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Genotypic and Phenotypic Markers of Livestock-Associated Methicillin-Resistant *Staphylococcus aureus* CC9 in Humans

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ABSTRACT

Use of antimicrobials in industrial food animal production is associated with the presence of multidrug-resistant *Staphylococcus aureus* among animals and humans. The livestock-associated (LA) methicillin-resistant *S. aureus* (MRSA) clonal complex 9 (CC9) is associated with animals and related workers in Asia. This study aimed to explore the genotypic and phenotypic markers of LA-MRSA CC9 in humans. We conducted a cross-sectional study of livestock workers and controls in Guangdong, China. The study participants responded to a questionnaire and provided a nasal swab for *S. aureus* analysis. The resulting isolates were assessed for antibiotic susceptibility, multilocus sequence type, and immune evasion cluster (IEC) genes. Livestock workers had significantly higher rates of *S. aureus* CC9 (odds ratio [OR] = 30.98; 95% confidence interval [CI], 4.06 to 236.39) and tetracycline-resistant *S. aureus* (OR = 3.26; 95% CI, 2.12 to 5.00) carriage than controls. All 19 *S. aureus* CC9 isolates from livestock workers were MRSA isolates and also exhibited the characteristics of resistance to several classes of antibiotics and absence of the IEC genes. Notably, the interaction analyses indicated phenotype-phenotype (OR = 525.7; 95% CI, 60.0 to 4,602.1) and gene-environment (OR = 232.3; 95% CI, 28.7 to 1,876.7) interactions associated with increased risk for livestock-associated *S. aureus* CC9 carriage. These findings suggest that livestock-associated *S. aureus* and MRSA (CC9, IEC negative, and tetracycline resistant) in humans are associated with occupational livestock contact, raising questions about the potential for occupational exposure to opportunistic *S. aureus*.

IMPORTANCE

This study adds to existing knowledge by giving insight into the genotypic and phenotypic markers of LA-MRSA. Our findings suggest that livestock-associated *S. aureus* and MRSA (CC9, IEC negative, and tetracycline resistant) in humans are associated with occupational livestock contact. Future studies should direct more attention to exploring the exact transmission routes and establishing measures to prevent the spread of LA-MRSA.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the leading causes of antibiotic-resistant nosocomial infections, causing diseases ranging from minor skin infections to severe septicemia and pneumonia, and is of particular concern because few antibiotics are effective at treating infections caused by the pathogen. The epidemiology of MRSA has changed with the increasing emergence of community-associated MRSA (1, 2). Recently, another MRSA clone emerged in the community, which was observed in livestock and related workers and was referred to as livestock-associated MRSA (LA-MRSA) (3). Livestock, especially pigs, can serve as reservoirs for LA-MRSA, and the bacteria can also be transmitted to humans in close contact with MRSA-colonized animals (4, 5).

LA-MRSA isolates have unique molecular characteristics that distinguish them from community-associated MRSA and health care-associated MRSA, and these characteristics vary according to the geographic area. Sequence type 398 (ST398) has been referred to as the most pandemic LA-MRSA in Europe and North America, while ST9 is the most prevalent LA-MRSA in most Asian countries (3). However, persons living in areas of high livestock density were also found to have a greater risk of LA-MRSA carriage even if they lacked direct contact with livestock (6, 7). Thus, the possibility of direct and indirect livestock contact as a potential source of human MRSA infection has become a growing public health concern.

Few reports have described the epidemiology and molecular characteristics of LA-MRSA in developing countries in Asia. In China, MRSA has been isolated from pigs and pig workers (8, 9). However, there is still very limited information on LA-MRSA infection among healthy people. In addition, few studies examining human MRSA carriage have attempted to differentiate human- from livestock-associated isolates based on genotypic and phenotypic markers. The goals of this study, therefore, were to determine the prevalence of MRSA (including LA-MRSA) in livestock workers and control workers in Guangdong, as well as to use the multifactor dimensionality reduction method to detect the genotypic and phenotypic markers for LA-MRSA.

MATERIALS AND METHODS

Ethics statement. This study was approved by the Ethics Committee of Guangdong Pharmaceutical University, and it was performed in accordance with the approved guidelines. All study participants signed an informed consent form.

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TABLE 1 Odds ratios for *S. aureus* carriage according to occupational livestock contact stratified by types of livestock in Guangdong, China

Source of contact[a]	*n*	*S. aureus* CC9		TRSA	
		Unadjusted OR (95% CI)	Adjusted[b] OR (95% CI)	Unadjusted OR (95% CI)	Adjusted[b] OR (95% CI)
Livestock					
No	1,178	1.00	1.00	1.00	1.00
Yes	682	33.73 (4.50–252.53)	30.98 (4.06–236.39)	3.02 (2.01–4.56)	3.26 (2.12–5.00)
Pigs					
No	1,178	1.00	1.00	1.00	1.00
Yes	591	39.10 (5.22–292.77)	35.98 (4.69–276.18)	3.18 (2.09–4.83)	3.42 (2.20–5.32)
Poultry					
No	1,178	1.00	1.00	1.00	1.00
Yes	45	–[c]	–	2.85 (0.97–8.35)	2.73 (0.92–8.11)
Other animals					
No	1,178	1.00	1.00	1.00	1.00
Yes	45	–	–	1.36 (0.32–5.81)	1.77 (0.40–7.78)

[a] No, no occupational contact with any type of livestock.
[b] Adjusted for gender, age (15 to 24, 25 to 34, 35 to 44, and 45 to 60 years), antimicrobial use in the previous month, and hospitalization in the previous month.
[c] –, no estimate is provided due to lack of occurrence of the outcome of interest in the two groups.

Study design and population. A cross-sectional study was conducted between November 2013 and November 2014 in Guangdong Province, China. The methods of this survey have been described in detail previously (10). Briefly, a multistage sample design was employed to obtain an independent, representative sample, including workers with occupational livestock contact (i.e., farm workers, veterinarians, slaughterhouse workers, and butchers) and control workers with no occupational livestock contact (i.e., workers from the hardware factory or the biscuit factory). After obtaining informed consent, a face-to-face questionnaire was administered to collect information about sex, age, etc.

Bacterial strains. Two nasal swabs were taken from each participant. The swabs were enriched in enrichment broth with 7.5% NaCl at 35 ± 1°C for 24 h and then streaked onto mannitol salt agar and incubated at 37°C for 24 h. From each plate, one representative colony of each different suspected morphology was selected and purified on 5% sheep blood agar plates and incubated at 35°C overnight. Presumptive *S. aureus* colonies were confirmed by colony morphology, Gram staining, catalase test, DNase test, coagulase tests, and PCR for 16S rRNA and *nuc* and *mecA* genes (11).

Antibiotic susceptibility test. All *S. aureus* isolates were assessed for susceptibility to a panel of 11 antibiotics: cefoxitin, clindamycin, tetracycline, erythromycin, ciprofloxacin, rifampin, chloramphenicol, gentamicin, trimethoprim-sulfamethoxazole (SXT), linezolid, and nitrofurantoin. The Kirby-Bauer disk diffusion method was used to test susceptibility to all the antibiotics, and diameter interpretations were based on the protocol of the Clinical and Laboratory Standards Institute (CLSI) 2013 guidelines (12). Quality control was achieved with *S. aureus* ATCC 25923 simultaneously. *S. aureus* isolates with zone sizes of less than 21 mm for cefoxitin were identified as suspect MRSA and further tested through PCR for the *mecA* gene.

Molecular characterization. We performed PCR tests targeting the immune evasion cluster (IEC) genes (*scn*, *chp*, *sak*, and *sep*), the Panton-Valentine leukocidin (*pvl*) toxin gene, and the staphylococcal cassette chromosome *mec* element (SCC*mec*) type, using previously described primers (13–15). Multilocus sequence typing (MLST) of the seven housekeeping genes was conducted using previously described primers and protocols (16). The sequence type (ST) for each isolate was determined by comparing the sequence obtained to known alleles at each locus in the MLST database (http://saureus.mlst.net), and clonal complexes (CCs) were determined using the eBURST algorithm (accessible at http://eburst.mlst.net) and the stringent group definition (6/7 shared alleles) (17).

Data analysis. Categorical variables were compared by Pearson's chi-squared test or the Fisher exact test when appropriate. The relationships between livestock exposure and *S. aureus* CC9 and tetracycline-resistant *S. aureus* (TRSA) carriage were examined using univariable and multivariable logistic regression models. These analyses were performed using STATA version 13.0 (StataCorp LP, College Station, TX, USA), and a two-sided *P* value for statistical significance was defined as a *P* value of ≤0.05. The open-source multifactor dimensionality reduction (MDR) software (version 2.0, beta 8.1; Computational Genetics Laboratory) was used to assess potential interactions between genotypic and phenotypic markers with statistically significant impacts on livestock-associated *S. aureus* CC9. The fitness of an MDR model was estimated by determining the testing balanced accuracy (TBA) and its cross-validation consistency (CVC). The single best model normally has the maximal TBA and CVC.

RESULTS

Study population. We enrolled 1,860 participants. Of those, 682 had occupational contact with livestock (defined as livestock workers) and 1,178 were control workers with no occupational livestock contact. There were statistically significant differences between the two groups with regard to sex ($\chi^2 = 103.16$; $P < 0.001$) and age ($\chi^2 = 22.40$; $P < 0.001$), and these discrepancies were adjusted by applying the multivariable logistic regression models.

Overall, 36.7% (682/1,860) of the participants reported occupational exposure to livestock. Specifically, 31.8% (591/1,860), 2.4% (45/1,860), and 2.4% (45/1,860) reported exposure to pigs, poultry, and other animals, respectively (Table 1). Of 1,860 participants, 200 (10.8%; 95% CI, 9.4% to 12.2%) carried *S. aureus*, 64 (3.4%; 95% CI, 2.7% to 4.4%) carried MRSA, 103 (5.5%; 95% CI, 4.5% to 6.7%) carried TRSA, and 20 (1.1%; 95% CI, 0.7% to 1.7%) carried *S. aureus* CC9.

Relationships between livestock exposure and *S. aureus* CC9 and TRSA carriage. In contrast to unexposed individuals, those with occupational exposure to any type of livestock had significantly higher rates of *S. aureus* CC9 (OR = 30.98; 95% CI, 4.06 to 236.39) and TRSA (OR = 3.26; 95% CI, 2.12 to 5.00) carriage (Table 1). Similar positive associations were observed among

TABLE 2 STs of *S. aureus* and MRSA isolates in different groups in Guangdong, China

Resource[a]	CC	ST[b]	No. with indicated bacterial isolate			
			S. aureus		MRSA	
			Controls ($n = 109$)	Livestock workers ($n = 91$)	Controls ($n = 16$)	Livestock workers ($n = 48$)
LA	CC9	ST9	0	16	0	16
LA	CC9	ST27	0	1	0	1
LA	CC9	ST63	0	1	0	1
LA	CC9	ST2359	1	1	0	1
LA	CC398	ST398	3	1	0	0
HA	CC6	ST6	17	10	1	3
HA	CC7	ST7	24	18	7	5
HA	CC7	ST943	0	1	0	0
HA	CC59	ST59	10	11	2	7
HA	CC59	ST338	0	1	0	1
HA	CC59	ST951	2	0	1	0
HA	CC188	ST188	15	5	1	1
HA	CC1	ST1	5	1	1	0
HA	CC1	ST2125	0	1	0	0
HA	CC1	ST2518	0	1	0	1
HA	CC5	ST5	3	1	1	0
HA	CC5	ST1863	1	0	0	0
HA	CC8	ST8	1	0	0	0
HA	CC10	ST10	2	1	1	0
HA	CC15	ST15	3	4	0	2
HA	CC22	ST22	0	1	0	0
HA	CC22	ST217	2	0	0	0
HA	CC45	ST45	3	5	0	5
HA	CC72	ST72	2	1	0	0
HA	CC88	ST88	0	4	0	2
HA	CC88	ST95	3	0	0	0
HA	CC182	ST944	0	1	0	0
HA	CC509	ST1985	1	0	0	0
HA	CC1719	ST2238	1	0	0	0
HA	CC2483	ST2259	5	1	0	0
		UT	5	3	1	2

[a] HA, human associated.
[b] UT, untypeable.

workers with occupational exposure to pigs (for *S. aureus* CC9, OR = 35.98 and 95% CI, 4.69 to 276.18; for TRSA, OR = 3.42 and 95% CI, 2.20 to 5.32). However, there were no significant differences in *S. aureus* CC9 and TRSA carriage in terms of occupational exposure to poultry or other animals.

STs of *S. aureus* and MRSA isolates in different participants. We identified 30 unique STs from 192 *S. aureus* isolates (Table 2). Sequence types for the 8 remaining isolates could not be determined. *S. aureus* isolates from livestock workers demonstrated the greatest ST diversity. ST6, ST7, ST59, and ST188 were common among *S. aureus* isolates from livestock workers and controls. Livestock-associated ST9 was common among livestock workers (17.6%; 16/91) but absent from controls. Notably, four isolates were identified as ST398, including three isolates from controls and one from a livestock worker. Among the MRSA isolates, the predominant sequence type was ST7 for controls (43.8%; 7/16) and ST9 for livestock workers (33.3%; 16/48).

Genotypic and phenotypic markers of livestock association. The most common SCC*mec* type of MRSA CC9 in livestock workers was type IV, followed by type V and untypeable SCC*mec*. The virulence gene *pvl* was absent from CC9 isolates in livestock workers and controls. Absence of the IEC genes was more prevalent among *S. aureus* CC9 than *S. aureus* non-CC9 isolates (*scn* gene, 95% versus 30.8%, $P < 0.001$; *chp* gene, 95% versus 63.4%, $P = 0.005$; *sak* gene, 95% versus 36.1%, $P < 0.001$; *sep* gene, 100.0% versus 82.6%, $P = 0.048$) (Table 3). Absence of the IEC genes (*scn*, *chp*, and *sak*) was also more prevalent among MRSA CC9 than MRSA non-CC9 isolates. All *S. aureus* CC9 isolates detected among livestock workers tested negative for the *scn*, *chp*, *sak*, and *sep* genes, whereas the single *S. aureus* CC9 (ST2359) isolate from controls tested negative only for the *sep* gene (Table 4).

The resistance rates of all the antimicrobial agents were significantly higher in *S. aureus* CC9 isolates than in *S. aureus* non-CC9 isolates (Table 3). Comparing the differences in resistance rates between MRSA CC9 and MRSA non-CC9 isolates, statistical significance was noted for clindamycin, tetracycline, erythromycin, chloramphenicol, ciprofloxacin, trimethoprim-sulfamethoxazole, and gentamicin. All *S. aureus* CC9 isolates detected in livestock workers were resistant to at least seven classes of antibiotics, and the most common pattern of multiple resistance was nonsusceptible to cefoxitin, clindamycin, tetracycline, and erythromycin, whereas the single *S. aureus* CC9 isolate (ST2359) from controls was susceptible to all the antibiotics (Table 4).

Notably, all 19 *S. aureus* CC9 isolates from livestock workers were MRSA isolates and also exhibited the characteristics of resistance to several classes of antibiotics and absence of the IEC genes, indicating an overlap between phenotypic and molecular markers of livestock association (Table 4). No overlap in these characteristics of livestock association was observed for the single *S. aureus* CC9 isolate (ST2359) from controls.

MDR analysis for livestock-associated *S. aureus* prediction. An MDR analysis was carried out to evaluate all possible combinations of the IEC genes, antibiotic resistance phenotypes, and livestock exposures proven to be associated with a risk of livestock-associated *S. aureus* CC9 carriage. As shown in Table 5 and Fig. 1, we observed the highest TBA (0.9576) and CVC (10/10) in the three-factor interaction model, which shows an interaction among phenotypic characteristics (cefoxitin resistant, gentamicin resistant, and SXT resistant). This phenotype-phenotype interaction was associated with a 525-fold-increased risk for livestock-associated *S. aureus* CC9 carriage (95% CI, 60.0 to 4,602.1; $P < 0.001$). To explore the possible gene-environment interactions, we included only the IEC genes and livestock exposure in the interaction model. The four-factor (*scn*, *chp*, and *sak* genes and livestock exposure) interaction model proved to be the most accurate model, with the highest TBA (0.9394) and CVC (10/10). This gene-environment interaction was associated with a 232-fold-increased risk for *S. aureus* CC9 carriage (95% CI, 28.7 to 1,876.7; $P < 0.001$) (Table 5 and Fig. 2).

DISCUSSION

Our study adds to existing knowledge by providing insight into the genotypic and phenotypic markers of livestock-associated *S. aureus* CC9 in humans. The study showed that livestock workers had significantly higher rates of both *S. aureus* CC9 and TRSA carriage than controls. The most striking finding was that all 19 MRSA CC9 isolates from livestock workers exhibited the characteristics of resistance to several classes of antibiotics and absence of the IEC genes, indicating an overlap between genotypic and phenotypic markers of livestock association. The MDR analysis also

TABLE 3 Genotypic and phenotypic characteristics of *S. aureus* and MRSA carriage among study participants in Guangdong, China

Characteristic	No. (%) of isolates with characteristic					
	S. aureus			MRSA		
	Non-CC9 (*n* = 172)	CC9 (*n* = 20)	*P* value[a]	Non-CC9 (*n* = 42)	CC9 (*n* = 19)	*P* value
Phenotypic						
Cefoxitin resistant	42 (24.4)	19 (95.0)	<0.001	42 (100.0)	19 (100.0)	–[b]
Clindamycin resistant	78 (45.4)	19 (95.0)	<0.001	29 (69.1)	19 (100.0)	0.006
Tetracycline resistant	72 (41.9)	19 (95.0)	<0.001	21 (50.0)	19 (100.0)	<0.001
Erythromycin resistant	68 (39.5)	19 (95.0)	<0.001	27 (64.3)	19 (100.0)	0.003
Chloramphenicol resistant	28 (16.3)	15 (75.0)	<0.001	12 (28.6)	15 (79.0)	<0.001
Ciprofloxacin resistant	18 (10.5)	15 (75.0)	<0.001	7 (16.7)	15 (79.0)	<0.001
SXT resistant	16 (9.3)	17 (85.0)	<0.001	6 (14.3)	17 (89.5)	<0.001
Rifampin resistant	14 (8.1)	8 (40.0)	<0.001	8 (19.1)	8 (42.1)	0.069
Gentamicin resistant	3 (1.7)	13 (65.0)	<0.001	1 (2.4)	13 (68.4)	<0.001
Nitrofurantoin resistant	10 (5.8)	5 (25.0)	0.011	5 (11.9)	5 (26.3)	0.261
Linezolid resistant	8 (4.7)	6 (30.0)	0.001	4 (9.5)	6 (31.6)	0.057
Genotypic						
scn negative	53 (30.8)	19 (95.0)	<0.001	17 (40.5)	19 (100.0)	<0.001
chp negative	109 (63.4)	19 (95.0)	0.005	24 (57.1)	19 (100.0)	<0.001
sak negative	62 (36.1)	19 (95.0)	<0.001	22 (52.3)	19 (100.0)	<0.001
sep negative	142 (82.6)	20 (100.0)	0.048	36 (85.7)	19 (100.0)	0.164

[a] The *P* values were calculated with Fisher's exact test.
[b] –, no estimate of the *P* value is provided due to lack of occurrence of the outcome of interest in at least one group.

indicated a phenotype-phenotype interaction and a gene-environment interaction associated with increased risk for *S. aureus* CC9 carriage.

MRSA is a significant pathogen in human and veterinary medicine. Notably, the novel MRSA isolates from livestock have become a growing public health concern. Among livestock workers in our study, the most common *S. aureus* genotypes were ST7, ST9, ST59, and ST6, with ST9 (33.3%; 16/48) as the predominant MRSA ST. Importantly, *S. aureus* ST9 was absent from controls with no occupational livestock contact, and workers with pig exposure were significantly more likely to carry *S. aureus* CC9 than those lacking exposure. Similarly, studies from other countries reported CC9 (ST9 and single-locus variants) as the predominant *S. aureus* and MRSA genotype in pigs and related workers in Asia,

TABLE 4 Genotypic and phenotypic characteristics of livestock-associated *S. aureus* CC9 carried by the study participants in Guangdong, China

Participant category	ST	SCC*mec* type[a]	Gene presence[b]					MRSA	Antibiotic resistance pattern[c]
			pvl	*scn*	*chp*	*sak*	*sep*		
Control (*n* = 1)	ST2359		–	+	+	+	–	No	Susceptible to all antibiotics
Livestock workers (*n* = 19)	ST9	UT	–	–	–	–	–	Yes	FOX-CLI-TET-ERY-RIF-GEN-NIT-LZD
	ST9	IV	–	–	–	–	–	Yes	FOX-CLI-TET-ERY-CIP-SXT-GEN
	ST9	V	–	–	–	–	–	Yes	FOX-CLI-TET-ERY-CIP-SXT-GEN
	ST9	UT	–	–	–	–	–	Yes	FOX-CLI-TET-ERY-CHL-SXT-RIF-NIT-LZD
	ST9	IV	–	–	–	–	–	Yes	FOX-CLI-TET-ERY-CHL-CIP-GEN
	ST9	UT	–	–	–	–	–	Yes	FOX-CLI-TET-ERY-CHL-CIP-SXT-NIT-LZD
	ST9	IV	–	–	–	–	–	Yes	FOX-CLI-TET-ERY-CHL-CIP-SXT-GEN
	ST9	IV	–	–	–	–	–	Yes	FOX-CLI-TET-ERY-CHL-CIP-SXT-GEN
	ST9	V	–	–	–	–	–	Yes	FOX-CLI-TET-ERY-CHL-CIP-SXT-GEN
	ST9	IV	–	–	–	–	–	Yes	FOX-CLI-TET-ERY-CHL-CIP-SXT-GEN-LZD
	ST9	IV	–	–	–	–	–	Yes	FOX-CLI-TET-ERY-CHL-CIP-SXT-RIF-NIT-LZD
	ST9	IV	–	–	–	–	–	Yes	FOX-CLI-TET-ERY-CHL-CIP-SXT-RIF-GEN
	ST9	IV	–	–	–	–	–	Yes	FOX-CLI-TET-ERY-CHL-CIP-SXT-RIF-GEN
	ST9	IV	–	–	–	–	–	Yes	FOX-CLI-TET-ERY-CHL-CIP-SXT-RIF-GEN
	ST9	IV	–	–	–	–	–	Yes	FOX-CLI-TET-ERY-CHL-CIP-SXT-RIF-GEN
	ST9	IV	–	–	–	–	–	Yes	FOX-CLI-TET-ERY-CHL-CIP-SXT-RIF-GEN
	ST27	IV	–	–	–	–	–	Yes	FOX-CLI-TET-ERY-CHL-CIP-SXT-RIF-GEN-NIT-LZD
	ST63	IV	–	–	–	–	–	Yes	FOX-CLI-TET-ERY-CHL-CIP-SXT-GEN
	ST2359	IV	–	–	–	–	–	Yes	FOX- CLI-TET-ERY- CHL-CIP-SXT-RIF

[a] UT, untypeable.
[b] +, positive; –, negative.
[c] FOX; cefoxitin; CLI, clindamycin; TET, tetracycline; ERY, erythromycin; CHL, chloramphenicol; CIP, ciprofloxacin; RIF, rifampin; GEN, gentamicin; NIT, nitrofurantoin; SXT, trimethoprim-sulfamethoxazole; LZD, linezolid.

TABLE 5 MDR analysis for livestock-associated *S. aureus* CC9 prediction

Best model	TBA	CVC	OR (95% CI)	*P* value
Phenotype-phenotype interaction[a]				
SXT	0.8785	10/10	55.3 (14.6–209.0)	<0.001
SXT and GEN	0.9035	9/10	185.3 (23.2–1,476.5)	<0.001
FOX, SXT, and GEN	0.9576	10/10	525.7 (60.0–4,602.1)	<0.001
FOX, CLI, SXT, and GEN	0.9547	9/10	798.0 (84.8–7,511.7)	<0.001
Gene-environment interaction				
scn	0.8209	10/10	42.7 (5.6–327.0)	<0.001
scn and livestock exposure	0.9169	10/10	144.4 (18.3–1,137.8)	<0.001
chp, *sak*, and livestock exposure	0.9198	7/10	198.9 (24.9–1,591.0)	<0.001
scn, *chp*, *sak*, and livestock exposure	0.9394	10/10	232.3 (28.7–1,876.7)	<0.001

[a] Antibiotic resistance.

indicating the potential for transmission of *S. aureus* (including MRSA) between livestock and humans (3, 18). Our recent study also revealed that there are significant frequency-risk and short-term duration–risk relationships between occupational pig contact and MRSA carriage, suggesting the probability of LA-MRSA spread via animal contact, a scenario demonstrated for LA-MRSA transmission in Europe and Asia (10). Rather than CC9, CC398 is the most prevalent LA-MRSA genotype associated with various animals and humans across European countries and North America (19, 20). We did not identify MRSA ST398 (belonging to CC398) in this study, although several cases of ST398 infection in hospitalized patients have been reported in Hong Kong and China (21, 22).

In recent years, much attention has been focused on the role of pet animals as reservoirs of antimicrobial-resistant bacteria and on the potential transfer of resistance genes from pets to humans (23–25). A monitoring study of members of dog-owning households in Spain reported cocarriage of *S. aureus* ST398 in owners and dogs (23), and another investigation in Canada revealed a high prevalence of concurrent MRSA colonization, as well as identifying indistinguishable strains in humans and pet dogs and cats in the same household, suggesting that interspecies transmission of *S. aureus* and MRSA is possible (24). A recent review concluded that available data on MRSA transmission between pet animals and humans are limited and that the public health impact of such transmission needs to be subjected to more detailed epidemiological studies (25). Note that in our study, a methicillin-susceptible *S. aureus* isolate belonging to CC9 (ST2359) was detected in a control worker who contacted pets in homes, but the results do not suggest that pet animals play a role in the transmission process of MRSA CC9. Whether this finding is due to a lack of power, lack of colonization of companion animals, or lack of transmission unfortunately cannot be determined. Therefore, further studies are needed to identify the potential risk of LA-MRSA transmission between pet animals and humans.

In our study, 7 of 11 *S. aureus* ST59 isolates from livestock workers were MRSA, whereas only 2 of 10 *S. aureus* ST59 isolates from controls were MRSA; *S. aureus* ST88 isolates (including MRSA ST88) were found, not in controls, but in livestock workers. Recently, a study from Taiwan found that 2% of 100 pig-related workers carried MRSA ST59 (26), which was consistent with our study. The MRSA ST59 and ST88 clones were also found in chicken samples and handmade food in China (27, 28) but were human associated in previous studies (29, 30), indicating that contamination may originate from poor hygiene of workers during food preparation. In addition, MRSA ST88 clones were found

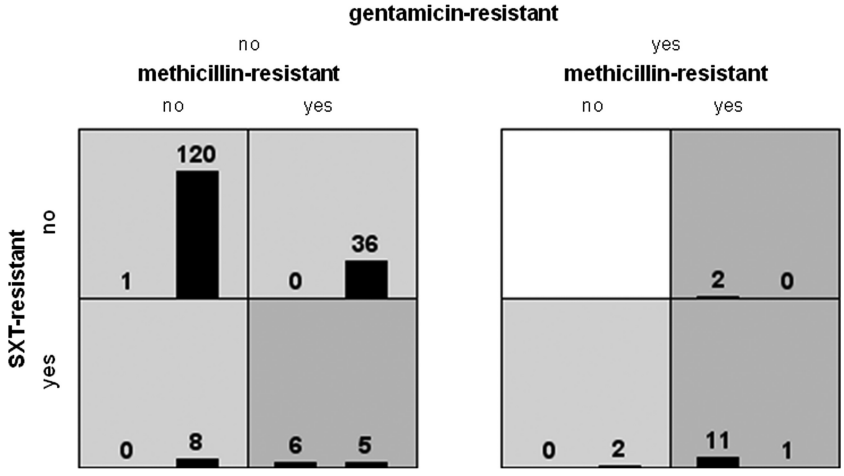

FIG 1 Multifactor dimensionality reduction analysis of the three-factor (methicillin, gentamicin, and SXT) phenotype-phenotype interaction associated with the risk of *S. aureus* CC9 carriage, with the corresponding distribution of *S. aureus* CC9 isolates (left bar in each box) and of *S. aureus* non-CC9 isolates (right bar in each box).

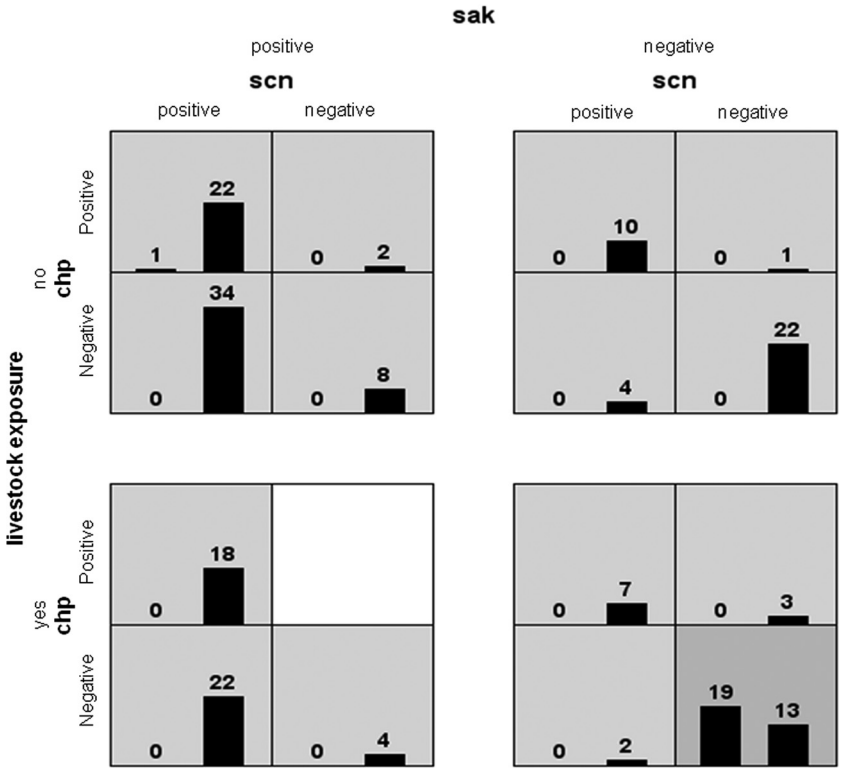

FIG 2 Multifactor dimensionality reduction analysis of the four-factor (*scn*, *sak*, *chp*, and livestock exposure) gene-environment interaction associated with the risk of *S. aureus* CC9 carriage, with the corresponding distribution of *S. aureus* CC9 isolates (left bar in each box) and of *S. aureus* non-CC9 isolates (right bar in each box).

in pigs in Dakar (31) but have been described as major MRSA lineages responsible for human infections in Dakar (32). These observations highlight the need for further surveillance data (including information on humans and animals) to better understand the epidemiology and transmission of human-associated MRSA in both hospital and community settings.

LA-MRSA isolates have unique characteristics that distinguish them from community-associated MRSA and health care-associated MRSA. In most Asian countries, LA-MRSA isolates belong to ST9; however, they have different types of SCC*mec*, such as type IV in Hong Kong (33), type V in Malaysia (34), type IX in Thailand (35), and untypeable SCC*mec* in Taiwan (36). In the present study, we observed that the most common SCC*mec* type of MRSA CC9 from livestock workers was type IV, similar to the results from Hong Kong (33). MRSA CC9 with untypeable SCC*mec* was also found in our study, similar to the results from Taiwan (36).

The virulence gene *pvl* was absent from MRSA ST9 isolates in our study, which is similar to results from Hong Kong (37), Taiwan (38), and Thailand (35). Despite lack of virulence factors, ST9 strains have been found to cause infectious diseases in humans (39). Note that in our study, all 19 *S. aureus* CC9 isolates from livestock workers were MRSA isolates and also exhibited the characteristics of resistance to several classes of antibiotics and absence of the IEC genes (including *scn*, *chp*, *sak*, and *sep*), indicating an overlap between phenotypic and genotypic markers of livestock association. Additionally, the MDR analysis revealed that the phenotype-phenotype (OR = 525.7) and gene-environment (OR = 232.3) interactions were associated with higher rates of livestock-associated *S. aureus* CC9 carriage, which indicated potential multidrug-resistant and species-specific virulence-related characteristics of *S. aureus* CC9 and provided more evidence for potential transmission of livestock-associated *S. aureus* from livestock to humans. Previous studies reported a low incidence of the IEC genes in cattle isolates of *S. aureus* (40, 41). Recent work has demonstrated that absence of the *scn* gene may aid in the differentiation of the animal origin of *S. aureus* carried by humans exposed to livestock (42–45). Recent comparative-genomics studies of human versus animal isolates have revealed that animal-related isolates are significantly less likely to possess the IEC genes (*chp*, *scn*, and *sak*) carried on β-hemolysin-converting bacteriophages (42). Other comparative-genomics studies of *S. aureus* CC398 also indicated that the best genetic markers of human-associated CC398 were IEC genes (*scn* and *chp*), while the best genetic marker of the livestock-associated CC398 was *tet*(M) (43, 44). In addition, a study of industrial hog operation workers in the United States reported that 82% of livestock-associated *S. aureus* isolates demonstrated resistance to tetracycline and that livestock-associated CC398 was not detected among *scn*-positive isolates (45). These findings highlight the need for further whole-genome analysis and comparative-genomics analysis to better identify genetic markers of livestock-to-human transmission of *S. aureus* CC9, which has been referred to as the most pandemic livestock-associated clone in Asia.

Antimicrobial agent use in animals is common in most Asian countries. In our study participants, the high prevalence of resistance of *S. aureus* and MRSA isolates to cefoxitin, clindamycin, tetracycline, erythromycin, chloramphenicol, ciprofloxacin, and trimethoprim-sulfamethoxazole may be related to the imprudent

use of these drugs in humans and food animals in China (27, 46). Consistent with our study, a report in four Chinese provinces indicated that all MRSA isolates from farm workers were resistant to cefoxitin, ciprofloxacin, clindamycin, and tetracycline (9). A study of pig carcasses in Hong Kong markets also revealed that porcine MRSA isolates were resistant to chloramphenicol, ciprofloxacin, clindamycin, SXT, erythromycin, gentamicin, and tetracycline (47). Among the tetracycline antibiotics, tetracycline, chlortetracycline, and oxytetracycline are commonly used in food animal production in Chinese pig farming. Consistent with information about the use of tetracycline in China, we observed that individuals with pig exposure were significantly more likely to carry TRSA than those lacking exposure. Although clindamycin is not allowed to be used in pig farming, lincomycin is widely used. Use of these antibiotics in pig farming may affect the antibiotic resistance patterns of MRSA and multidrug-resistant isolates from pigs and associated workers.

Our study adds to existing knowledge by providing insight into the phenotypic and molecular markers of livestock-associated *S. aureus* CC9 (including MRSA CC9) in humans. In addition, we used the MDR method to detect potential phenotype-phenotype and gene-environment interactions associated with the risk of *S. aureus* CC9 carriage. However, some limitations should be considered when interpreting our results. First, the study design is cross-sectional, conducted at only one time point, so we could not determine whether MRSA carriage among livestock workers and controls was transient or persistent. Second, there were statistically significant differences between livestock workers and controls with regard to sex and age, which might introduce bias. Therefore, multivariable logistic models were used to adjust for these potential covariates. Third, for those with LA-MRSA carriage, we did not further obtain nasal samples from their household members to determine whether the transmission of LA-MRSA occurred in the households. Finally, we did not detect association of carriage of *S. aureus* CC9 with pet contacts in the small sample of pet-owning workers, so larger studies are required to better understand the epidemiology of MRSA cross-transmission occurring between pet animals and humans.

In conclusion, this study contributes to the literature by revealing the overlap between phenotypic markers (resistance to several classes of antibiotics) and genotypic markers (IEC negative) of livestock association. Our results suggest a need for surveillance of antimicrobial-resistant *S. aureus* and MRSA in populations with direct or indirect exposure to livestock. More research is required to establish the exact transmission routes and to explore measures for preventing the spread of the bacterium in the farming environment.

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