

Phenotypic and Molecular Characteristics of Community-Associated *Staphylococcus aureus* Infection in Neonates

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Background: *Staphylococcus aureus* is an important human pathogen causing a wide variety of diseases ranging from mild skin infections to life-threatening bacteremia. This study aimed to provide insight into the phenotype–genotype characteristics of *S. aureus* isolated from neonates.

Methods: A hospital-based retrospective study was conducted to recruit neonatal patients with community-associated *S. aureus* infection. All 92 *S. aureus* clinical isolates were tested for antimicrobial susceptibility, resistance genes, virulence genes, and multilocus sequence typing.

Results: A total of 92 *S. aureus* isolates were included in this study, including 29 (31.5%) MRSA isolates. Most *S. aureus* isolates were susceptible to gentamicin, and most methicillin-resistant *S. aureus* (MRSA) isolates were susceptible to vancomycin and linezolid. The predominant *S. aureus* genotypes were CC188 (ST188), CC59 (ST338), CC5 (ST5), CC88 (ST8), and CC6 (ST6), with CC59 (ST338) as the most prevalent MRSA. We observed a significantly corresponding relationship between clonal complexes and resistance patterns (such as CC45/CC59/CC121/CC630 associated with multidrug resistance) or the number of virulence genes (such as CC1/CC5/CC45/CC59/CC88 associated with harboring 5–7 virulence genes, and CC22/CC25/CC121 associated with carrying 8–13 genes). Moreover, both univariate and multivariate analyses indicated that invasive *S. aureus* isolates were significantly associated with resistance to several classes of antibiotics and carrying specific virulence factors.

Conclusion: Our findings provide insight into phenotype–genotype characteristics for *S. aureus* isolates so as to provide new ideas for differentiating potential genetic backgrounds and monitoring new epidemiologic trends.

Keywords: *Staphylococcus aureus*, antimicrobial susceptibility, molecular characterization, virulence genes

Introduction

Staphylococcus aureus (*S. aureus*) is an important human pathogen causing a wide variety of diseases ranging from mild skin infections to life-threatening bacteremia and contributes to both community-associated and healthcare-associated infections with a high clinical burden for infants worldwide.¹ Nearly 120,000 *S. aureus* bloodstream infections and 20,000 associated deaths occurred in the United States in 2017, indicating that *S. aureus* infections account for significant morbidity and mortality.² A multicenter study in China showed that the incidence of community-associated *S. aureus* from skin and soft tissue infections was significantly higher in pediatric patients than in adult patients, suggesting that *S. aureus* is an important

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causative agent in children.³ Moreover, several studies have reported that outbreaks and transmission events due to *S. aureus* and methicillin-resistant *S. aureus* (MRSA) occurred in neonatal intensive care units (NICUs).^{4–6} Therefore, it is urgent to study phenotypic and genotypic characteristics of *S. aureus* in neonates, which may promote an increased understanding of its genetic backgrounds and pathogenesis.

The incidence of *S. aureus* (especially for MRSA) infection and colonization have been explored for neonates across the globe.^{7–10} For example, MRSA colonization was detected in 6.7% of infants, and MRSA infection occurred in 2.0% of infants in NICUs of the US.⁷ Similarly, rates of MRSA colonization and infection were found to be 5.2% and 2.6% in Chinese NICUs, respectively.⁸ These findings suggest that active surveillance may detect MRSA affected neonates earlier and limit nosocomial spread. To note, previous studies have mainly focused on MRSA isolates in neonates or infants,^{6,9,11,12} whereas little is known about methicillin-sensitive *S. aureus* (MSSA). In terms of virulence and pathogenicity, *S. aureus* infection is characterized by virulence factors and antibiotic resistance.¹³ However, limited data on phenotypic and molecular characteristics of neonatal *S. aureus* are available for China, and the potential relationship between clonal complexes (CCs) and phenotype–genotype characteristics of *S. aureus* is still unclear. Therefore, we undertook a hospital-based retrospective study of neonatal *S. aureus* infection in Liuzhou, China. The primary purpose of this study was to describe antimicrobial susceptibility, virulence genes, and molecular typing of both MRSA and MSSA isolates. The second purpose was to reveal the potential corresponding relationships between CCs and phenotype–genotype characteristics of *S. aureus* isolates.

Materials and Methods

Patient Enrollment and Clinical Data Collection

This hospital-based retrospective study was conducted from April 2014 to May 2017 at the sole tertiary children's hospital in Liuzhou, China. Our children's hospital has around 10,000–20,000 admissions of children annually. The target population was neonates ≤ 28 days of age and with community-associated *S. aureus* infections. The eligibility criteria for community-associated *S. aureus* infections included: (1) a culture sample was obtained within 48h of hospitalization or during an outpatient visit; (2) the

patient had no history of known risk factors for *S. aureus* infections in the past year; and (3) the patient had no history of medical facility visits in the past year.¹⁴ Infections in which any positive culture was obtained from cerebrospinal fluid (CSF), blood, sterile fluid, or an abscess were considered to be invasive. Infections in which all positive cultures were obtained from the trachea, urine, conjunctiva, or a wound were considered to be non-invasive. We used a case report form for the collection of demographic information (sex and age), clinical symptoms, types of infection, antibiotic use, and clinical outcomes.

Bacterial Isolation and Identification

Specimens were collected from infection sites of each neonatal patient enrolled and cultured on blood agar. To avoid overrepresentation, we included only the first isolate from each patient. Preliminary *S. aureus* identification was performed based on a combination of bacterial morphology, Gram staining, hemolysis, catalase test, and tube coagulase test. Then, Polymerase Chain Reaction (PCR) assays were used to test the staphylococci 16SrRNA, *nuc*, and *mecA* genes ([Supplementary Table S1](#)).¹⁵ All *S. aureus* isolates carried the 16S rRNA and *nuc* genes. The *mecA*-positive or ceftiofloxacin-resistant *S. aureus* was identified as MRSA. So MRSA included genotypic (*mecA*-positive) and phenotypic (ceftiofloxacin-resistant) MRSA isolates.

Antimicrobial Susceptibility Tests

The minimum inhibitory concentrations of antimicrobial agents were determined according to the Clinical and Laboratory Standards Institute.¹⁶ The following drugs were tested: penicillin, ceftiofloxacin, erythromycin, clindamycin, tetracycline, trimethoprim-sulfamethoxazole, rifampin, chloramphenicol, ciprofloxacin, gentamicin, vancomycin, and linezolid. *S. aureus* ATCC 29,213 was used as a control strain. Multidrug-resistant (MDR) *S. aureus* (MDRSA) was defined as being non-susceptible to ≥ 1 agent in ≥ 3 antimicrobial categories.¹⁷ The resistance pattern was categorized into three groups: susceptible to all antibiotics, non-MDR (non-susceptible to 1–2 antimicrobial categories), and MDR (non-susceptible to ≥ 3 antimicrobial categories).

MLST Typing and SCC_{mec} Analysis

For all *S. aureus* isolates, multilocus sequence typing (MLST) was analyzed by sequencing seven housekeeping genes.¹⁸ Sequence types (STs) were confirmed by comparing with the

MLST database (<http://saureus.mlst.net>). Clonal complexes (CCs) were inferred using the eBURST algorithm, based on our data and a list of distinct ST types with six of the seven loci.¹⁹ MRSA isolates carrying the *mecA* gene were subjected to SCC*mec* typing, which is based on a set of multiplex PCR reactions ([Supplementary Table S1](#)).²⁰

Antibiotic-Resistant Genes and Virulence Genes

For *S. aureus* isolates, specific PCR tests were used to detect the presence of tetracycline-resistant genes including *tet* (M) and *tet* (K), erythromycin-resistant genes including *erm* (A) and *erm* (C), and virulence genes including the Pantone–Valentine leucocidin (*pvl*) toxin gene, the toxic shock syndrome toxin-1 gene (*tsst1*), the staphylococcal enterotoxin genes (*sea*, *seb*, *sec*, *sed*, *see*, *seg*, *seh*, *sei*, *sej*, *sek*, *sel*, *sem*, *sen*, *seo*, *sep*, *seq*, *ser*, and *seu*), the immune evasion cluster (IEC) genes (*scn*, *chp*, and *sak*), and the hemolysin genes (*hla*, *hlb*, and *hld*).^{21–26} The detailed information for PCR tests was presented in the [supplementary Table S1](#).

Statistical Analyses

Briefly, a two-stage analysis process was employed to explore specific characteristics for invasive isolates. First, Pearson's chi-squared test or Fisher's exact test was used to test the differences in phenotype–genotype characteristics between invasive and non-invasive isolates. Second, the random forest model (machine learning approach) was used to evaluate the importance of each characteristic variable, which may be associated with invasive *S. aureus* isolates. Variable importance scores (mean decrease Gini, MDG) for characteristic variables were estimated and ranked in a descending order. A greater MDG value indicated that the degree of impurity arising from a category could be reduced the most by a single variable, suggesting an important variable. The random forest was implemented in the R package (Breiman and Cutler's Random Forests for Classification and Regression, version 4.6–14).

In addition, we used the correspondence analysis to test two-dimensional relationships and the cluster analysis to explore high-dimensional relationships. Correspondence analysis provides a graphic and statistical method for exploring the internal relationship between categorical variables, so we used the correspondence analysis to explore potential corresponding relationships between CCs and phenotypic or genotypic characteristics of *S. aureus* isolates.

Correspondence analyses were performed using STATA version 14.0 (StataCorp LP, College Station, TX, USA), and a two-sided *P*-value of <0.05 was defined as being of statistical significance. Moreover, the unweighted pair-group method with arithmetic means (UPGMA) cluster analysis was used to construct the UPGMA tree based on the mismatch matrix of alleles between ST types, simultaneously labeling with multiple molecular characteristics.²⁷ The UPGMA tree was also marked and visualized by iTOL (<https://itol.embl.de/>) to compare the phenotype–genotype characteristics of *S. aureus* isolates.²⁸

Ethics Statement

This study was approved by the Ethics Committee of Guangdong Pharmaceutical University and was conducted in accordance with the approved guidelines (No. 2015–22). Before participating, all parents or legal guardians of the neonates signed an informed consent form regarding the goals of the study and the willingness to participate. So this study complied with the Declaration of Helsinki.

Results

S. aureus and MRSA Detection

A total of 92 *S. aureus* isolates (13 invasive isolates and 79 non-invasive isolates) were identified from the study neonates during the study period, including 29 MRSA isolates. A total of 52 (56.5%) neonates were male, the average age was 15.8±7.4 days old, and the average length of hospital stay was 10.3±5.4 days. A few neonates (8 of 92 neonates, 8.7%) were transferred to the Neonatal Intensive Care Unit (NICU).

Antimicrobial Susceptibility Testing

All *S. aureus* isolates were susceptible to vancomycin, and most of *S. aureus* isolates were susceptible to chloramphenicol and linezolid ([Table 1](#)). But most isolates were resistant to penicillin (96.7%), and nearly half of the isolates were resistant to erythromycin (40.2%) and clindamycin (40.2%). Notably, 33 (35.9%) isolates were classified as MDRSA, with the most common resistance pattern being non-susceptible to clindamycin, erythromycin, and cefoxitin (9/33, 27.3%). In addition, one linezolid-resistant isolate was classified as MDRSA, which was non-susceptible to cefoxitin, erythromycin, clindamycin, tetracycline, and linezolid. For 26 phenotypic tetracycline-resistant isolates, 17 (65.4%) carried *tet* (K). But the *tet*

(M) gene was absent from all phenotypic tetracycline-resistant isolates. For 37 phenotypic erythromycin-resistant isolates, 19 (51.4%) carried *erm* (C) and 1 (2.7%) carried *erm* (A).

Molecular Typing and Virulence Genes

Among all *S. aureus* isolates, we observed 28 unique STs belonging to 18 CCs (Figure 1), except for three non-typeable isolates. The most common *S. aureus* CCs were CC188, CC59, CC5, and CC88, with the predominant MRSA being CC59-MRSA. The most prevalent *S. aureus* STs were ST188, ST338, ST5, ST88, and ST6, with the predominant MRSA being ST338-MRSA. There were 29 MRSA isolates, including 9 genotypic (*mecA*-positive) and 20 phenotypic (cefoxitin-resistant) MRSA isolates. Among all 9 genotypic MRSA isolates, the predominant SCC*mec* type was SCC*mec* IVd (n=3), followed by SCC*mec* IVa (n=2), SCC*mec* I (n=2), and untypeable (n=2).

As to the IEC genes (Table 1), the predominant IEC gene was *sak* (95.7%), followed by *chp* (21.7%) and *scn* (14.1%). In terms of the enterotoxin genes, the most abundant gene was *seg* (22.8%), followed by *sei* (20.7%), *sek* (17.4%), *seb* (14.1%), *sem* (14.1%) and *seo* (12.0%). Note that several enterotoxin genes (including *see*, *sel* and *ser*) were absent from all *S. aureus* isolates. With regard to the hemolysin genes, *hla* was detected in 97.8%, *hly* in 53.3%, and *hld* in 94.6% of *S. aureus* isolates. In addition, *tsst1* was present in 24 (26.1%) isolates, and *pvl* was found in 16 (17.4%) isolates.

Relationships Between CCs and Phenotype–Genotype Characteristics

The first correspondence analysis indicated a significantly corresponding relationship between CCs and resistance patterns ($\chi^2=56.00$, $P=0.003$; Figures 2A and 3), suggesting that CC45/CC59/CC121/CC630 isolates were associated with being MDR, CC7/CC8/CC12/CC25/CC509 isolates were associated with being non-MDR, and CC1/CC5/CC6/CC22/CC88/CC188/CC398 isolates were associated with being susceptible to all antibiotics. The second correspondence analysis revealed a significantly corresponding relationship between CCs and the number of virulence genes (such as CC6/CC7/CC8/CC188/CC398/CC509 and harboring 1–4 virulence genes, CC1/CC5/CC12/CC45/CC59/CC88/CC630 and harboring 5–7 virulence genes, and CC22/CC25/CC121

and carrying 8–13 genes; $\chi^2=67.97$, $P<0.001$; Figures 2B and 3). According to Figure 1, CC6/CC188 isolates were associated with harboring *sak-hla-hld*, CC59 (including ST338, ST59 and ST537) isolates were associated with harboring *sak-seb-sek-hla-hly-hld-pvl*, CC5 isolates were associated with harboring *sak-seg-sei-sej-sem-seo-hla-hly-hld*, and CC88 isolates were associated with harboring *sak-sep-hla-hly-hld-pvl*. In addition, CC59/CC88/CC25 isolates were associated with carrying *pvl*, and CC630 and CC45 isolates were associated with carrying *tsst1*.

Differences in Phenotype–Genotype Characteristics Between Invasive and Non-Invasive *S. aureus* Isolates

When comparing the resistance characteristics between invasive and non-invasive isolates (Table 1), we observed that invasive *S. aureus* isolates were significantly associated with higher rates of resistance to cefoxitin (69.2% vs 22.8%, $P=0.001$) and tetracycline (53.8% vs 24.1%, $P=0.027$). When comparing the virulence genes between invasive and non-invasive isolates, invasive *S. aureus* isolates were significantly associated with carrying *sak* (76.9% vs 98.7%, $P=0.008$), *seg* (0.0% vs 26.6%, $P=0.034$), *sei* (0.0% vs 24.1%, $P=0.047$), *sek* (46.2% vs 12.7%, $P=0.003$), and *pvl* (38.5% vs 13.9%, $P=0.031$). Notably, invasive *S. aureus* isolates were significantly associated with CC59 (46.2% vs 10.1%, $P=0.001$). Among 13 invasive *S. aureus* isolates, the predominant lineage was MRSA CC59 (ST338) carrying several virulence genes (Table 2).

In addition, random forest model was used to evaluate the importance of phenotypic and molecular characteristics influencing invasive *S. aureus* isolates (Figure 4). The results of random forest showed that the top-10 important variables were *sak*, cefoxitin, *sek*, CC59, *tsst1*, *sea*, *pvl*, *chp*, rifampin, and tetracycline.

Discussion

The antimicrobial resistance of neonatal *S. aureus* and MRSA has become an important public health issue. The World Health Organization recommends penicillin in combination with gentamicin as first-line antibiotics for newborns with serious bacterial infection or sepsis.²⁹ But, worryingly, we observed a high rate of resistance to penicillin (96.7%) among *S. aureus* isolates, which is in line with recent reports on neonatal *S. aureus* septicemia in

Table 1 Phenotypic and Molecular Characteristics of Invasive and Non-Invasive *S. aureus* Isolates

Characteristics	<i>S. aureus</i> (n=92)	Invasive Isolates (n=13)	Non-Invasive Isolates (n=79)	P value
Phenotype (resistance) ^a				
penicillin	89(96.7)	13(100.0)	76(96.2)	0.999 ^c
cefoxitin	27(29.3)	9(69.2)	18(22.8)	0.001
erythromycin	37(40.2)	8(61.5)	29(36.7)	0.091
clindamycin	37(40.2)	7(53.8)	30(38.0)	0.279
tetracycline	26(28.3)	7(53.8)	19(24.1)	0.027
trimethoprim-sulfamethoxazole	8(8.7)	1(7.7)	7(8.9)	0.890
gentamicin	7(7.6)	0(0.0)	7(8.9)	0.331 ^c
rifampin	4(4.3)	2(15.4)	2(2.5)	0.094 ^c
ciprofloxacin	5(5.4)	0(0.0)	5(6.3)	0.999 ^c
chloramphenicol	1(1.1)	1(7.7)	0(0.0)	0.141 ^c
linezolid	1(1.1)	1(7.7)	0(0.0)	0.141 ^c
Immune evasion cluster genes (positive)				
<i>scn</i>	13(14.1)	0(0.0)	13(16.5)	0.118 ^c
<i>chp</i>	20(21.7)	5(38.5)	15(19.0)	0.115
<i>sak</i>	88(95.7)	10(76.9)	78(98.7)	0.008 ^c
Enterotoxin genes (positive) ^b				
<i>sea</i>	1(1.1)	1(7.7)	0(0.0)	0.141 ^c
<i>seb</i>	13(14.1)	2(15.4)	11(13.9)	0.889
<i>sec</i>	7(7.6)	1(7.7)	6(7.6)	0.999 ^c
<i>sed</i>	4(4.3)	1(7.7)	3(3.8)	0.462 ^c
<i>seg</i>	21(22.8)	0(0.0)	21(26.6)	0.034
<i>seh</i>	3(3.3)	0(0.0)	3(3.8)	0.630 ^c
<i>sei</i>	19(20.7)	0(0.0)	19(24.1)	0.047
<i>sej</i>	7(7.6)	0(0.0)	7(8.9)	0.264 ^c
<i>sek</i>	16(17.4)	6(46.2)	10(12.7)	0.003
<i>sem</i>	13(14.1)	1(7.7)	12(15.2)	0.472
<i>sen</i>	3(3.3)	1(7.7)	2(2.5)	0.370 ^c
<i>seo</i>	11(12.0)	1(7.7)	11(13.9)	0.609
<i>sep</i>	9(9.8)	0(0.0)	9(11.4)	0.200
<i>seq</i>	3(3.3)	2(15.4)	1(1.3)	0.051 ^c
<i>seu</i>	4(4.3)	0(0.0)	4(5.1)	0.538 ^c
Hemolysin genes (positive)				
<i>hla</i>	90(97.8)	13(100.0)	77(97.5)	0.736 ^c
<i>hlb</i>	49(53.3)	8(61.5)	41(51.9)	0.519
<i>hld</i>	87(94.6)	12(92.3)	75(94.9)	0.542 ^c
<i>pvl</i> (positive)	16(17.4)	5(38.5)	11(13.9)	0.031
<i>tsstI</i> (positive)	24(26.1)	3(23.1)	21(26.6)	0.790
CC typing				
CC59	14(15.2)	6(46.2)	8(10.1)	0.001
non-CC59	78(84.8)	7(53.8)	71(89.9)	

Notes: ^aAll isolates were susceptible to vancomycin. ^bAll isolates were negative for some enterotoxin genes, including *see*, *sel* and *ser*. ^cEstimated by the Fisher's exact test.

China (96.0%) and Gambia (92.5%).^{30,31} Fortunately, the present study demonstrated low-level resistance to gentamicin (7.6%), which is similar to the results from China

(0.8%) and Gambia (5.5%).^{31,32} These findings suggest that gentamicin is still the first choice for the treatment of *S. aureus* infections, especially for serious bacterial

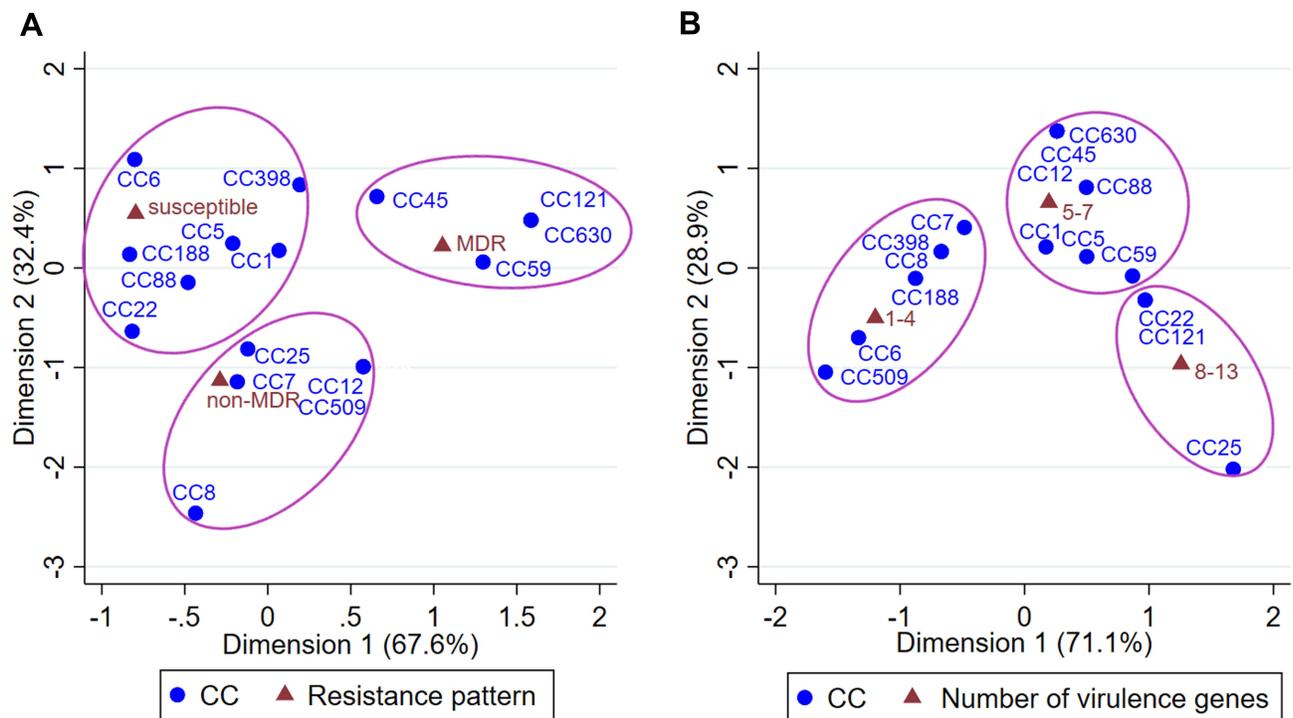


Figure 2 Correspondence analysis for testing the relationship between CCs and resistance patterns (A) or the number of virulence genes (B) of *S. aureus* isolates. **Abbreviations:** CCs, clonal complexes; MDR, multidrug-resistant.

infection or sepsis. MRSA has become a significant cause of morbidity and mortality in infants worldwide. It is well known that vancomycin and linezolid are the few clinically effective agents against MRSA infections. In this study, most MRSA isolates were susceptible to vancomycin and linezolid, which is consistent with previous studies in China, Japan, and Colombia.^{12,30,33} These findings suggest that vancomycin and linezolid are still used as the first-line antibiotic treatment for MRSA infections. Notably, MDRSA infections are associated with worse health outcomes and higher expenditures. In this study, 35.9% *S. aureus* isolates were classified as MDRSA, with the most common resistance pattern being non-susceptible to clindamycin, erythromycin, and ceftiofloxacin. Similar rates of MDRSA among *S. aureus* isolates were found in Guangzhou (30.5%), but significantly higher rates were observed in Shanghai (51.3%) and Iran (53.1%).^{32,34,35} Therefore, the prevalence and resistance patterns of MDRSA isolates must be monitored in prospective surveillance programs so as to guide rational use of antibiotics.

The results of molecular characteristics may broaden our insights into neonatal *S. aureus* and potential

transmission mechanisms. In this study, the most common *S. aureus* genotypes were CC188 (ST188), CC59 (ST338), CC5 (ST5), CC88 (ST8), and CC6 (ST6), with CC59 (ST338) as the predominant MRSA, which is similar to the latest reports from China.^{32,34} However, the most common *S. aureus* CCs in Germany were CC15, CC8, CC25, and CC7, and the dominant CCs in the US were CC30, CC5, and CC8.^{36,37} These findings exhibit marked clonal diversity and regional differences in genotype distribution. Notably, the present study found that ST188 was the most prevalent in neonatal isolates, which was also reported as the major host-adapted lineage causing human and livestock infections in China.³⁸ These findings suggest that the burden of *S. aureus* ST188 infections in humans has been increasing, especially in neonates. Besides, ST398/ST7/ST59 were the most prevalent *S. aureus* isolated from breast milk samples,³⁹ and CC5/CC59/CC45 were the most predominant clones observed in both mother and neonate isolates,⁴⁰ which were also found in our study. These findings may provide evidence for *S. aureus* transmission through breastfeeding and the birth canal.

The potential relationships between CCs and phenotype-genotype characteristics of *S. aureus* isolates may

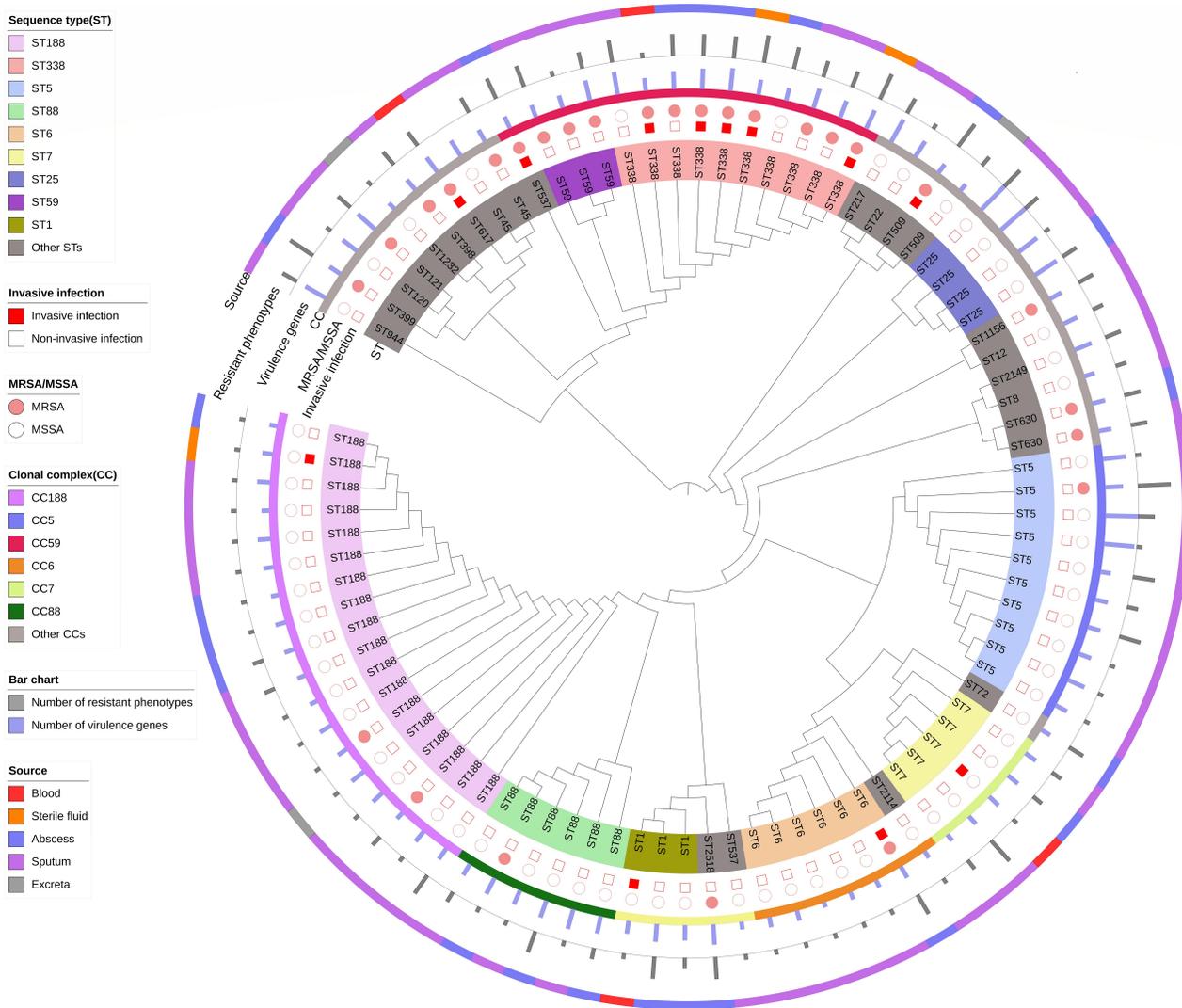


Figure 3 Clonal dendrogram for describing the relationship between genotypes and detailed phenotype–genotype characteristics of 89 ST-typeable *S. aureus* isolates.

provide more implications for virulent properties. However, previous studies were limited to use descriptive tables or figures only, so this study builds on previous literature to use correspondence analysis to test the potential relationships between two-dimensional characteristic variables. Our correspondence analyses revealed significantly corresponding relationships between CCs and resistance patterns or the number of virulence genes, suggesting that CC45/CC59/CC121/CC630 isolates were associated with being MDR, CC6/CC188 were associated with carrying 1–4 virulence genes (*sak-hla-hld*), and CC1/CC5/CC45/CC59/CC88 were associated with harboring 5–7 virulence genes (*sak-seb-sek-hla-hlb-hld-pvl* for CC59, *sak-seg-sei-sej-sem-seo-hla-hlb-hld* for CC5, and *sak-sep-hla-hlb-hld-pvl* for CC88). Strikingly, the CC5/USA100, CC8/USA300, CC1/USA400, and CC45/

USA600 lineages are frequently associated with life-threatening *S. aureus* infections and widespread in both the community and hospital settings,⁴¹ which are also found in neonatal infections in our study. Importantly, CC5/USA100 is a leading cause of invasive diseases and represents the majority of vancomycin-resistant/-intermediate isolates,⁴¹ and CC5 isolates in the present study also carried several virulence genes but were susceptible to all antibiotics. Moreover, multidrug-resistant CC5 isolates reported as livestock-associated MRSA have been identified in livestock and related workers in China and Germany.^{42,43} Conflicting outcomes obscure the identification of virulence and resistance of *S. aureus* isolates, which may lead to increase morbidity and mortality. Notably, the toxic shock syndrome toxin gene *tst1* was mainly observed in CC45 isolates, the *pvl* gene was mainly

Table 2 Genotypic and Phenotypic Characteristics of 13 Invasive *S. aureus* Isolates

CCs	STs	MRSA	Antibiotics Resistance Pattern	Virulence Gene Profile
CC59	ST338	Yes	FOX-ERY-CLI-TET-LZD	<i>chp-sak-sep-seb-sek-hla-hlb-hld-pvl</i>
CC59	ST338	Yes	FOX-ERY-TET-RIF-CHL	<i>chp-sak-hla-hlb-hld-pvl</i>
CC59	ST338	Yes	FOX-CLI-TET	<i>chp-seb-sek-seq-hla-hlb-hld-pvl</i>
CC59	ST338	Yes	FOX-CLI-TET	<i>chp-seb-sek-sen-seq hla-hlb-hld-pvl</i>
CC59	ST338	Yes	FOX	<i>sak-sek-hla-hlb-hld</i>
CC59	ST537	Yes	FOX-ERY-CLI-TET	<i>sak-hla-hlb-hld-pvl</i>
CC45	ST617	Yes	FOX-ERY-CLI	<i>sak-sec-sem-seo-hla-hld-tsstI</i>
CC509	ST509	Yes	FOX-ERY-CLI-SXT-TET	<i>sak-sek-hla-hld</i>
CC6	ST6	Yes	FOX-ERY-CLI-TET	<i>sak-sea-sea-hla-hlb-hld</i>
CC188	ST188	No	Susceptible to all antibiotics	<i>sak-hla-hld-tsstI</i>
CC7	ST7	No	Susceptible to all antibiotics	<i>chp-sak-sea-hla-hld-tsstI</i>
CC1	ST1	No	Susceptible to all antibiotics	<i>sak-sea-sek-hla-hlb-hld</i>
NT	NT	No	TET	<i>hla</i>

Abbreviations: CCs, clonal complexes; STs, sequence types; MRSA, methicillin-resistant *S. aureus*; NT, non-typeable; FOX, ceftioxin; ERY, erythromycin; CLI, clindamycin; TET, tetracycline; SXT, trimethoprim-sulfamethoxazole; RIF, rifampin; CHL, chloramphenicol; LZD, linezolid.

found in CC59 and CC88 isolates, and IEC genes were mainly found in CC88, which are consistent with the latest reports.^{32,41,44,45} These findings reveal the necessity of differentiating *S. aureus* clones based on multiple phenotype-genotype characteristics, which may provide new ideas for monitoring new epidemiologic trends.

S. aureus (especially for MRSA) is a major cause of community-associated and hospital-associated invasive infections globally. When exploring invasive *S. aureus* and MRSA isolates in depth, we observed a significant relationship between CC59 carrying multiple virulence factors and invasive MRSA infections, and the random forest also revealed that CC59 isolates play an important role in neonatal invasive infections. Similarly, other studies found an interaction between specific CCs and clinical

outcomes.^{46,47} For example, there was a strong association between CC1/CC121/CC30 and invasive *S. aureus* infections.⁴⁶ These findings suggest that distinct combinations of virulence genes may be present in specific invasive CCs. However, few studies have systematically compared phenotypic and molecular characteristics between invasive and non-invasive *S. aureus* isolates. This study adds to existing knowledge by providing insight into the genotypic and phenotypic characteristics for invasive isolates. The results from univariate analyses revealed that invasive *S. aureus* isolates were significantly associated with resistance to specific classes of antibiotics (including ceftioxin and tetracycline) and carrying or non-carrying specific virulence factors (including *sak*, *seg*, *sei*, *sek*, and *pvl*). In addition, the random forest indicated that

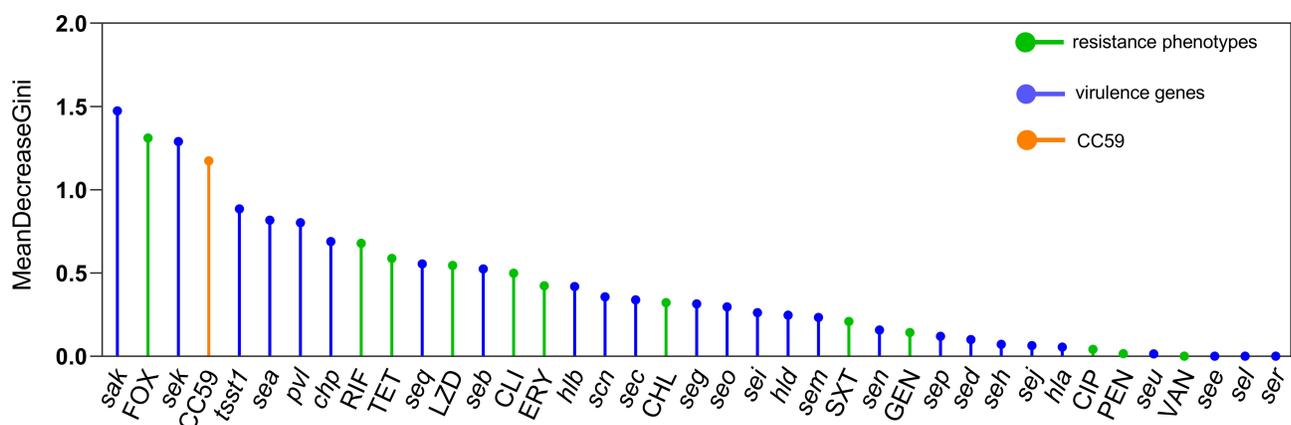


Figure 4 Importance rank of phenotypic and molecular characteristics influencing invasive *S. aureus* isolates.

Abbreviations: FOX, ceftioxin; RIF, rifampin; TET, tetracycline; LZD, linezolid; CLI, clindamycin; ERY, erythromycin; CHL, chloramphenicol; GEN, gentamicin; CIP, ciprofloxacin; SXT, trimethoprim-sulfamethoxazole; PEN, penicillin; VAN, vancomycin.

invasive *S. aureus* isolates were associated with resistance to several classes of antibiotics and carrying specific virulence factors, which provides more evidence for revealing the specific phenotype–genotype characteristics for invasive *S. aureus* isolates. These findings provide implications for differentiating the genetic background and pathogenicity of invasive isolates based on multiple phenotype–genotype characteristics.

This study is a new attempt to reveal potential relationships between CCs and phenotype–genotype characteristics of *S. aureus* isolates by using both correspondence analysis and cluster analysis, which provides implications for monitoring new epidemiologic trends. Moreover, this study provides insight into the phenotypic and molecular characteristics for invasive *S. aureus* by using the multivariate random forest model, which provides implications for the genetic background and pathogenicity of invasive isolates. However, there are some limitations to this study. First, *S. aureus* carriage of healthcare workers could not be taken into consideration because of limited funding, so it could not determine whether exists the risk of neonatal *S. aureus* transmission by healthcare workers. Second, no samples were obtained from mothers, so it could not determine whether exists the risk of *S. aureus* transmission from mothers to neonates. Future multi-object cohort studies are required to better understand the transmission routes of neonatal infections.

Conclusion

This study demonstrated that most MRSA isolates were susceptible to vancomycin and linezolid, suggesting that vancomycin and linezolid are still used as the first-line antibiotic treatment for MRSA infections. Notably, this study contributes to the literature by revealing a significantly corresponding relationship between CCs and phenotype–genotype characteristics, which provides new ideas for monitoring new epidemiologic trends. Interestingly, we found that invasive *S. aureus* isolates were significantly associated with resistance to several classes of antibiotics and carrying specific virulence factors, which provides implications for differentiating the genetic background and pathogenicity of invasive *S. aureus* isolates based on multiple phenotype–genotype characteristics.

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Disclosure

The authors have no conflicts of interest to disclose.

References

1. Tong SYC, Davis JS, Eichenberger E, Holland TL, Fowler VG. *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management. *Clin Microbiol Rev*. 2015;28:603–661. doi:10.1128/CMR.00134-14
2. Kourtis AP, Hatfield K, Baggs J, Mu Y, See I, Epson E. Vital signs: epidemiology and recent trends in methicillin-resistant and in methicillin-susceptible *Staphylococcus aureus* bloodstream infections — united States. *MMWR Morb Mortal Wkly Rep*. 2019;68:214–219.
3. Liu Y, Xu Z, Yang Z, Sun J, Ma L. Characterization of community-associated *Staphylococcus aureus* from skin and soft-tissue infections: a multicenter study in China. *Emerg Microbes Infect*. 2016;5:e127. doi:10.1038/emi.2016.128
4. Harris SR, Cartwright EJP, Török ME, et al. Whole-genome sequencing for analysis of an outbreak of methicillin-resistant *Staphylococcus aureus*: a descriptive study. *Lancet Infect Dis*. 2013;13:130–136. doi:10.1016/S1473-3099(12)70268-2
5. Brown NM, Reacher M, Rice W, et al. An outbreak of methicillin-resistant *Staphylococcus aureus* colonization in a neonatal intensive care unit: use of a case-control study to investigate and control it and lessons learnt. *J Hosp Infect*. 2019;103(1):35–43. doi:10.1016/j.jhin.2019.05.009
6. Cheng VCC, Wong SC, Cao H, et al. Whole-genome sequencing data-based modeling for the investigation of an outbreak of community-associated methicillin-resistant *Staphylococcus aureus* in a neonatal intensive care unit in Hong Kong. *Eur J Clin Microbiol Infect Dis*. 2019;38:563–573.
7. Maraqa NF, Aigbivbalu L, Masnita-Iusan C, et al. Prevalence of and risk factors for methicillin-resistant *Staphylococcus aureus* colonization and infection among infants at a level III neonatal intensive care unit. *Am J Infect Control*. 2011;39:35–41. doi:10.1016/j.ajic.2010.07.013
8. de Lencastre H, Geng W, Qi Y, et al. Epidemiology of *Staphylococcus aureus* in neonates on admission to a Chinese neonatal intensive care unit. *PLoS One*. 2020;15:e0211845.
9. Washam M, Woltmann J, Haberman B, Haslam D, Staat MA. Risk factors for methicillin-resistant *Staphylococcus aureus* colonization in the neonatal intensive care unit: A systematic review and meta-analysis. *Am J Infect Control*. 2017;45:1388–1393. doi:10.1016/j.ajic.2017.06.021
10. Giuffrè M, Amodio E, Bonura C, et al. Methicillin-resistant *Staphylococcus aureus* nasal colonization in a level III neonatal intensive care unit: incidence and risk factors. *Am J Infect Control*. 2015;43:476–481. doi:10.1016/j.ajic.2014.12.027
11. Lavie-Nevo K, Srigley JA, Al-Rawahi GN, et al. Prevalence and clinical impact of methicillin-resistant *Staphylococcus aureus* colonization among infants at a level III neonatal intensive care unit. *Am J Infect Control*. 2019;47:1336–1339. doi:10.1016/j.ajic.2019.04.173
12. Murai T, Okazaki K, Kinoshita K, et al. Comparison of USA300 with non-USA300 methicillin-resistant *Staphylococcus aureus* in a neonatal intensive care unit. *Int J Infect Dis*. 2019;79:134–138. doi:10.1016/j.ijid.2018.11.020
13. Laabei M, Recker M, Rudkin JK, et al. Predicting the virulence of MRSA from its genome sequence. *Genome Res*. 2014;24:839–849. doi:10.1101/gr.165415.113

14. Centers for Disease Control and Prevention. Community-associated MRSA information for clinicians. infection control topics. centers for disease control and prevention. Atlanta, GA. Available from: http://www.cdc.gov/ncidod/dhqp/ar_mrsa_ca_clinicians.html#4. Accessed February 3, 2005.
15. Zhang K, Sparling J, Chow BL, et al. New quadriplex PCR assay for detection of methicillin and mupirocin resistance and simultaneous discrimination of *Staphylococcus aureus* from coagulase-negative staphylococci. *J Clin Microbiol*. 2004;42:4947–4955. doi:10.1128/JCM.42.11.4947-4955.2004
16. Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing; 27th Informational Supplement. M100-S28*. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.
17. Magiorakos AP, Srinivasan A, Carey RB, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect*. 2012;18:268–281.
18. Enright MC, Day NP, Davies CE, Peacock SJ, Spratt BG. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. *J Clin Microbiol*. 2000;38:1008–1015. doi:10.1128/JCM.38.3.1008-1015.2000
19. Feil EJ, Li BC, Aanensen DM, Hanage WP, Spratt BG. eBURST: inferring patterns of evolutionary descent among clusters of related bacterial genotypes from multilocus sequence typing data. *J Bacteriol*. 2004;186:1518–1530. doi:10.1128/JB.186.5.1518-1530.2004
20. Zhang K, McClure J-A, Elsayed S, Louie T, Conly JM. Novel multiplex PCR assay for characterization and concomitant subtyping of staphylococcal cassette chromosome mec types I to V in methicillin-resistant *Staphylococcus aureus*. *J Clin Microbiol*. 2005;43:5026–5033. doi:10.1128/JCM.43.10.5026-5033.2005
21. Lina G, Quaglia A, Reverdy ME, Leclercq R, Vandenesch F, Etienne J. Distribution of genes encoding resistance to macrolides, lincosamides, and streptogramins among staphylococci. *Antimicrob Agents Chemother*. 1999;43:1062–1066. doi:10.1128/AAC.43.5.1062
22. Aarestrup FM, Agerso Y, Gerner-Smidt P, Madsen M, Jensen LB. Comparison of antimicrobial resistance phenotypes and resistance genes in *Enterococcus faecalis* and *Enterococcus faecium* from humans in the community, broilers, and pigs in Denmark. *Diagn Microbiol Infect Dis*. 2000;37:127–137. doi:10.1016/S0732-8893(00)00130-9
23. Wamel W, Rooijackers SHM, Ruyken M, Kessel K, Strijp J. The innate immune modulators staphylococcal complement inhibitor and chemotaxis inhibitory protein of *Staphylococcus aureus* are located on beta-hemolysin-converting bacteriophages. *J Bacteriol*. 2006;188:1310–1315. doi:10.1128/JB.188.4.1310-1315.2006
24. Omoe K, Hu DL, Takahashi-Omoe H, Nakane A, Shinagawa K. Comprehensive analysis of classical and newly described staphylococcal superantigenic toxin genes in *Staphylococcus aureus* isolates. *FEMS Microbiol Lett*. 2005;246:191–198. doi:10.1016/j.femsle.2005.04.007
25. Jarraud S, Mougel C, Thioulouse J, et al. Relationships between *Staphylococcus aureus* genetic background, virulence factors, agr groups (alleles), and human disease. *Infect Immun*. 2002;70:631–641. doi:10.1128/IAI.70.2.631-641.2002
26. McClure J-A, Conly JM, Lau V, et al. Novel multiplex PCR assay for detection of the staphylococcal virulence marker Panton-Valentine leukocidin genes and simultaneous discrimination of methicillin-susceptible from -resistant staphylococci. *J Clin Microbiol*. 2006;44:1141–1144. doi:10.1128/JCM.44.3.1141-1144.2006
27. Jolley KA, Feil EJ, Chan MS, Maiden MC. Sequence type analysis and recombinational tests (START). *Bioinformatics*. 2001;17:1230–1231. doi:10.1093/bioinformatics/17.12.1230
28. Letunic I, Bork P. Interactive Tree Of Life (iTOL) v4: recent updates and new developments. *Nucleic Acids Res*. 2019;47:W256–W9. doi:10.1093/nar/gkz239
29. Pocket book of hospital care for children: guidelines for the management of common childhood illnesses. 2nd edition. Geneva: World Health Organization; 2013. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK154447/>. Accessed December 7, 2020.
30. Li JY, Chen SQ, Yan YY, et al. Identification and antimicrobial resistance of pathogens in neonatal septicemia in China—A meta-analysis. *Int J Infect Dis*. 2018;71:89–93. doi:10.1016/j.ijid.2018.04.794
31. Darboe S, Dobreniecki S, Jarju S, et al. Prevalence of Panton-Valentine Leukocidin (PVL) and antimicrobial resistance in community-acquired clinical *Staphylococcus aureus* in an urban gambian hospital: a 11-year period retrospective pilot study. *Front Cell Infect Microbiol*. 2019;9:170.
32. Liang B, Mai J, Liu Y, et al. Prevalence and characterization of *Staphylococcus aureus* isolated from women and children in Guangzhou, China. *Front Microbiol*. 2018;9:2790. doi:10.3389/fmicb.2018.02790
33. Salazar-Ospina L, Jiménez JN. High frequency of methicillin-susceptible and methicillin-resistant *Staphylococcus aureus* in children under 1 year old with skin and soft tissue infections. *J Pediatr*. 2018;94:380–389. doi:10.1016/j.jpeds.2017.06.020
34. Wang X, Liu Q, Zhang H, et al. Molecular characteristics of community-associated *Staphylococcus aureus* isolates from pediatric patients with bloodstream infections between 2012 and 2017 in Shanghai, China. *Front Microbiol*. 2018;9:1211. doi:10.3389/fmicb.2018.01211
35. Imani Fooladi AA, Ashrafi E, Tazandareh SG, et al. The distribution of pathogenic and toxigenic genes among MRSA and MSSA clinical isolates. *Microb Pathog*. 2015;81:60–66. doi:10.1016/j.micpath.2015.03.013
36. Deinhardt-Emmer S, Sachse S, Geraci J, et al. Virulence patterns of *Staphylococcus aureus* strains from nasopharyngeal colonization. *J Hosp Infect*. 2018;100:309–315. doi:10.1016/j.jhin.2017.12.011
37. Hulten KG, Mason EO, Lamberth LB, Forbes AR, Revell PA, Kaplan SL. Analysis of invasive community-acquired methicillin-susceptible *staphylococcus aureus* infections during a period of declining community acquired methicillin-resistant staphylococcus aureus infections at a large Children’s Hospital. *Pediatr Infect Dis J*. 2018;37:235–241. doi:10.1097/INF.0000000000001753
38. Wang Y, Liu Q, Liu Q, et al. Phylogenetic analysis and virulence determinant of the host-adapted *Staphylococcus aureus* lineage ST188 in China. *Emerg Microbes Infect*. 2018;7:45. doi:10.1038/s41426-018-0048-7
39. Li X, Zhou Y, Zhan X, Huang W, Wang X. Breast milk is a potential reservoir for livestock-associated staphylococcus aureus and community-associated *Staphylococcus aureus* in Shanghai, China. *Front Microbiol*. 2017;8:2639.
40. Lin J, Wu C, Yan C, et al. A prospective cohort study of *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* carriage in neonates: the role of maternal carriage and phenotypic and molecular characteristics. *Infect Drug Resist*. 2018;11:555–565. doi:10.2147/IDR.S157522
41. King JM, Kulhankova K, Stach CS, Vu BG, Salgado-Pabón W. Phenotypes and Virulence among *Staphylococcus aureus* USA100, USA200, USA300, USA400, and USA600 Clonal Lineages. *mSphere*. 2016;1:e00071–16. doi:10.1128/mSphere.00071-16
42. Guo D, Liu Y, Han C, Chen Z, Ye X. Phenotypic and molecular characteristics of methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* isolated from pigs: implication for livestock-association markers and vaccine strategies. *Infect Drug Resist*. 2018;11:1299–1307. doi:10.2147/IDR.S173624
43. Köck R, Schaumburg F, Mellmann A, et al. Livestock-associated methicillin-resistant *Staphylococcus aureus* (MRSA) as causes of human infection and colonization in Germany. *PLoS One*. 2013;8:e55040.
44. Eibach D, Nagel M, Hogan B, et al. Nasal Carriage of *Staphylococcus aureus* among children in the ashanti region of ghana. *PLoS One*. 2017;12:e0170320. doi:10.1371/journal.pone.0170320

45. Mrochen DM, Grumann D, Schulz D, et al. Global spread of mouse-adapted *Staphylococcus aureus* lineages CC1, CC15, and CC88 among mouse breeding facilities. *Int J Med Microbiol.* 2018;308:598–606. doi:10.1016/j.ijmm.2017.11.006
46. Williamson DA, Ritchie SR, Roberts SA, et al. Clinical and molecular epidemiology of community-onset invasive *Staphylococcus aureus* infection in New Zealand children. *Epidemiol Infect.* 2014;142:1713–1721. doi:10.1017/S0950268814000053
47. Kurt K, Rasigade J-P, Laurent F, et al. Subpopulations of *Staphylococcus aureus* clonal complex 121 are associated with distinct clinical entities. *PLoS One.* 2013;8:e58155.

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