings, 14.69% (21/143) were positive for MRSA at least once. Nearly 19.05% (4/21) exhibited persistent carriage among the 21 MRSA carriers, while the remaining 80.95% (17/21) displayed intermittent carriage.

SNP Analysis

The SNP analysis for *S. aureus* was conducted on the basis of the alignment of 94,963 core

Table 2 SPA, MLST, complex type of persistent colonization state people

Colonization state	ID	Genotyping	Sample time									
			Day 0	Day 3	Day 7	Day 10	Day 14	Day 17	Day 21	Day 42	Day 90	Day 180
Persistent carriers	8	SPA	NT1	NT1	NT1	NT1	NT1	NT1	NT1	NT1	NT1	NT1
		MLST	ST1920									ST1920
		Complex type	CC1									CC1
	82	SPA	t701	t701	t701	t701	t701	t701	t701	t701	t701	
		MLST	ST6								ST6	
		Complex type	CC5								CC5	
	96	SPA	NT6	NT6	NT6	NT6	NT6	NT6		NT6	NT6	
		MLST	ST88								ST88	
		Complex type										
	99	SPA	t015	t015	t015	t015		t015	t015	t015	t015	t015
		MLST	ST508									ST508
		Complex type	CC45									CC45
	102	SPA	t015	t015	t015	t015	t015	t015	t015	t015		
		MLST	ST508							ST508		
		Complex type	CC45							CC45		
	107	SPA	t164	t164	t164	t164	t164	t164	t164	t164	t164	t164
		MLST	ST1281									ST1281
		Complex type										
	125	SPA	t571	t571	t571	t571	t571	t571	t571	t571	t571	t571
		MLST	ST7644									ST7644
		Complex type										

Table 2 continued

Colonization state	ID	Genotyping	Sample time									
			Day 0	Day 3	Day 7	Day 10	Day 14	Day 17	Day 21	Day 42	Day 90	Day 180
	133	SPA	t034	t034	t034	t034	t034	t034	t034	t034	t034	t034
		MLST	ST398									ST398
		Complex type										
	49	SPA	t759	t899	t759	t437	t759	t437	t759		t759	t759
		MLST	ST25	ST6554	ST25	ST59	ST25	ST25	ST25		ST25	ST25
		Complex type										
	52	SPA	t1107	t1107	t1107	t1107	t1107	t1107	t1107	t189	t1107	t1107
		MLST	ST5						ST5	ST188	ST5	ST5
		Complex type	CC5						CC5	CC1	CC5	CC5
	62	SPA	t4358	t4358	t4358	t4358	t4358	t4358	t4358	t437		
		MLST	ST9						ST9	ST59		
		Complex type	CC1						CC1			
	75	SPA		t548	t548	t548	t548	t548	t548	t548	t731	t4562
		MLST		ST2144						ST2144	ST1281	ST6
		Complex type		CC5						CC5		CC5

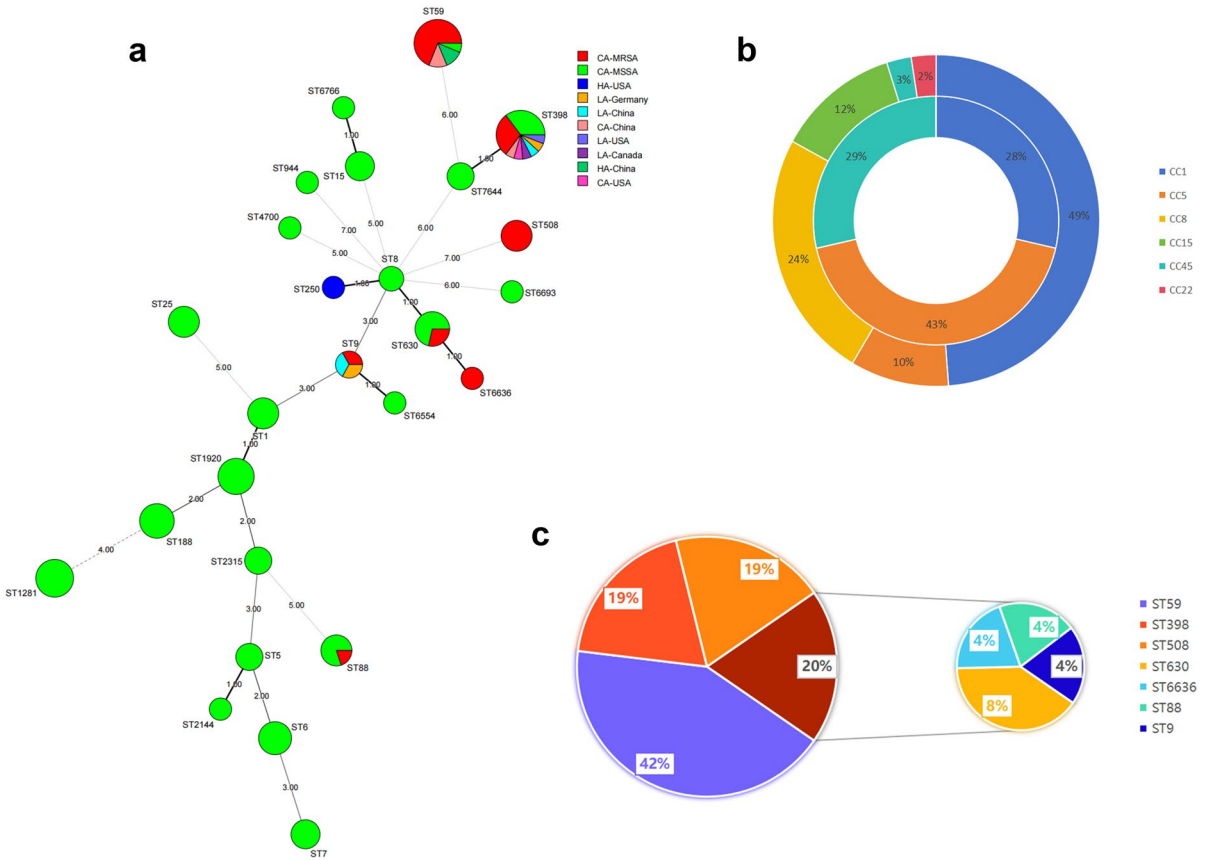

Fig. 3 **a** Genetic correlation of *S. aureus* based on MLST profile. The minimal spanning tree allows comparison of STs for MRSA and MSSA from various sources, including hospital-acquired, community-acquired, and livestock-associated settings. The size of each circle corresponds to the number of isolates sharing the same ST. Circles are color-coded to distinguish between MRSA (red) and MSSA (green) strains. **b** CCs of *S. aureus* from different colonization states. The inner concentric sector of the pie chart delineates the frequency distribution of CCs pertaining to persistent colonizers, while the outer concentric sector illustrates the frequency distribution of CCs corresponding to intermittent colonizers. **c** Distribution of the STs in MRSA strains. The prominent pie chart displayed on the left side illustrates the three STs categories harboring the highest abundance of MRSA strains

genome SNPs (0–54411). When comparing later-stage isolates with early-stage isolates from the same SPA/ST strains of the 12 persistent individuals, SNP distances were highly homologous (SNP < 10).

Using SNP analysis, we identified close relatedness among volunteers 66, 67, 68, 82, 83, 99, 101, 102, 123, and 125. Epidemiological investigations revealed that they belonged to four different families. The carriers within the same family exhibited identical SPA and MLST values (Fig. 4). In Family 1 and 2, ST398-MRSA and ST6-MSSA were isolated from the family members (66, 67, and 68; 82, 83) in the day 90 sampling, respectively, without any significant SNP differences between among the strains. In Family 3, a small number of SNP distances (1–7 SNPs) were observed between the five ST508-MRSA strains isolated from the family members.

VFs, ARs, and ICEs/IMEs

Clustering analysis was performed using SNPs, and the heat map in Fig. 5 illustrates the distribution of VFs, ARs, and ICEs/IMEs. Interestingly, the ST59 strains possessed the most VFs. Moreover, *seb-selk-selq* was exclusively found

Table 3 Drug resistance phenotypes of 107 *Staphylococcus aureus* strains

Antibiotic		Carrier state					
		Persistent carriage ($N=28$)			Intermittent carriage ($N=56$)		
Class	Drug	S	I	R	S	I	R
β-lactam	Ampicillin	0	2	26	0	9	70
	Penicillin G	0	2	26	0	9	70
	Oxacillin	21	0	7	60	0	19
	Ceftaroline	28	0	0	79	0	0
Aminoglycoside	Gentamicin	26	0	2	78	0	1
Tetracycline	Minocycline	28	0	0	79	0	0
	Tetracycline	26	0	2	68	8	3
Chloramphenicols	Chloramphenicol	26	2	0	74	2	3
Quinolone	Ciprofloxacin	26	0	2	72	0	7
	Moxifloxacin	26	1	1	72	0	7
	Levofloxacin	26	0	2	72	0	7
	Norfloxacin	25	2	1	66	7	6
OIazolidinones	Linezolid	28	0	0	79	0	0
Lincosamides	Clindamycin	19	0	9	53	0	26
Nitrofurans	Nitrofurantoin	28	0	0	79	0	0
Macrolide	Erythromycin	19	0	9	53	0	26
Glycylcyclines	Tigecycline	28	0	0	79	0	0
Glycopeptide	Vancomycin	28	0	0	79	0	0
	Teicoplanin	28	0	0	79	0	0
Cyclic lipopeptides	Daptomycin	28	0	0	79	0	0
Rifamycin broad-spectrum	Rifampin	27	0	1	79	0	0
Sulfonamide	Trimethoprim-sulfamethoxazole	26	0	2	77	2	0
Other antibiotics	Mupirocin (high level)	28	0	0	79	0	0

Drug resistance phenotype is indicated as R, I, S

R resistant, *I* intermediate, *S* susceptible

in the ST59 strains. Panton-Valentine leukocidin (PVL) genes (*lukF* and *lukS*) were detected in 70.09% (75/107) of the strains, including 38.46% (10/26) of MRSA isolates (Table 4). The frequencies of VFs carried by strains from the same family members showed a similar pattern. *tsst-1* was present only in volunteers from Family 3. No significant difference was observed in the VFs between persistent and intermittent carriers.

The ResFinder analysis predicted a total of 23 ARs (Table 5). The most prevalent ARs were

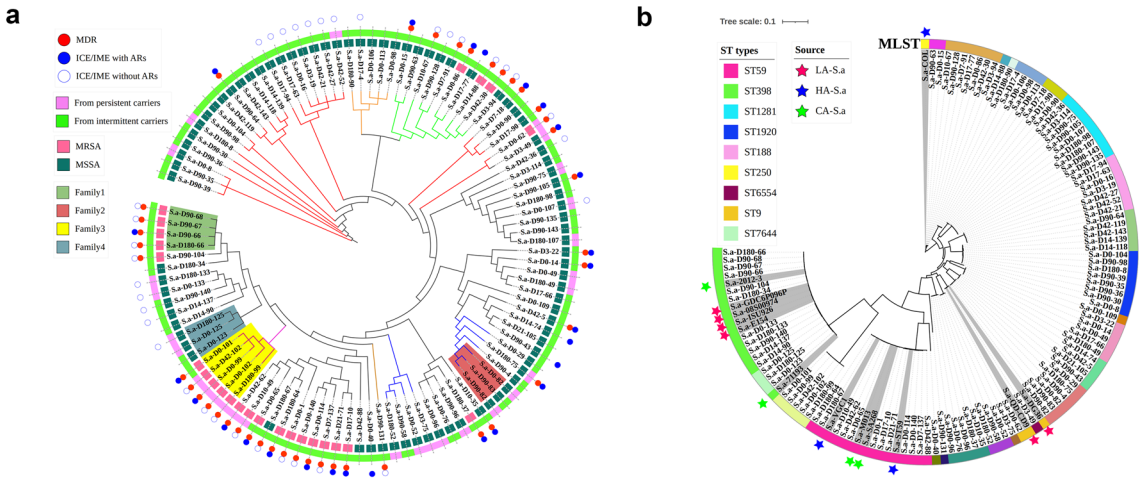

Fig. 4 **a** Genetic tree analysis based on SNPs of 107 *S. aureus*. CC1, CC8, CC5, CC15, CC45, and CC22 clones are represented by red, green, blue, orange, purple, and brown branches, respectively. Strains from volunteers belonging to the same family with similar SNPs are labeled as Family 1, Family 2, Family 3, and Family 4. The green boxes represent MSSA, while the red boxes represent MRSA. Continuous red-green outer ring indicates strains collected from persistent/intermittent carriers. Red circles denote whether the strains are MDR, while the blue circles represent strains carrying ICEs/IMEs with inserted ARs, and empty blue circles indicate strains carrying ICEs/IMEs without ARs. **b** 107 strains of *S. aureus* were compared with the retrieved 13 sequences. Gray branches indicate the retrieved *S. aureus*, which is distinguished from the *S. aureus* in this study. Different colors of the outer ring represent different STs. Stars and their colors represent different sources (community: red, hospital: blue, livestock: green)

blaZ, followed by *mec*A and *erm*(C). These ARs are associated with β-lactam, macrolides/lincosamides/streptomycins (MLS), and aminoglycoside antibiotics. All MRSA isolates harbored *mec*A. *Tet*K was detected in 84.62% (11/13) of the non-sensitive (R/I) tetracycline isolates. *Erm*(C) was found in 60% (21/35) of the erythromycin-R isolates, whereas *erm*(B) was detected in 83.33% (10/12) of the ST59 strains. Notably, ARs were relatively concentrated within the ST59 strains, with 91.7%(11/12) of the ST59 strains being MDRSA. 38 out of the 68 individuals carrying 47 ICEs and 10 IMEs. 13 strains (from 11 intermittent and 2 persistent carriers) possessed ARs in the ICEs (Supplementary Table 4).

DISCUSSION

To our knowledge, this is the first longitudinal study equivalent to 10 cross-sectional studies that provides a comprehensive insight into the prevalence of nasal carriage state, resistance phenotype, and genetic characteristics of *S. aureus* among the community population in China. The average nasal carriage rate of *S. aureus* was 16.78% among the 10 samplings. This finding coincides with the prevalence rates observed in the cross-sectional studies conducted in Northern China (16.5%) and Guangzhou (23.4%) [15, 16]. Cross-sectional studies conducted outside China found *S. aureus* prevalence ranging from 21.9% to 47.6% among different populations, with MRSA identified in 0.7–8.81% [17–20]. The prevalence rates of MRSA and MDRSA carriage in our first cross-sectional study were approximately 6.29% and 7.69%, respectively. Therefore, compared with other countries, the general Chinese population exhibits a lower rate of nasal carriage, but a higher prevalence of MRSA nasal carriage.

In our longitudinal nasal sampling, 14.69% of individuals harbored MRSA at least once, which is twice as the rate perceived in our single cross-sectional MRSA study, and significantly

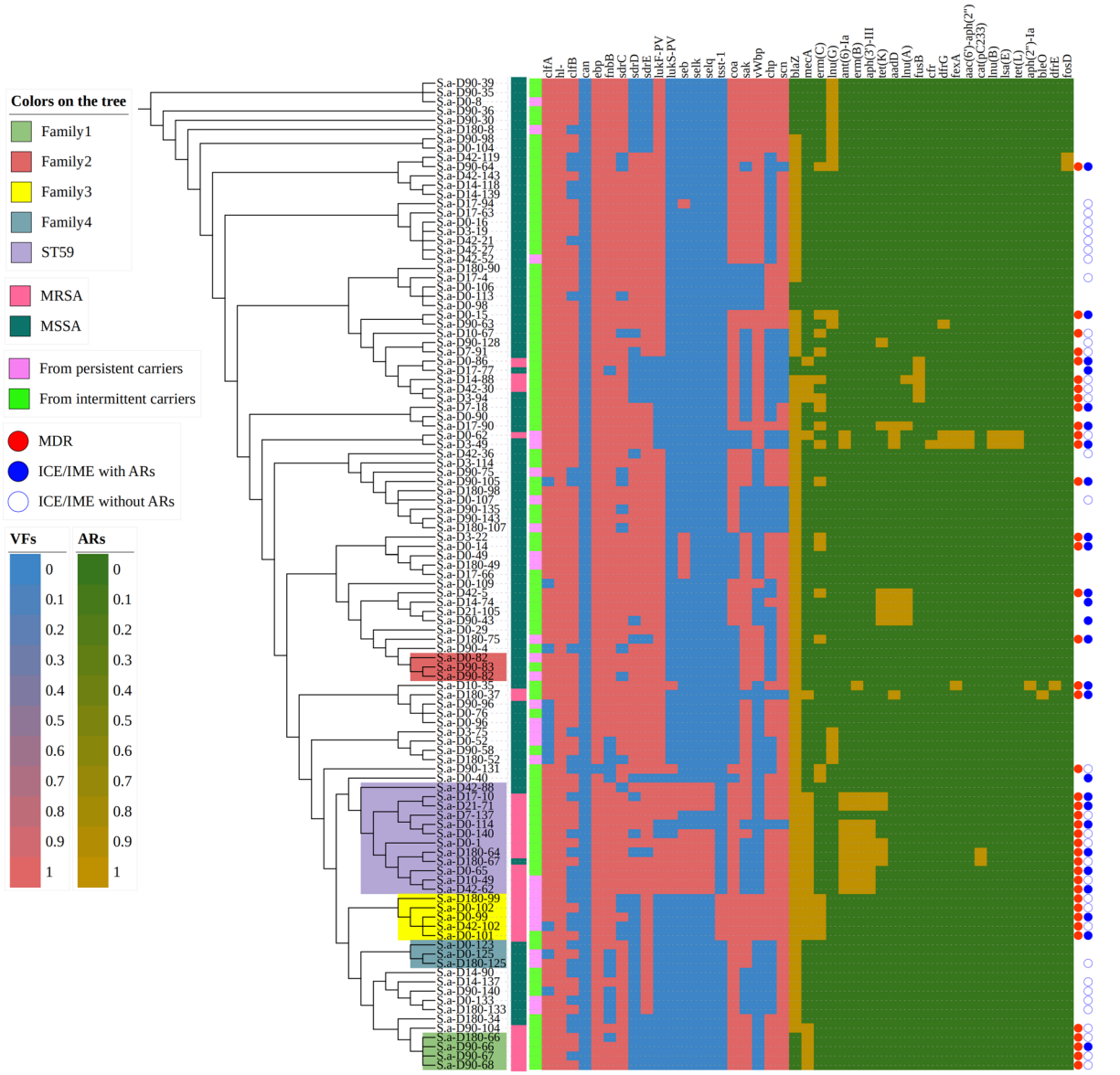

Fig. 5 Complex heatmap illustrating the distribution of VFs and ARs in all isolated *S. aureus* strains. The tree colors indicate strains from 4 families and ST59 strains. The right side of the dendrogram indicates whether the strain is MRSA or MSSA, carrier type, and a heatmap. Circles on the right side of the heatmap denote the carriage status of MDR or ICEs/IMEs. The left side of the heatmap shows the presence (red) or absence (blue) of VF in 107 *S. aureus* strains, indicating their carriage status. In contrast, the right side of the heatmap shows the presence (yellow) or absence (green) of AR genes, indicating their carriage status. Strains originating from volunteers within the same family are labeled as Family 1, Family 2, Family 3, and Family 4

surpassed those reported in both domestic and international cross-sectional studies. This suggested that relying solely on cross-sectional data to determine carrier status may underestimate true prevalence, thereby emphasizing the importance of collecting multiple consecutive samples to determine a more accurate carrier rate. However, accurately determining the persistent carriage rate of MRSA in a healthy community remains challenging because of the absence

Table 4 Distribution of virulence factors in persistent and intermittent colonizers

Virulence factors			% Positive colonization isolates		
Group	**Description**	**Gene**	**Persistent colonization (*n* = 28)**	**Intermittent colonization (*n* = 79)**	**Co-colonization (*n* = 107)**
Adherence	Clumping factor	*clfA*	75.00	92.41	87.85
		clfB	60.71	77.22	72.90
	Collagen binding protein	*cna*	10.71	3.80	5.61
	Elastin-binding protein	*ebp*	100.00	98.73	99.07
	Fibronectin binding proteins	*fnbA*	78.57	89.87	86.92
		fnbB	78.57	89.87	86.92
	Ser-Asp rich proteins	*sdrC*	75.00	88.61	85.05
		sdrD	60.71	62.03	61.68
		sdrE	89.29	75.95	79.44
Exotoxin	α-hemolysin	*hly*	100.00	100.00	100.00
		hla	100.00	100.00	100.00
	β-hemolysin	*hlb*	100.00	100.00	100.00
	δ-hemolysin	*hld*	100.00	100.00	100.00
	γ-hemolysin	*hlgA*	100.00	100.00	100.00
		hlgB	100.00	100.00	100.00
		hlgC	100.00	100.00	100.00
	PVL (Panton-Valentine leukocidin)	*lukF-PV*	64.29	72.15	70.09
		lukS-PV	7.14	12.66	11.21
	SE (Staphylococcal enterotoxin)	*sea*	25.00	20.25	21.50
		seb	14.29	15.19	14.95
		selk	7.14	10.13	9.35
		selq	7.14	10.13	9.35
	Toxic shock syndrome toxin-1	*tsst-1*	14.29	1.27	4.67
Exoenzyme	Staphylocoagulase	*coa*	57.14	79.75	73.83
	Staphylokinase	*sak*	71.43	64.56	66.36
	Von Willebrand factor-binding protein	*vWbp*	42.86	44.30	43.93
Immune modulation	Chemotaxis inhibitory protein of Staphylococcus	*chp*	50.00	50.63	50.47
	Staphylococcal complement inhibitor	*scn*	85.71	82.28	83.18

Table 5 Distribution of antibiotic resistance genes in persistent and intermittent colonizers

Antibiotic resistance gene	% Positive colonization isolates		
	Persistent colonization ($n = 28$)	Intermittent colonization ($n = 79$)	Co-colonization ($n = 107$)
blaZ	92.85	83.54	85.98
mecA	25.00	25.32	25.23
erm(C)	21.43	18.99	19.63
lnu(G)	17.86	13.92	14.95
ant(6)-Ia	14.29	10.13	11.21
erm(B)	7.14	11.39	10.28
aph(3')-III	7.14	10.13	9.35
tet(K)	0.00	13.92	10.28
aadD	7.14	7.59	7.48
lnu(A)	0.00	7.59	5.61
fusB	0.00	6.33	4.67
cfr	3.57	0.00	0.93
dfrG	7.14	1.27	2.80
fexA	7.14	1.27	2.80
aac(6')-aph(2")	7.14	0.00	1.87
cat(pC233)	0.00	2.53	1.87
lnu(B)	7.14	0.00	1.87
lsa(E)	7.14	0.00	1.87
tet(L)	7.14	0.00	1.87
aph(2")-Ia	0.00	1.27	0.93
bleO	0.00	1.27	0.93
dfrv	0.00	1.27	0.93
fosD	0.00	2.53	1.87

of comprehensive antibiotic susceptibility testing for all *S. aureus* isolates.

In this study 8.39% of individuals were identified as persistent carriers, 39.16% as intermittent carriers, and 52.45% as non-carriers. This contrasts with the findings of several longitudinal studies, which indicate 20% persistent carriers, 30% intermittent carriers, and 50% non-carriers [21]. This discrepancy may stem from the use of diverse culture techniques and intervals, different study populations, and varying colonization state definitions. Our research confirmed that the rates of persistent carriers and non-carriers decrease with longer follow-up periods and fewer culture intervals, suggesting potential misclassification of intermittent carriers as either persistent carriers or non-carriers if the follow-up period is brief or if only a few samples

are cultured [22]. Previous colonization studies simplified carriage phenotypes into just two categories: persistent carriers and intermittent/non-carriers; however, given the intricate nature of the disparities between persistent and intermittent carriers, we concur that the colonization status should be divided into three categories [23, 24].

Although not statistically significant ($p>0.05$), there was a trend for higher *S. aureus* colonization in the nasal cavity in women, younger individuals, and those with higher BMI. This result was consistent with the findings of Olsen et al., who observed a positive correlation between BMI and colonization in younger women [25]. This discrepancy highlights the need for further research to elucidate variations in carriage rates and patterns of *S. aureus* and MRSA among different populations.

Our study further demonstrated that persistent carriers are inclined to carry the same *S. aureus* genotype, while intermittent carriers may carry varying genotypes, owing to decolonization and recolonization over time [21, 22]. This dynamic nature underscores the complexity of *S. aureus* nasal carriage [26]. Once colonized by stubborn *S. aureus* for an extended period, decolonization becomes more difficult. Therefore, monitoring and treating nasal carriages is crucial to reduce nosocomial infection rates, thus emphasizing the need for expanded prehospitalization nasal swab testing.

We observed a correlation between the genotype, SNP distance, and SPA results, which confirmed that SPA typing can be used as a quick method for identifying *S. aureus* carriers. The correlation between SPA typing and MLST was previously assessed, and it was determined that SPA typing could effectively predict MLST clonal complexes, as defined by eBURST [27, 28]. Similarly, all t084 in our study corresponded to CC15. An intriguing discovery was made in both the aforementioned study and ours, indicating a connection between intermittent carriage and t084.

However, our study uncovered widespread resistance to penicillin and ampicillin in all strains, likely attributed to their extensive use. Furthermore, 24.30% of the strains demonstrated oxacillin resistance and all strains displayed sensitivity to ceftaroline. Oxacillin and ceftaroline were the most suitable choices for treating CA-MSSA. Linezolid, teicoplanin, and vancomycin were effective against all strains, indicating their potential as last-resort options for MRSA treatment. Clindamycin and erythromycin are commonly utilized to treat SSTIs [29]; but, our CA-MRSA strains exhibited substantial resistance to clindamycin and erythromycin, underscoring the critical need for judicious antibiotic selection. Furthermore, we identified 41 MDR strains, of which approximately 68.29% of the MDRSA strains originated from the persistent carriers. Prolonged colonization by MDRSA poses an increased potential risk for carriers as it renders treatment more challenging once infected. This finding underscores the need for regular testing of healthy individuals and timely eradication of staphylococcal carriage to prevent *S. aureus* infection.

Our study found that ARs are consistent with resistance phenotypes, such as *tet(L)* and *erm(C)* genes, which are associated with tetracycline and MLS antibiotic resistance, respectively, and consistent with previous research [30]. Besides, all 26 MRSA strains carried *mecA* and were resistant to oxacillin, which aligns with the definition of MRSA. β-lactam resistance profiles are generally biased by MRSA status. In addition, our findings suggest that the presence of ARs in ICEs/IMEs may contribute to the spread of antibiotic resistance among bacterial populations. Overall, our study emphasizes the need for a more comprehensive approach to address antibiotic resistance, considering the role of ICEs/IMEs [31].

ST59 was identified as the most prevalent ST, closely followed by ST398. Notably, 91.7% of the ST59 strains were identified as MRSA. ST59-MRSA has long been acknowledged as one of the most successful and enduring CA-MRSA clones in Asia, including China [32]. Furthermore, ST59 strains frequently harbor ARs to macrolides and β-lactams (*ermB* and *mecA*, respectively), and present significant challenges for treating infections caused by these strains [32]. All ST59 strains belonged to MDRSA, which is consistent with previous findings. Additionally, the ST59 strains carried the highest number of VFs. ST59-t437, a prominent strain in our study, has been extensively documented in China and

has been associated with severe inflammatory reactions, ultimately leading to patient mortality [33]. Therefore, our findings underscore the importance of maintaining heightened vigilance regarding ST59 colonization in our healthy population. Our study revealed that the proportion of ST398-MRSA strains was comparable to that of ST398-MSSA strains, indicating an increasing prevalence of ST398-MRSA among healthy adults in Jiangsu. Furthermore, we noted a predominance of ST398 strains among intermittent colonizers, with a significant proportion of these strains carrying ICEs, thus reinforcing our hypothesis regarding the association between intermittent colonization and ICE carriage. We noted an interesting phenomenon among the intermittent colonizers (67, 137, and 140), wherein ST59 and ST398 were observed as alternating strains carried by these individuals. This observation prompts further investigations into the potential connection between these two STs.

The pathogenesis of toxic shock syndrome (TSS) is closely linked to *S. aureus*, which produces tsst-1. All the ST508-t015-CC45 isolates in our study carried this toxin and were classified as MDRSA. However, previous studies conducted in China have primarily associated CC5 and CC398 genotypes with *tsst-1* [34]. A strong association between *PVL* and CA-MRSA has already been demonstrated [35, 36]. However, contrary to these findings, our results revealed that only 38.46% of CA-MRSA strains carried the PVL toxin. Similarly, during the same period as our research in Germany, 40.4% of CA-MRSA isolates harbored the virulence factor PVL [37], indicating a probability similar to ours. Therefore, we believe that solely relying on the presence of *PVL* cannot definitively determine MRSA strain as CA-MRSA. MRSA strains possessing both *lukS* and *lukF* genes are considered more virulent and have been associated with the development of severe necrotizing pneumonia [38]. Notably, all MRSA strains carrying both *lukS* and *lukF* genes in this study belonged to the ST59-MRSA lineage and also carried the *seb-selk-selq* genes, highlighting their pathogenic potential within the ST59 lineage.

Notably, *S. aureus* collected from individuals within the same household exhibited close genetic relatedness, suggesting the possibility of intra-household transmission, and therefore, aligning with findings from previous research [5]. Within these households, we identified both persistent and intermittent carriers, with persistent colonizers potentially serving as sources of infection, thereby facilitating transmission within the household setting. Consequently, cohabitation with persistent carriers represents a significant risk factor for healthy individuals in communities, which increases the likelihood of *S. aureus* infection.

CONCLUSION

On the basis of our findings, longitudinal multiple nasal sampling offers a more accurate depiction of *S. aureus* nasal carriage than cross-sectional sampling. The high carriage rates among intermittent carriers underscore the importance of regular community testing. Continuous monitoring is critical to understand and mitigate the prevalence of *S. aureus*, particularly the widespread ST59-MRSA strain, in healthy individuals in the Jiangsu community. SPA typing presents itself as a rapid method to determine *S. aureus* colonization. The potential for intrafamilial transmission of *S. aureus* is evident, with persistent carriers being the primary sources of infection. Oxacillin and ceftaroline are preferred treatment options for CA-MSSA. Linezolid, teicoplanin, and vancomycin demonstrated efficacy against all the strains, indicating their potential as 11th-hour treatment options for CA-MRSA. Our study revealed a notable prevalence of antimicrobial resistance in healthy individuals. This underscores the need for vigilant monitoring of *S. aureus* colonization and infection, along with timely alerts regarding the evolving trend of MSSA resistance to β-lactam antibiotics.

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Data Availability. All sequence data were submitted to the NCBI. Accession numbers were obtained at the time of submission and will be provided during review.

Declarations

Conflict of Interest. Wenjing Hu, Yang Wang, Lu Zhou, Kai Chu, Pengfei Jin, Qi Liang, Jingxin Li, Zhongming Tan, and Fengzai Zhu report no conflict of interest in this work.

Ethics Statement. This study used samples from routine monitoring conducted previously, and the Ethics Review Committee of the Jiangsu Provincial Center for Disease Control and Prevention determined that a special ethical review was not necessary. The study was conducted in accordance with the principles of the Declaration of Helsinki. We ensured to protect the privacy of the participants involved and followed all the ethical guidelines during the publication of the article.

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