

Phenotypic and molecular characteristics of methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* isolated from pigs: implication for livestock-association markers and vaccine strategies

Dan Guo
Yangqun Liu
Changlin Han
Zhiyao Chen
Xiaohua Ye

Laboratory of Molecular
Epidemiology, School of Public Health,
Guangdong Pharmaceutical University,
Guangzhou, China

Background: Routine non-therapeutic antimicrobial use and overcrowding in animal farming may facilitate the propagation of methicillin-resistant *Staphylococcus aureus* (MRSA). This study aimed to examine the carriage prevalence and phenotype–genotype characteristics of MRSA and methicillin-susceptible *S. aureus* isolated from pigs.

Methods: Nasal swabs were collected from 1,458 pigs in 9 pig farms and 3 slaughterhouses. All strains were tested for antimicrobial susceptibility, resistance genes, and virulence genes, and characterized by multilocus sequence typing. The correspondence analysis was conducted to explore the relationships between multiple phenotypic and molecular characteristics of *S. aureus* isolates.

Results: In the 1,458 pigs, the carriage prevalence was 9.5% for *S. aureus*, 3.3% for MRSA, and 9.3% for multidrug-resistant *S. aureus*. Notably, 97.1% *S. aureus* isolates were multidrug resistant, and the predominant resistance pattern was non-susceptible to clindamycin, tetracycline, and erythromycin. The predominant genotype was CC9 (ST9) for *S. aureus* and MRSA isolates. Importantly, all *S. aureus* isolates were negative for the *scn* gene and resistant to tetracycline. Notably, all 9 linezolid-resistant isolates were classified as multidrug resistance, including 1 expressing the *cfr* gene and 6 expressing the *optrA* gene. The correspondence analysis showed a significant relationship between clonal complexes and resistance pattern or virulence genes. For example, CC9 was associated with extensive drug-resistance and co-carrying *chp*, *sak*, and *hlb*, and CC1 was associated with multidrug resistance and co-carrying *sak* and *hlb*.

Conclusion: The significant correspondence relationship between multiple characteristics provides some implication for vaccine strategies and new ideas for monitoring new epidemiologic clones.

Keywords: livestock, animals, *Staphylococcus aureus*, antimicrobial susceptibility, multidrug resistance, molecular characterization

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important cause of nosocomial infections worldwide.¹ Human-related MRSA is usually divided into 2 types according to epidemiological and molecular characteristics, including community-associated and hospital-associated MRSA. However, latest evidence has revealed that the epidemiological trend has changed with the increasing appearance of livestock-

Correspondence: Xiaohua Ye
Laboratory of Molecular Epidemiology,
School of Public Health, Guangdong
Pharmaceutical University, 283 Jianghai
Street, Guangzhou 510310, China
Tel +86 203 405 5121
Fax +86 203 405 5355
Email smalltomato@163.com

associated MRSA (LA-MRSA). In Asia, the most common lineage of LA-MRSA is ST9; while in Europe and North America, ST398 isolates are most prevalent.² Notably, there is increasing emergence of outbreaks of LA-MRSA in hospitals and invasive LA-MRSA infections in humans.³ Therefore, LA-MRSA has become an important public health issue that warrants close monitoring.

More and more evidence revealed that non-therapeutic antimicrobial use and overcrowding in animal farming may facilitate the spread of LA-MRSA.^{4,5} Additionally, recent reports found that LA-MRSA could colonize in multiple animals and related workers.^{2,6–9} Notably, MRSA epidemiology in livestock is increasing and the resulting food products may become contaminated through the MRSA-positive livestock, polluted environment, and related workers.^{10–12} Foodstuffs contaminated by MRSA have been found recently, suggesting the possibility of spread to humans through consumption and meat handling.^{13–15} Therefore, there is a growing concern that MRSA of animal origin may be transmitted by the food chain or contact with colonized animals. It is noteworthy that specific markers for *S. aureus* species association are still uncertain. Although some studies have identified LA-MRSA from farm and slaughterhouse animals in Asia,² there is still little evidence of potential genetic markers for livestock association of MRSA isolates, and not much is known about potential relationships between molecular typing and phenotype–genotype characteristics of MRSA isolates using statistical testing methods. Therefore, we undertook a cross-sectional study in pig farms and slaughterhouses to sample pigs for *S. aureus* analysis. This study aimed to examine the carriage prevalence, antimicrobial susceptibility, virulence genes, and molecular typing of *S. aureus* isolates. Additionally, this study builds on previous research to explore the high-dimensional relationships between molecular typing and phenotype–genotype characteristics of *S. aureus* isolates using correspondence analysis.

Methods

Study design and sample collection

This cross-sectional study was conducted from May to July 2015 in Jiangmen, China. The target population was pigs, which were sampled by the method of multistage sampling process. First, 3 county-level cities were randomly sampled from 7 county-level city in Jiangmen city. Second, in each county-level city, 3 pig farms and 1 slaughterhouse were selected to reach a sample size of about 140 farm pigs and 350 slaughterhouse pigs. In all, 1,458 pigs were sampled in this survey, including 411 pigs from 9 pig farms and 1,047

pigs from 3 slaughterhouses. Of these, 194 were piglets (<3 months) and 1,264 were grower pigs (≥3 months). Non-duplicate swabs were obtained from both nares of each pig.

Bacterial isolation and identification

Swabs were soaked into 5-mL enrichment broth (0.25% yeast extract, 1% mannitol, 1% tryptone, and 7.5% NaCl) at 4°C during transportation, and incubated at 35°C ± 1°C for 24 hours. Then a loopful of the broth was plated onto mannitol salt agar and incubated at 37°C for 24–48 hours. Suspected colonies were selected and subcultured to 5% sheep blood agar plates and incubated at 35°C ± 1°C overnight. *S. aureus* or MRSA isolates were confirmed by a combination of morphology, Gram staining, catalase test, tube coagulase test, DNase test, and polymerase chain reaction (PCR) assays for the carriage of the staphylococci 16S rRNA, *nuc* and *mecA* (or *mecC*) genes.^{16,17} All *S. aureus* isolates carried the 16S rRNA and *nuc* genes, and all MRSA isolates carried the 16S rRNA, *nuc* and *mecA* (or *mecC*) genes.

Antimicrobial susceptibility testing and resistant genes

Antimicrobial susceptibility testing was performed by the disk diffusion method according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI, 2015).¹⁸ Susceptibility was tested to the following antibiotic disks: cefoxitin (30 µg), erythromycin (15 µg), clindamycin (2 µg), tetracycline (30 µg), trimethoprim-sulfamethoxazole (25 µg), rifampin (5 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), gentamicin (10 µg), quinupristin-dalfopristin (15 µg), and linezolid (30 µg). According to the CLSI guidelines, *S. aureus* isolates were classified as susceptible, intermediate, or resistant to each antibiotic. Multidrug-resistant (MDR) *S. aureus* (MDRSA) was defined as being non-susceptible to ≥1 agent in ≥3 antimicrobial categories, extensively drug-resistant (XDR) *S. aureus* was defined as being non-susceptible to ≥1 agent in all but ≤2 categories, and pan-drug-resistant (PDR) *S. aureus* was defined as being non-susceptible to all antimicrobial agents listed.¹⁹ For all *S. aureus* isolates, the presence of erythromycin-resistant genes (*erm*[A] and *erm*[C]) and tetracycline-resistant genes (*tet*[M] and *tet*[K]) was determined by PCR tests described previously.²⁰ For linezolid-resistant isolates, the presence of resistant genes (*cfr* and *optrA*) was determined by PCR tests.²¹

Molecular characterization

For all *S. aureus* isolates, multilocus sequence typing (MLST) was performed as previously described.²² Alleles and

sequence types (STs) were assigned by submitting the DNA sequences to the MLST database (<http://saureus.mlst.net>). BURST analysis was conducted to analyze clonal complexes (CCs) using eBURST V3 software program (Department of Infectious Disease Epidemiology, Imperial College London, London, UK; <http://eburst.mlst.net>). We conducted specific PCR tests for the presence of the Pantone–Valentine leucocidin (*pvl*) toxin gene, the immune evasion cluster (IEC) genes (*scn*, *chp*, *sea*, *sak*, and *sep*), the beta-hemolysin gene (*hly*) and the staphylococcal cassette chromosome *mec* element (SCC*mec*) type.^{23–25}

Data analysis

The differences in *S. aureus* (including MRSA and MDRSA) carriage between groups were determined using the Pearson's chi-squared test or Fisher's exact test when appropriate. Since correspondence analysis provides a useful graphic and statistical method for exploring the internal relationship between categorical variables, we used the correspondence analysis to explore potential relationships between CC and phenotype–genotype characteristics of *S. aureus* isolates. These analyses were performed using STATA version 14.0 (StataCorp LP, College Station, TX, USA), and a 2-sided *P* value of <0.05 was defined as being of statistical significance.

Ethics statement

Ethics approval for the study was obtained from the Ethics Committee of Guangdong Pharmaceutical University, Guangzhou, China.

Results

S. aureus, MRSA, and MDRSA detection in pigs

Of 1,458 pigs sampled in this survey (Table 1), 139 (9.5%) carried *S. aureus*, including 135 (9.3%) MDRSA and 48

(3.3%) MRSA isolates. All MRSA isolates carried the *mecA* gene, but all these isolates were absent of the *mecC* (the novel *mecA* homolog). When comparing the carriage rates between farm and slaughterhouse pigs, there were statistically significant differences in the carriage of *S. aureus* (6.8% vs 10.6%; *P*=0.027) and MDRSA (6.8% vs 10.2%; *P*=0.043). When comparing the carriage rates between piglets and grower pigs, there were statistically significant differences in the carriage of *S. aureus* (4.1% vs 10.4%; *P*=0.006).

Antibiotic susceptibility and resistance genes

Among 139 *S. aureus* isolates, most of isolates were resistant to clindamycin (97.1%), tetracycline (96.4%), erythromycin (92.8%), and trimethoprim-sulfamethoxazole (82.7%) (Table 2). Notably, 87 isolates were resistant to ceftiofur, including 48 MRSA and 39 non-MRSA isolates. Additionally, 135 (97.1%) isolates were classified as MDRSA, with the most common resistance pattern being non-susceptible to clindamycin, tetracycline, and erythromycin (Figure 1). In terms of the macrolide-resistant genes (Table 2), the most predominant gene was *erm(C)* (79.1%), followed by *erm(A)* (5.0%). Additionally, there were 6 (4.3%) isolates co-expressing *erm(C)* and *erm(A)*. In terms of the tetracycline-resistant genes, 87 (62.6%) carried the *tet(K)* and 81 (58.3%) carried the *tet(M)*. In addition, 51 (36.7%) isolates co-expressed the *tet(K)* and *tet(M)*. Notably, all linezolid-resistant isolates (9 isolates) were classified as MDRSA, including 1 expressing the *cfr* gene and 6 expressing the *optrA* gene.

Molecular characteristics

Among 139 *S. aureus* isolates (Table 3), we observed 40 unique STs belonging to 11 CCs. The most common *S. aureus* CCs were CC9 (73 isolates), CC1 (17 isolates), and CC5 (15 isolates), with the predominant MRSA being CC9 (28

Table 1 Prevalence of *S. aureus*, MRSA, and MDRSA among 1,458 pigs in Jiangmen, China

Characteristics	No. of samples (%)	<i>S. aureus</i>		MRSA		MDRSA	
		<i>n</i> ₁ (%)	<i>P</i> -value	<i>n</i> ₂ (%)	<i>P</i> -value	<i>n</i> ₃ (%)	<i>P</i> -value
Total	1,458 (100.0)	139 (9.5)		48 (3.3)		135 (9.3%)	
Sample type							
Farm pigs	411 (28.2)	28 (6.8)	0.027	17 (4.1)	0.258	28 (6.8)	0.043
Slaughterhouse pigs	1,047 (71.8)	111 (10.6)		31 (3.0)		107 (10.2)	
Age group							
Piglets (<3 months)	194 (13.3)	8 (4.1)	0.006	5 (2.6)	0.549	8 (4.1)	0.215
Grower pigs (≥3 months)	1,264 (86.7)	131 (10.4)		43 (3.4)		127 (6.4)	

Abbreviations: *S. aureus*, *Staphylococcus aureus*; MRSA, methicillin-resistant *S. aureus*; MDRSA, multidrug-resistant *S. aureus*; *n*₁, number of *S. aureus* isolates; *n*₂, number of MRSA isolates; *n*₃, number of MDRSA isolates.

Table 2 Antibiotic susceptibility and resistance genes of 139 *Staphylococcus aureus* isolates

Phenotype (resistance)	Genotype (positive)	No. of positive isolates (%)
Clindamycin		135 (97.1)
Tetracycline		134 (96.4)
	tet(M)	81 (58.3)
	tet(K)	87 (62.6)
Erythromycin		129 (92.8)
	erm(A)	7 (5.0)
	erm(C)	110 (79.1)
Trimethoprim-sulfamethoxazole		115 (82.7)
Gentamicin		65 (46.8)
Chloramphenicol		87 (62.6)
Cefoxitin		87 (62.6)
Rifampin		51 (36.7)
Ciprofloxacin		47 (33.8)
Quinupristin-dalfopristin		17 (12.2)
Linezolid		9 (6.5)

isolates) and CC1 (10 isolates). The most common *S. aureus* STs were ST9 (33 isolates), ST2931 (13 isolates), ST920 (12 isolates), ST2454 (11 isolates), and ST1 (8 isolates). Among 48 MRSA isolates, the predominant STs were ST9 (19 isolates, including 12 isolates for SCCmec IV, 5 for non-types I–VII, 1 for SCCmec III, and 1 for SCCmec V) and ST1 (5 isolates, including 2 isolates for SCCmec IV, 2 for non-types I–VII, and 1 for SCCmec V). As to the IEC genes, 112 (80.6%) isolates expressed the *sak*, followed by the *chp* (60.4%), *sea* (15.8%), and *sep* (8.6%). Notably, *scn* gene was absent in all the *S. aureus* isolates. Additionally, *hly* was present in 82 (59.0%) isolates, but *pvl* was only found in 1 isolate.

Relationships between predominant CCs and phenotype–genotype characteristics

The correspondence analysis showed good corresponding relationships between CC typing and resistance pattern of *S. aureus* isolates (chi-squared=54.98, $P<0.001$; Figure 2A). For example, CC398 was associated with PDR, CC9 with XDR, and CC1/CC5/CC8/CC12/CC97 isolates were associated with MDR. As shown in Table 3, CC398 was associated with nonsusceptibility to almost all these antibiotics; CC9 (including ST9, ST2931, and ST2454) was associated with nonsusceptibility to 9 antibiotics; CC1 (including ST1) and CC5 (including ST920) were associated with nonsusceptibility to 7 antibiotics; and CC8/CC12/CC97 isolates were nonsusceptible to 4 or 5 antibiotics.

Another correspondence analysis revealed good corresponding relationships between CC typing and the number of

virulence genes (including *scn*, *chp*, *sea*, *sak*, *sep*, and *hly*) of *S. aureus* isolates (chi-squared=38.03, $P=0.004$; Figure 2B). For example, CC9 and CC8 were associated with carrying 3 virulence genes, CC1/CC5/CC12/CC97 were associated with carrying 1 or 2 virulence genes, and CC398 was associated with absence of these virulence genes. As shown in Table 3, CC9 (including ST9, ST2931, and ST2454) and CC8 were associated with carrying *chp*, *sak*, and *hly*; CC1 was associated with carrying *sak* and *hly*; and CC5 (including ST920), CC12 and CC97 were associated with carrying *chp* and *sak*.

Discussion

This study of pigs indicated that the carriage rate was 9.5% for *S. aureus* and 3.3% for MRSA, which is similar to results from previous studies (4.6%–15.9% for *S. aureus* and 0.9%–7.7% for MRSA).^{9,26–31} However, disparity results also have been reported. For example, *S. aureus* carriage rate was significant high in India (71.4%) and Ireland (26%–73%),^{32,33} and MRSA carriage rate was significant high in Germany (49%–70.8%) and Spain (46%).^{34,35} There may be several reasons for this difference, such as geographical location, sampling methods, bacterial isolation and identification methods (conventional biochemical methods or PCR test; enrichment or no enrichment), livestock density, and antibiotic usage. In this study, *S. aureus* (including MDRSA) prevalence rate in slaughterhouse pigs was significantly higher than that observed in farm pigs. Similarly, a particularly high MRSA carriage rate was reported in slaughterhouse pigs in Shanghai and Spanish,^{36,37} which may be explained by co-transportation with MRSA-colonized animals.³⁸ Additionally, we observed that the prevalence of *S. aureus* carriage in grower pigs was higher than piglets, which is consistent with the report from Trinidad.²⁷ This situation may be due to the abuse of antimicrobial agents, and grower pigs may have been exposed to antibiotics for much longer than piglets.

The antimicrobial resistance of animal *S. aureus* and MRSA has become an important public health issue. We observed high rates of resistance to erythromycin, tetracycline, and clindamycin among *S. aureus* isolates, which may be due to the abuse of antimicrobial agents in animals in China.³⁹ Similarly, recent reports on animal-related MRSA in China, Portugal, and Japan showed that most isolates were resistant to clindamycin (88%–100%), tetracycline (47%–100%), and erythromycin (96%–100%).^{29,40,41} Notably, in this study, about 97.1% isolates were MDR. A study in Hongkong demonstrated that almost all of livestock *S. aureus* were MDR, suggesting that antimicrobial abuse and overcrowding in animal farming may facilitate the spread of

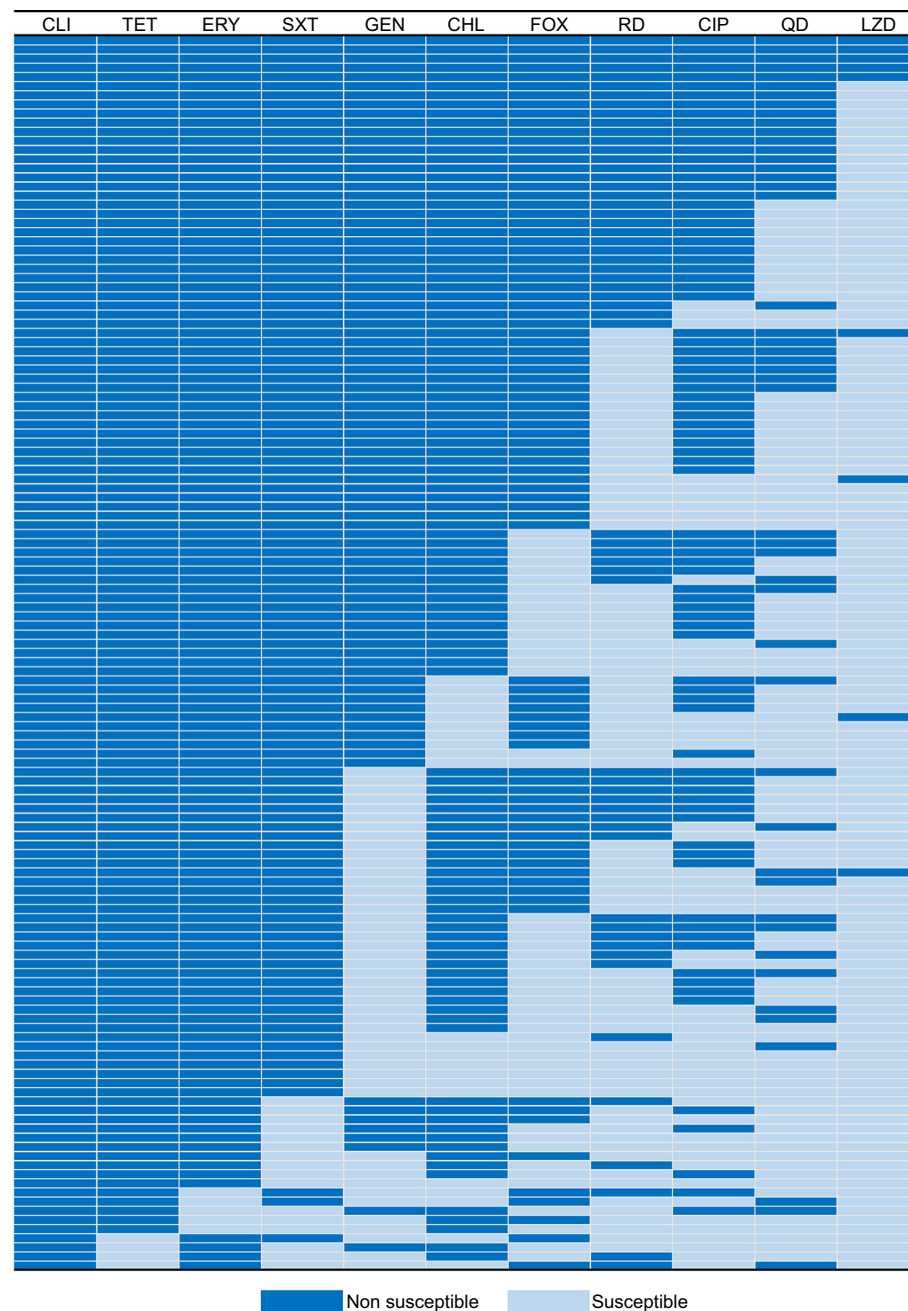


Figure 1 Heat map showing antibiotic resistance profiles of all multidrug-resistant *S. aureus* isolates.

Abbreviations: CLI, clindamycin; TET, tetracycline; ERY, erythromycin; SXT, trimethoprim-sulfamethoxazole; GEN, gentamicin; CHL, chloramphenicol; FOX, ceftioxin; RD, rifampin; CIP, ciprofloxacin; QD, quinupristin-dalfopristin; LZD, linezolid; *S. aureus*, *Staphylococcus aureus*.

MDRSA isolates.⁴² The most predominant resistance pattern observed in this study was co-nonsusceptible to clindamycin, tetracycline, and erythromycin, which is similar to the findings from China and USA.^{8,28} Notably, we observed that all linezolid-resistant isolates were classified as MDRSA, including 1 expressing the multi-resistance *cfr* gene. Similarly, the latest studies in China and Germany reported the emergence of *cfr*-mediated multi-resistance in staphylococci from animals and livestock-related humans.^{43,44} In contrast

to *cfr*, a novel *optrA* gene confers cross-resistance only to oxazolidinones and phenicols. Presence of the *optrA* gene has been detected in *Staphylococcus sciuri* and *Enterococcus* of human and animal origin,^{21,45} and we also found 6 linezolid-resistant isolates expressing the *optrA* gene. Therefore, both linezolid-resistant and MDR isolates must be monitored in prospective surveillance programs.

LA-MRSA has been reported worldwide. In Asia, of particular interest is livestock-associated CC9. The present

Table 3 Relationships between predominant CCs/STs and phenotype-genotype characteristics of *S. aureus* isolates

CC/ST(n ^a)	Resistance phenotypes (non-susceptible)													Resistance genes (positive)				Virulence genes (positive)					
	GEN	CIP	SXT	FOX	TET	QD	ERY	LZD	CHL	RD	CLI	tet (M)	tet (K)	erm(A)	erm(C)	scn	chp	sea	sak	sep	hib		
CC1(17)	9	3	10	11	16	3	14	2	14	2	17	11	13	1	11	0	9	5	13	0	12		
ST1(8)	4	2	4	5	7	2	7	1	7	2	8	5	5	1	6	0	7	3	5	0	7		
CC5(15)	9	9	12	5	13	2	13	1	13	6	15	6	10	1	10	0	11	0	11	0	2		
ST920(12)	7	7	10	3	10	2	11	1	10	5	12	5	8	0	8	0	10	0	8	0	0		
CC9(73)	50	51	68	49	73	27	71	1	64	37	73	45	38	0	64	0	40	17	60	6	56		
ST9(33)	26	27	31	25	33	13	32	1	30	15	33	17	10	0	30	0	17	15	26	2	27		
ST2931(13)	7	8	13	8	13	4	13	0	11	6	13	10	9	0	11	0	7	0	10	1	9		
ST2454(11)	5	8	10	8	11	4	10	0	10	5	11	8	8	0	10	0	8	0	9	0	6		
CC398(2)	2	2	2	2	2	2	2	1	2	1	2	2	1	1	2	0	1	0	1	1	1		
C97(3)	1	1	2	1	2	1	3	0	1	1	3	1	3	0	1	0	2	0	2	0	1		
CC12(4)	2	1	3	1	4	1	4	1	3	1	4	3	3	2	3	0	3	0	2	0	1		
CC8(2)	1	1	2	2	2	0	2	0	1	0	2	2	2	0	2	0	2	0	2	0	1		

Notes: Values are expressed as number of isolates. ^aNumber of *S. aureus* isolates.
Abbreviations: CC, clonal complexes; ST, sequence types; GEN, gentamicin; CIP, ciprofloxacin; SXT, trimethoprim-sulfamethoxazole; FOX, ceftiofur; TET, tetracycline; QD, quinupristin-dalfopristin; ERY, erythromycin; LZD, linezolid; CHL, chloramphenicol; RD, rifampin; CLI, clindamycin; S. aureus, *Staphylococcus aureus*.

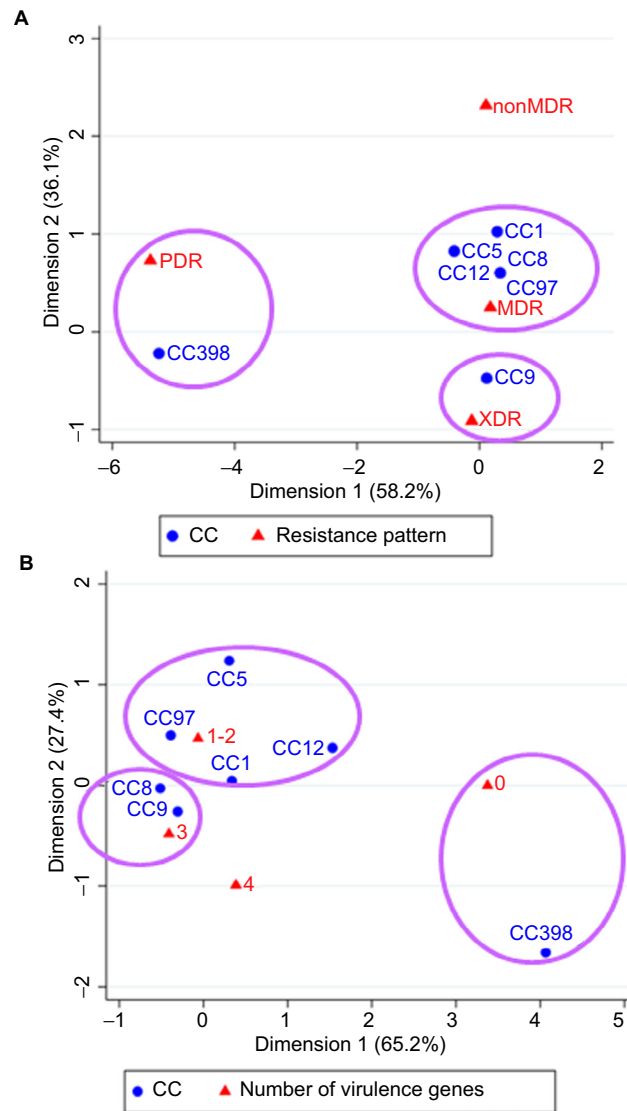


Figure 2 Correspondence analysis for the relationship between CC and resistance pattern (A) or number of virulence genes (B) of *S. aureus* isolates.
Abbreviations: PDR, pan-drug-resistant; MDR, multidrug-resistant; XDR, extensively drug-resistant; CC, clonal complex; *S. aureus*, *Staphylococcus aureus*.

study revealed that the predominant genotype of *S. aureus* and MRSA isolates was CC9, including ST9, ST2931, and ST2454. MRSA ST9 was first detected from pig finishing holdings in Italy⁴⁶ and has also been reported to predominate in pigs in Asian countries.^{9,30,47,48} Additionally, LA-MRSA CC9 can colonize in retail meat and other host species (including cows, sheep, and poultry).^{2,29,49,50} Notably, previous studies revealed that pig workers carried LA-MRSA ST9 and patients without contact with animals were also tested with MRSA ST9, suggesting the potential LA-MRSA transmission from animals to humans by direct and indirect animal exposure.^{8,51} Notably, LA-MRSA CC9 isolates express

different types of SCC*mec*, including type IX for Thailand, type V for Malaysia, types IVb/V for Hong Kong, and type III for China.² However, MRSA ST9 in this study mainly carried SCC*mec* IV, followed by non-types I–VII. These findings show some regional characteristics in LA-MRSA ST9.

Another important LA-MRSA is CC398, which prevails in animals and related workers in North America and European countries.^{6,52} In our study, no MRSA CC398 was found, but 2 MSSA CC398 isolates were isolated from pigs. Although CC398 isolates are rare in Asia, the study from South Korea found a high rate of ST398 isolated from breeding pigs since these pigs were imported from other countries.² Besides, CC5 and CC97 have been identified in this study, which were also reported as LA-MRSA in Germany and Senegal.^{11,26} Therefore, MRSA-carrying animals pose a potential threat to human health. Surprisingly, ST1 and ST59 as the typical human-associated MRSA clones were detected in pigs in this study, which is consistent with the reports in Africa and China.^{29,53} These findings may add to the evidence of inter-species MRSA transmission between humans and animals.

It should be noted that a few studies have attempted to explore the specific markers for *S. aureus* species association. Recent evidence of European–American CC398 has revealed that absence of the bacteriophage-encoded IEC genes may be associated with animal specificity and presence of IEC genes may be related with human specificity.^{54,55} This animal-specificity relation for Asian CCs is still uncertain. The present study first revealed that all *S. aureus* were absence of *scn* gene, suggesting that absence of *scn* may be associated with pig specificity. Additionally, susceptibility to tetracycline was observed only in isolates from workers without animal contact, but tetracycline resistance was only found in CC9 from animals and animal-related workers.⁸ Similarly, we observed that all pig *S. aureus* (including MRSA) isolates were resistant to tetracycline, indicating that phenotypic resistance to tetracycline may be associated with pig specificity.

The potential relationship between CCs and phenotype–genotype characteristics of *S. aureus* is still unclear. Recent evidence is limited to use the statistical description to explore potential relations between molecular characteristics. For example, the latest studies of LA-MRSA indicated that all MRSA CC97 isolates were MDR and about 90% of MRSA ST9 isolates were MDR.^{56,57} These findings suggest that there may be differences in resistance patterns between different clones. This study adds to existing literature to reveal a significant correspondence between CC and resistance pattern of *S. aureus* isolates, indicating that LA CC9 and

CC398 were associated with severe XDR or PDR. Notably, latest studies found that absence of IEC genes may be associated with animal specificity.^{8,58} For example, all MDRSA CC9 and MRSA CC9 carried by pig-related workers were absent of IEC genes.⁸ This study first revealed a significant correspondence between CCs and the number of virulence genes of *S. aureus* isolates; CC9/CC8 isolates were associated with carrying 3 virulence genes (*chp*, *sak*, and *hly*) and CC5/CC12/CC97 isolates with carrying 2 virulence genes (*chp* and *sak*). These findings provide new ideas for monitoring new epidemiologic trends and provide implication for facilitating vaccine developments.

This study is a new attempt to reveal potential relationships between CC typing and phenotype–genotype characteristics of *S. aureus* isolates using the correspondence analysis. However, the current study has some limitations. First, the research design was cross-sectional, so it could not determine whether the nasal colonization was persistent or transient. Future longitudinal study may reveal more information about colonization dynamics of *S. aureus*. Second, the present study observed 39 isolates resistant to cefoxitin but absent of the *mecA*, suggesting that the MRSA definition based on *mecA* in this study may underestimate the true prevalence. Third, no nasal samples were obtained from livestock-related workers, so it could not determine whether exists the risk of LA-MRSA transmission from animals to livestock-related workers.

Conclusion

All pig-related *S. aureus* isolates were negative for *scn* gene and resistant to tetracycline, suggesting that absence of *scn* and presence of tetracycline resistance may be associated with pig specificity. Additionally, the correspondence analysis found good corresponding relationships between CC typing and phenotype–genotype characteristics, which provides some implication for vaccine strategies and new ideas for monitoring new epidemiologic trends. Notably, the proportion of MDRSA was high in 97.1%, supporting growing concern about the potential hazards of non-therapeutic antibiotic use.

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Author contributions

All authors contributed toward data analysis, drafting and revising the paper and agreed to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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