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ORIGINAL RESEARCH

Livestock-Associated and Non-Livestock-Associated *Staphylococcus aureus* Carriage in Humans is Associated with Pig Exposure in a Dose–Response Manner

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Background: The distinction between livestock-associated and human-associated methicillin-resistant *Staphylococcus aureus* (MRSA) has become more and more blurred. This study aimed to reveal the transmission risk of livestock-associated and non-livestock-associated *S. aureus* (including MRSA and multidrug-resistant *S. aureus* [MDRSA]) by occupational pig exposure.

Methods: A total of 591 pig-exposed workers and 1178 non-exposed workers were enrolled in this study. All nasal *S. aureus* isolates were tested for antibiotic susceptibility and molecular characteristics. Logistic regression models were used to examine the dose–response relationships between occupational pig exposure and *S. aureus* carriage.

Results: Pig-exposed workers had significantly higher carriage rates of MRSA (OR=6.29, 95% CI: 3.38~11.68) and MDRSA (OR=3.17, 95% CI: 2.03~4.96) than non-exposed workers. Notably, we found dose–response relationships between occupational pig exposure and MRSA or MDRSA carriage. Using genotypic and phenotypic markers for differentiating livestock-associated and non-livestock-associated *S. aureus*, we also revealed dose–response relationships occupational pig exposure and livestock-associated or non-livestock-associated *S. aureus* carriage.

Conclusion: Our findings provide sufficient epidemiological evidence for revealing the high transmission risk of livestock-associated *S. aureus* and the low transmission risk of non-livestock-associated *S. aureus* by occupational pig exposure.

Keywords: livestock, human, methicillin-resistant *S. aureus*, multidrug-resistant *S. aureus*, transmission

Introduction

Staphylococcus aureus, especially methicillin-resistant *S. aureus* (MRSA) have been regarded as a highly virulent pathogen in humans, causing a wide variety of diseases ranging from mild superficial skin infections to severe invasive infections such as toxic shock and sepsis.[1–3] According to previous healthcare-associated exposure and molecular characteristics, human-associated MRSA has been classified into healthcare-associated MRSA (HA-MRSA) and community-associated MRSA (CA-MRSA).[4] Recently, the epidemiology of MRSA has changed with the increasing emergence of livestock-associated (LA) MRSA (LA-MRSA) clones in a variety of animals, from domesticated livestock to companion animals to wild

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animals.[5,6] Therefore, ongoing surveillance is needed to detect changes in the epidemiology of *S. aureus* and MRSA infection in humans and animals.

The spread of LA-MRSA has become a serious health problem worldwide. Currently, the most worrying aspect of LA-MRSA appears to be the capability of spread to humans. Increasing reports have revealed that LA-MRSA clones may emerge in a variety of livestock-related workers with occupational livestock contact. For example, LA-MRSA CC9 isolated from pig-related workers (such as farm workers, slaughterhouse workers, and pig handlers) has been reported in most Asian countries,[5,7] and LA-MRSA CC398 isolated from veterinarians and pig-related workers has been reported in North America and European countries.[8,9] Moreover, persons living in livestock-dense communities had an increased risk for LA-MRSA carriage even if they lacked occupational contact with livestock.[10,11] More worryingly, LA-MRSA emerged rapidly in hospitals during the past decade and there are ongoing outbreaks of invasive LA-MRSA infections in hospital patients.[12–14] These findings suggest the risk of cross-species transmission of livestock-associated *S. aureus* (LA-SA) and LA-MRSA from livestock reservoirs to humans.

Previous studies mainly focused on LA-MRSA CC398 isolates in North America and European countries, but not much is known about LA-MRSA CC9, especially in China. At present, the potential risk and mechanism of LA-MRSA CC9 cross-species transmission is still uncertain, partly due to the lack of host-specific markers and detailed epidemiological investigations. Notably, host-specific markers may aid in differentiating LA-MRSA from human-associated MRSA. More and more studies have defined LA-MRSA based on molecular and phenotypic characteristics, including the clonal complexes (CCs), immune evasion cluster (IEC) genes, and antimicrobial resistance patterns. For example, LA-MRSA CC9 predominates in most Asian countries, whereas CC398 is the overwhelmingly dominant lineage in Europe as well as Northern America, indicating that CC9 and CC398 may be useful molecular markers for livestock association.[5,15] It has been noted that the IEC gene *scn* was found in human isolates but not in livestock isolates, suggesting that the *scn* gene is associated with human specificity.[16,17] Furthermore, tetracycline resistance was observed in 100% LA-SA but absent from human-associated *S. aureus*,[16,18] suggesting that tetracycline resistance may aid in determining the epidemiological origin of MRSA isolates. Therefore, this study used multiple phenotype-genotype markers to explore the frequency-risk and duration–risk relationships between occupational pig exposure and *S. aureus* (including LA-SA and non-LA-SA) carriage in humans, so as to reveal the risk of cross-species transmission.

Methods

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, written informed consent forms were obtained from all study participants or parents of participants under the age of 18 years. So this study complied with the Declaration of Helsinki.

Study Design and Population

This cross-sectional study was conducted from November 2013 to November 2014 in Guangdong Province, China. Briefly, a two-stage sampling process was used to obtain an independent, representative sample. First, four cities (Shenzhen, Dongguan, Jiangmen, and Foshan) were randomly sampled from a total of 21 cities in Guangdong province. Second, in each sampled city, a sample size of about 150 workers with occupational pig exposure (defined as the pig-exposed workers) were sampled from livestock-related venues including pig farms, slaughterhouses, and vet markets. At the same time, a sample size of about 300 workers without occupational pig or livestock exposure (defined as the non-exposed workers) were sampled from biscuit factories and hardware factories in each sampled city. The eligibility criteria for workers included: (1) being able to speak and understand Chinese; (2) being ≥15 years old; (3) not working at medical institutions; and (4) having no occupational pig or livestock exposure for non-exposed workers. After obtaining informed consent, eligible participants were asked to complete a face-to-face questionnaire by trained interviewers. In all, there were 1769 workers sampled in this study, including 591 pig-exposed workers and 1178 non-exposed workers.

Bacterial Isolation and Identification

After completing the questionnaire, study personnel obtained a nasal swab from both nares of each study participant. Swabs were soaked into 7.5% NaCl

enrichment broth at 4°C during transportation and incubated at 35°C ± 1°C for 24 hours. Then, a loopful of the broth was streaked onto mannitol salt agar and incubated at 37°C for 24–48 hours. All presumptive *S. aureus* isolates were tested by colony morphology, gram staining reaction, *β*-hemolysis, catalase test, DNase test, tube coagulase tests, and polymerase chain reaction (PCR) assays for the carriage of the staphylococci *16S rRNA, nuc* and *mecA* (or *mecC*) genes.[19,20] *S. aureus* isolates were confirmed based on the above-mentioned positive tests. *S. aureus* isolates with zone sizes of less than 21 mm for cefoxitin were identified as suspect MRSA and further confirmed by PCR for the *mecA* (or *mecC*) gene.

Antimicrobial Susceptibility Testing

All *S. aureus* isolates were tested for their susceptibility to antimicrobials by standard disk diffusion method, according to guidelines and breakpoints of the Clinical and Laboratory Standards Institute (CLSI).[21] The antimicrobial disks tested were penicillin (10 units), trimethoprim-sulfamethoxazole (25 μg), clindamycin (2 μg), erythromycin (15 μg), tetracycline (30 μg), cefoxitin (30 μg), chloramphenicol (30 μg), rifampin (5 μg), quinupristin-dalfopristin (15 μg), gentamicin (10 μg), ciprofloxacin (5 μg), and linezolid (30 μg) (Table S1). The *S. aureus* ATCC 29213 and *S. aureus* ATCC 25923 were included as a control. *S. aureus* isolates were classified as multidrug-resistant *S. aureus* (MDRSA) if they were MRSA isolates or non-susceptible (including both intermediate and resistant) to ≥3 classes of antimicrobials.[22]

Molecular Characterization

All *S. aureus* isolates were molecularly characterized by multilocus sequence typing (MLST). The sequence types (STs) were assigned by comparing the DNA 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 (http://eburst.mlst.net).[23] All *S. aureus* isolates were also tested through PCR strategy for carriage of the *scn* gene, using previously described primers.[24]

Study Variables

The main outcome variables were antimicrobial resistance (the numbers of antimicrobial classes to which *S. aureus* isolates were resistant) and *S. aureus* carriage (eg, MRSA, MDRSA, LA-MRSA, non-LA-MRSA, LA-MDRSA, and non-LA-MDRSA). *S. aureus* isolates (including MRSA and MDRSA) were classified as LA isolates if they were CC9 and negative for *scn* and resistant to tetracycline, and the others were classified as non-LA isolates.

The main independent variable was self-reported occupational pig exposure, including binary exposure (yes or no), continuous frequency of exposure (hours of exposure per day), and continuous duration of exposure (years of exposure). These three independent variables were analyzed in three different models to explore binary associations, frequency–risk relationships, and duration–risk relationships, respectively. To determine the binary pig exposure, study participations were asked whether they had been occupationally exposed to pigs (yes [defined as the pig-exposed workers] or no [defined as the non-exposed workers]). To determine the frequency and duration of pig exposure, pig-exposed workers were asked how many hours per day (frequency of exposure) and how many years (duration of exposure) they had been exposed to pigs. Covariates in this study were sex, age (15–24, 25–34, 35–44, and ≥45 years), antibiotic use in the last month (yes or no), skin infections in the last month (yes or no), and hospitalization in the last month (yes or no).

Data Analysis

Categorical variables were compared by Pearson chi-squared (χ^2) test. Univariable and multivariable logistic regression models were used to examine the potential relationships between occupational pig exposure and the risk of MRSA, MDRSA, LA-MRSA, non-LA-MRSA, LA-MDRSA, and non-LA-MDRSA carriage. When lack of occurrence of the outcome in one group (such as the case where all non-exposed workers are observed to have a negative outcome of LA-SA), exact logistic regression models were used to produce more-accurate inference. Univariable and multivariable Poisson regression models were used to explore the potential relationships between occupational pig exposure and the average number of antimicrobial classes to which a *S. aureus* isolate was resistant (based on the CLSI definition). Linear trends of livestock exposure were assessed by modeling frequency of exposure or duration of exposure as continuous variables (arithmetic or logarithmic scale) in the models, with a better fit for the model using the logarithmic scale. Based on a *priori* assumptions, all multivariable models were adjusted for sex, age groups, antibiotic use in the last month, skin infections in the last month, and hospitalization in the last month by including these covariates into the model. All statistical analyses were performed using Stata 14.0 version (StataCorp LP, College Station, Texas, USA). The logit command was used to fit the logistic regression model, the exlogistic command was used to fit the exact

logistic regression model, and the poisson command was used to fit the poisson regression model. Generally, a two-sided *P*-value of <0.05 was considered as being of statistical significance.

Results

Characteristics of Study Population

A total of 1769 participants were enrolled in this study (Table 1). Of those, 591 participants were pig-exposed workers with occupational pig exposure and 1178 were non-exposed workers without occupational pig or livestock exposure. Among 591 pig-exposed workers, the mean frequency of exposure (±standard deviation) was 8.5±2.2 hours per day with the median of 8 hours per day, and the mean duration of exposure was 6.9±7.4 years with the median of 5.0 years. There were statistically significant differences between two groups with regard to gender (χ^2=115.21, *P*<0.001), age (χ^2=24.64, *P*<0.001), antibiotic use in the last month (χ^2=5.88, *P*=0.015), and skin infections in the last month (χ^2=78.16, *P*=0.015). The overall prevalence of *S. aureus*, MRSA and MDRSA nasal carriage among study participants were 10.7% (189/1769), 3.3% (59/1769) and 5.4% (96/1769, including 59 MRSA isolates), respectively, and were significantly higher in pig-exposed workers than in non-exposed workers (13.5% vs 9.3%, χ^2=7.57, *P* =0.006, for *S. aureus*; 7.3% vs 1.4%, χ^2=42.75, *P* <0.001, for MRSA; 9.5% vs 3.4%, χ^2=28.35, *P* <0.001, for MDRSA). There were similar significant differences of LA-MRSA (*P* <0.001), non-LA-MRSA (*P* <0.001), LA-MDRSA (*P* <0.001) and non-LA-MDRSA (*P* =0.005) between pig-exposed workers and non-exposed workers. All MRSA isolates carried the *mecA* gene, but all these isolates were

Table 1 Demographic Characteristics of Study Population and Prevalence of *S. aureus*

Characteristics	Total (n=1769)	Non-Exposed Workers (n=1178)	Pig-Exposed Workers (n=591)	χ^2	*P*-value
Gender					
Male	1134 (64.1)	653 (55.4)	481 (81.4)	115.21	<0.001
Female	635 (35.9)	525 (44.6)	110 (18.6)		
Age (years)					
15–24	198 (11.2)	138 (11.7)	60 (10.1)	24.64	<0.001
24–34	519 (29.3)	305 (25.9)	214 (36.2)		
35–44	607 (34.3)	440 (37.4)	167 (28.3)		
≥45	445 (25.2)	295 (25.0)	150 (25.4)		
Antibiotic use in the last month					
Yes	847 (47.9)	540 (45.8)	307 (52.0)	5.88	0.015
No	922 (52.1)	638 (54.2)	284 (48.0)		
Skin infections in the last month					
Yes	390 (22.0)	187 (15.9)	203 (34.3)	78.16	<0.001
No	1379 (78.0)	991 (94.1)	388 (65.7)		
Hospitalization in the last month					
Yes	68 (3.8)	44 (3.7)	24 (4.1)	0.11	0.737
No	1701 (96.2)	1134 (96.3)	567 (95.9)		
Prevalence					
S. aureus	189 (10.7)	109 (9.3)	80 (13.5)	7.57	0.006
MRSA	59 (3.3)	16 (1.4)	43 (7.3)	42.75	<0.001
MDRSA	96 (5.4)	40 (3.4)	56 (9.5)	28.35	<0.001
LA-MRSA	19 (1.1)	0 (0.0)	19 (3.2)	38.28	<0.001
Non- LA-MRSA	40 (2.3)	16 (1.4)	24 (4.1)	13.01	<0.001
LA-MDRSA	19 (1.1)	0 (0.0)	19 (3.2)	38.28	<0.001
Non- LA-MDRSA	77 (4.4)	40 (3.4)	37 (6.3)	7.76	0.005

Note: Values are expressed as number of participants (the proportion of participants surveyed), except where specified otherwise.

Abbreviations: MRSA, methicillin-resistant *S. aureus*; MDRSA, multidrug-resistant *S. aureus*; LA-MRSA, livestock-associated methicillin-resistant *S.aureus*; non-LA-MRSA, non-livestock -associated methicillin-resistant *S.aureus*; LA-MDRSA, livestock-associated multidrug-resistant *S. aureus*; non-LA-MDRSA, non-livestock-associated multidrug-resistant *S. aureus*.

absent of the *mecC*. Among 96 MDRSA isolates, the most common resistance pattern was non-susceptible to clindamycin, erythromycin, and tetracycline (Figure 1).

Group Differences in *S. aureus* Molecular Typing

Of 80 *S. aureus* isolates from 591 pig-exposed workers (Figure 2), the predominant genotypes were CC9 (19 isolates, including 16 for ST9) and CC7 (19 isolates, including 18 for ST7), followed by CC6 (9 isolates), CC59 (6 isolates), CC188 (5 isolates), and CC45 (5 isolates). Of 109 *S. aureus* isolates from 1178 non-exposed workers (Figure 2), the most prevalent genotypes were CC7 (24 isolates), CC6 (17 isolates), CC188 (15 isolates), and CC59 (12 isolates). Comparing the molecular typing of *S. aureus* isolates between two groups, we found that LA-SA CC9

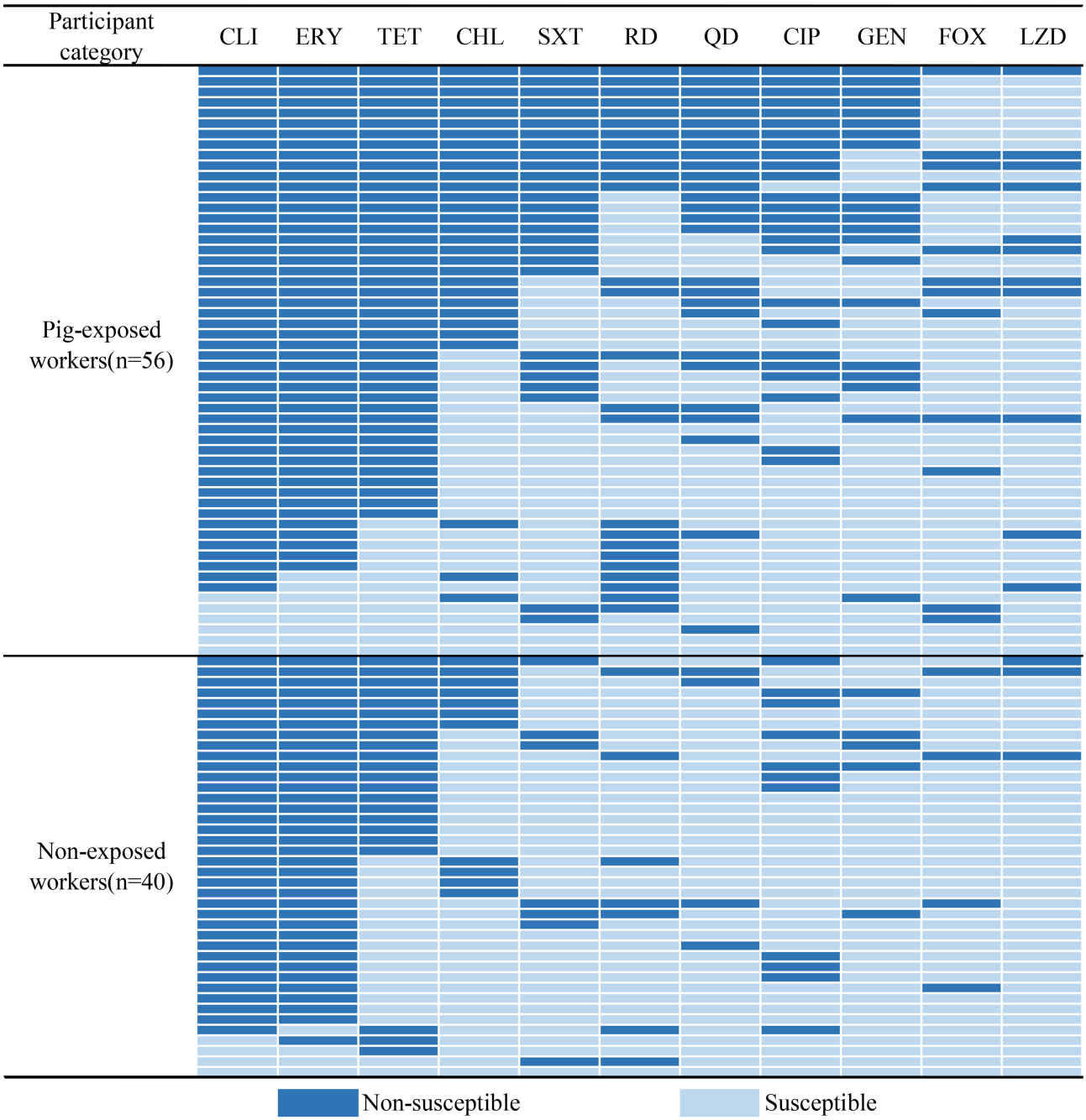

Figure 1 Heat map showing antibiotic resistance profiles of all multidrug-resistant *S. aureus* isolates.
Abbreviations: CLI, clindamycin; ERY, erythromycin; TET, tetracycline; CHL, chloramphenicol; SXT, trimethoprim-sulfamethoxazole; RD, rifampin; QD, quinupristin-dalfopristin; CIP, ciprofloxacin; GEN, gentamicin; FOX, cefoxitin; LZD, linezolid.

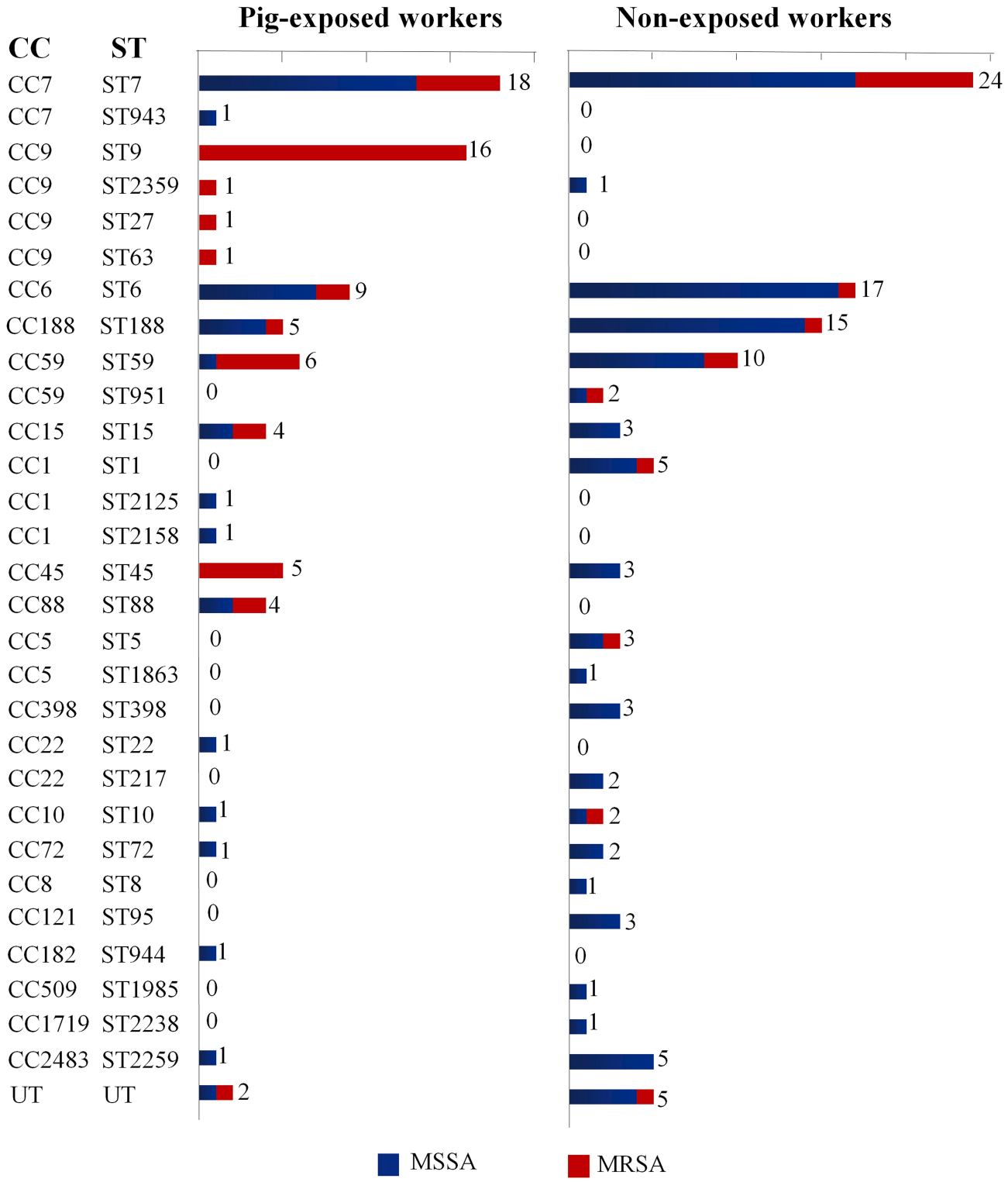

Figure 2 *S. aureus* sequence type diversity and distribution of *S. aureus* isolates among pig-exposed workers (80 isolates) and non-exposed workers (109 isolates).
Note: Each bar represents the number of *S. aureus* isolates for each sequence type.
Abbreviations: CC, clonal complex; ST, sequence type; UT, untypeable; MRSA, methicillin-resistant *S. aureus*; MSSA, methicillin-sensitive *S. aureus*.

isolates were mainly observed in pig-exposed workers, but human-associated *S. aureus* CC7/CC6/CC188/CC59 isolates were mainly observed in both pig-exposed and non-exposed workers. The single methicillin-susceptible *S. aureus* CC9 (ST2359) observed in a non-exposed worker was susceptible to tetracycline and carried the *scn* gene.

Relationships Between Occupational Pig Exposure and *S. aureus* Antimicrobial Resistance

The numbers of antimicrobial classes to which *S. aureus* isolates were resistant differed between groups (Table 2). Compared with isolates from non-exposed workers, *S. aureus* isolates from pig-exposed workers were on average resistant to 2.35 times more antimicrobial classes (IRR=2.35, 95% CI: 1.81~3.04). Moreover, there were monotonically increasing dose–response relationships between frequency of pig exposure and the average number of antimicrobial classes to which a *S. aureus* isolate was resistant (IRR= 1.48, 95% CI: 1.32~1.67) and between duration of pig exposure and the average number of antimicrobial classes to which a *S. aureus* isolate was resistant (IRR = 1.12, 95% CI: 1.09~1.16).

Relationships Between Occupational Pig Exposure and MRSA or MDRSA Carriage

Table 3 presents the relationships between occupational pig exposure and MRSA or MDRSA carriage. Compared with non-exposed workers, pig-exposed workers experienced significantly higher carriage rates of MRSA (OR=6.29, 95% CI: 3.38~11.68) and MDRSA (OR=3.17, 95% CI: 2.03~4.96). Notably, there were monotonically increasing dose–response relationships between frequency of occupational pig exposure (hours/day) and the carriage of MRSA (OR=2.28, 95% CI: 1.72~3.02; Figure 3A) and MDRSA (OR=1.72, 95% CI: 1.40~2.12; Figure 3B). Similarly, there were increasing dose–response relationships between duration of occupational pig exposure (years) and the carriage of MRSA (OR=1.24, 95% CI: 1.15~1.34, Figure 3C) and MDRSA (OR=1.17, 95% CI: 1.10~1.24, Figure 3D).

Relationships Between Occupational Pig Exposure and LA-MRSA or Non-LA-MRSA Carriage

Table 4 shows the relationships between occupational pig exposure and LA-MRSA or non-LA-MRSA carriage. Notably, pig-exposed workers had significantly higher carriage rates of LA-MRSA (OR=52.80, 95% CI: 8.79~∞) and non-LA-MRSA (OR=3.73, 95% CI:

Table 2 Relationships Between Occupational Pig Exposure and the Number of Antimicrobial Classes to Which a *S. aureus* Was Resistant

Source of Exposure	Unadjusted IRR (95% CI)	*P*-value	Adjusted IRR (95% CI)[b]	*P*-value
Pig exposure				
No[a]	1.00		1.00	
Yes	2.37 (1.87~3.00)	<0.001	2.35 (1.81~3.04)	<0.001
Frequency of pig exposure (hours/day, logarithmic)	1.48 (1.33~1.65)	<0.001	1.48 (1.32~1.67)	<0.001
Duration of pig exposure (years, logarithmic)	1.12 (1.09~1.15)	<0.001	1.12 (1.09~1.16)	<0.001

Notes: [a]No occupational exposure with any types of livestock. [b]Adjusted for sex, age (15–24, 25–34, 35–44, and ≥45 years), antibiotic use in the last month, skin infections in the last month, and hospitalization in the last month.
Abbreviation: IRR, incidence-rate ratio.

Table 3 Relationships Between Occupational Pig Exposure and MRSA or MDRSA Carriage

Source of Exposure	MRSA		MDRSA	
	Unadjusted OR (95% CI)	Adjusted OR (95% CI)[b]	Unadjusted OR (95% CI)	Adjusted OR (95% CI)[b]
Pig exposure				
No[a]	1.00	1.00	1.00	1.00
Yes	5.70 (3.18~10.21)	6.29 (3.38~11.68)	2.98 (1.96~4.53)	3.17 (2.03~4.96)
Frequency of pig exposure (hours/day, logarithmic)	2.14 (1.65~2.78)	2.28 (1.72~3.02)	1.66 (1.37~2.01)	1.72 (1.40~2.12)
Duration of pig exposure (years, logarithmic)	1.22 (1.14~1.31)	1.24 (1.15~1.34)	1.15 (1.09~1.22)	1.17 (1.10~1.24)

Notes: [a]No occupational exposure with any types of livestock. [b]Adjusted for sex, age (15–24, 25–34, 35–44, and ≥45 years), antibiotic use in the last month, skin infections in the last month, and hospitalization in the last month.
Abbreviations: MRSA, methicillin-resistant *S. aureus*; MDRSA, multidrug-resistant *S. aureus*; OR, odds ratio; CI, confidence interval.

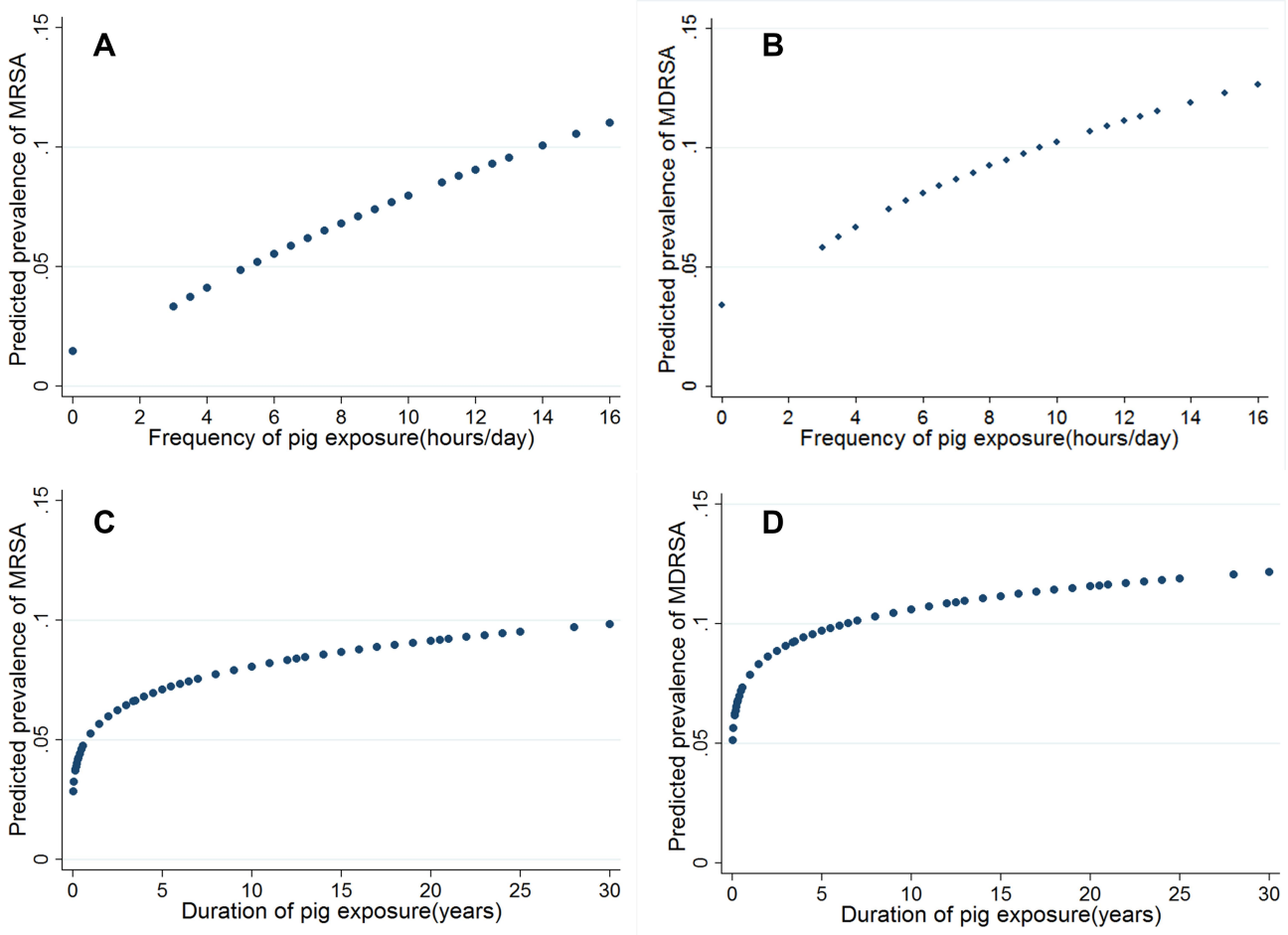

Figure 3 Predicted prevalence of MRSA ((**A**) hours of pig exposure per day; (**C**) years of pig exposure) and MDRSA isolates ((**B**) hours of pig exposure per day; (**D**) years of pig exposure) based on pig exposure.

1.88~7.39) as compared with non-exposed workers. Notably, there were monotonically increasing dose–response relationships between frequency of pig exposure and LA-MRSA (OR=7.49, 95% CI: 2.38~23.57) or non-LA-MRSA (OR=1.80, 95% CI: 1.31~2.46) carriage. In addition, we observed similar dose–response relationships between duration of pig exposure and LA-MRSA (OR=1.61, 95% CI: 1.28~2.02) or non-LA-MRSA (OR=1.16, 95% CI: 1.06~1.27) carriage.

Table 4 Relationships Between Occupational Pig Exposure and LA-MRSA or Non-LA-MRSA Carriage

Source of Exposure	LA-MRSA		Non-LA-MRSA	
	Unadjusted OR (95% CI)	**Adjusted OR (95% CI)[b]**	**Unadjusted OR (95% CI)**	**Adjusted OR (95% CI)[b]**
Pig exposure				
No[a]	1.00	1.00	1.00	1.00
Yes	56.92 (9. 84~∞)[c]	52.80 (8.79~∞)[c]	3.18 (1.68~6.04)	3.73 (1.88~7.39)
Frequency of pig exposure (hours/day, logarithmic)	5.23 (2.37~11.50)	7. 49 (2.38~23.57)	1.67 (1.24~2.23)	1.80 (1.31~2.46)
Duration of pig exposure (years, logarithmic)	1.48 (1.25~1.75)	1.61 (1.28~2.02)	1.14 (1.05~1.24)	1.16 (1.06~1.27)

Notes: [a]No occupational exposure with any types of livestock. [b]Adjusted for sex, age (15–24, 25–34, 35–44, and ≥45 years), antibiotic use in the last month, skin infections in the last month, and hospitalization in the last month. [c]Exact logistic regression models were used due to lack of occurrence of the outcome in one group.
Abbreviations: LA-MRSA, livestock-associated methicillin-resistant *S. aureus*; non-LA-MRSA, non-livestock-associated methicillin-resistant *S. aureus*; OR, odds ratio; CI, confidence interval.

Relationships Between Occupational Pig Exposure and LA-MDRSA or Non-LA-MDRSA Carriage

Table 5 presents the relationships between occupational pig exposure and LA-MDRSA or non-LA-MDRSA carriage. Interestingly, pig-exposed workers experienced a significantly higher proportion of LA-MDRSA (OR=53.39, 95% CI: 8.85~∞) and non-LA-MDRSA (OR=2.15, 95% CI: 1.32~3.50) carriage than non-exposed workers. Moreover, we observed increasing dose–response relationships between frequency of pig exposure and LA-MDRSA (OR=7.56, 95% CI: 2.40~23.86) or non-LA-MDRSA (OR=1.45, 95% CI: 1.15~1.81) carriage. Similarly, there were increasing dose–response relationships between duration of pig exposure and LA-MDRSA (OR=1.61, 95% CI: 1.28~2.02) or non-LA-MDRSA (OR=1.11, 95% CI: 1.04~1.19) carriage.

Discussion

S. aureus (including MRSA) is a commensal and opportunistic pathogen of livestock and humans.[5] In Asia, CC9 (ST9) has been referred to as the most prevalent LA-MRSA; while for human-associated isolates, ST59 and ST30 are the most common CA-MRSA, and ST239 and ST5 are the predominant HA-MRSA.[4,5] Notably, the increasing appearance of LA-MRSA in community and hospitals has been of growing concern.[4,5,12,13] Currently, the most worrying aspect is the potential risk of MRSA transmission from livestock to human beings in the community and hospital. Moreover, previous studies have revealed that LA-MRSA CC9 predominates in pigs and related workers in most Asian countries,[4,5] which reveals the potential risk of cross-species transmission of LA-MRSA. In the present study, typical LA-MRSA isolates (CC9 and absence of the *scn* gene and tetracycline resistance) were observed only in pig-exposed workers but absent from non-exposed workers, and the single methicillin-susceptible *S. aureus* CC9 (ST2359) observed in a non-exposed worker was susceptible to tetracycline and carried the *scn* gene, indicating that substantial overlap in livestock-associated characteristics occurred only in pig-exposed workers. These findings provide genetic evidence for revealing the risk of cross-species transmission of LA-MRSA.

Note that there are significant differences in antimicrobial resistance between human and animal *S. aureus* isolates, so resistance analysis on different sources of *S. aureus* may provide important epidemiological evidence for revealing the potential transmission risk of resistant *S. aureus* between livestock and humans. Notably, the surprisingly high antimicrobial resistance of animal-related *S. aureus* has become an important public health issue. For example, the latest study in China revealed that 97.1% pig-related *S. aureus* be characterized as MDRSA,[25] and another study in Hongkong demonstrated that almost all of animal *S. aureus* were MDRSA.[26] The present study builds on previous literature to reveal that the prevalence of MRSA and MDRSA carriage was significantly higher in pig-exposed workers than in non-exposed workers. In addition, we found monotonically increasing frequency-risk and duration–risk relationships between occupational pig exposure and human MDRSA or MRSA carriage, suggesting that occupational livestock exposure consistently increases the risk of MDRSA and MRSA carriage in humans. Moreover, this study contributed additionally to the literature by finding monotonically

Table 5 Relationships Between Occupational Pig Exposure and LA-MDRSA or Non-LA-MDRSA Carriage

Source of Exposure	LA-MDRSA		Non-LA-MDRSA	
	Unadjusted OR (95% CI)	Adjusted OR (95% CI)[b]	Unadjusted OR (95% CI)	Adjusted OR (95% CI)[b]
Pig exposure				
No[a]	1.00	1.00	1.00	1.00
Yes	57.09 (9.87~∞)[c]	53.39 (8.85~∞)[c]	1.97 (1.24~3.11)	2.15 (1.32~3.50)
Frequency of pig exposure (hours/day, logarithmic)	5.31 (2.39~11.77)	7.56 (2. 40~23.86)	1.38 (1.12~1.71)	1.45 (1.15~1.81)
Duration of pig exposure (years, logarithmic)	1.48 (1.25~1.76)	1.61 (1.28~2.02)	1.10 (1.03~1.17)	1.11 (1.04~1.19)

Notes: [a]No occupational exposure with any types of livestock. [b]Adjusted for sex, age (15–24, 25–34, 35–44, and ≥45 years), antibiotic use in the last month, skin infections in the last month, and hospitalization in the last month. [c]Exact logistic regression models were used due to lack of occurrence of the outcome in one group.
Abbreviations: LA-MDRSA, livestock-associated multidrug-resistant *S. aureus*; non-LA-MDRSA, non-livestock-associated multidrug-resistant *S. aureus*; OR, odds ratio; CI, confidence interval.

increasing frequency-risk and duration–risk relationships between occupational pig exposure and the average number of antimicrobial classes to which a *S. aureus* isolate was resistant. In all, the above findings provide sufficient epidemiological evidence for revealing a high transmission risk of MRSA and MDRSA by occupational livestock exposure. It is worth noting that pig-exposed workers had significantly higher rates of antibiotic use in the last month (52.0% vs 45.8%, P =0.015) and skin infections in the last month (34.3% vs 15.9%, P <0.001) as compared with non-exposed workers. These findings support growing concern about high antibiotic use and skin infections in pig-exposed workers.

It is well known that specifically genotypic and phenotypic markers may aid in differentiating LA-MRSA from human-associated MRSA. Note that increasing studies have demonstrated that the most epidemic lineages of LA-MRSA are CC9 (ST9) predominated in most Asian countries and CC398 (ST398) predominated in European and American countries, suggesting that CC9 and CC398 may be useful molecular markers for livestock association.[4,5,18] The latest comparative-genomics of human versus animal *S. aureus* isolates have shown that the human-specific IEC genes (*scn*) were carried only in human CC398 isolates but absence from pig CC398 isolates, suggesting that the *scn* gene is associated with human specificity.[16,17] Additionally, tetracycline resistance gene *tet*(M) is common in livestock isolates but rare in human isolates, suggesting that the presence of tetracycline resistance or *tet* (M) gene may be a useful marker for livestock association.[16,27,28] Therefore, the above markers for livestock association (CC9, absence of the *scn* gene, and tetracycline resistance) may aid in differentiating LA-MRSA from human-associated MRSA isolates, which may provide important evidence for revealing a cross-species transmission risk.

Studies on LA-MRSA CC398 isolates have been frequently reported, but not much is known about LA-MRSA CC9, especially in China. An interesting aspect of this study is to clarify the transmission risk of LA-MRSA and non-LA-MRSA by occupational pig exposure based on a large-sample investigation. Increasing studies including the present study have revealed strong associations between livestock exposure and human MRSA carriage,[28,29] indicating the potential risk of MRSA transmission by livestock exposure. This study builds on previous literature to demonstrate that pig-exposed workers had a significantly higher risk of LA-MRSA (OR=52.80) and LA-MDRSA (OR=53.39) carriage than non-exposed workers, suggesting that pig exposure increases the risk of LA-MRSA and LA-MDRSA carriage in humans. More importantly, we found monotonically increasing frequency-risk and duration–risk relationships between occupational pig exposure and LA-MRSA or LA-MDRSA carriage in humans. When examining these relationships for non-LA-MRSA and non-LA-MDRSA, there was still evidence of significant frequency-risk and duration–risk relationships. In summary, the above results provide sufficient epidemiological evidence for revealing the transmission risk of LA-MRSA, non-LA-MRSA, LA-MDRSA, and non-LA-MDRSA by occupational livestock exposure.

It is a large-sample investigation on this topic. However, potential limitations also needed to be considered in this study. First, there may be differences between pig-exposed and non-exposed workers with regard to sex, age, history of antibiotic use, history of skin infections, and history of hospitalization, which might introduce bias. Therefore, multivariable regression models were used to adjust for these potential covariates by including covariates into the model. Second, since NaCl concentrations >6% may interfere with *S. aureus* detection, the nasal swabs soaked into 7.5% NaCl enrichment broth in the present study may underestimate the detection rate of *S. aureus*.

Conclusion

This study revealed monotonically increasing dose–response relationships between occupational pig exposure and MRSA or MDRSA carriage in humans, and also found monotonically increasing dose–response relationships for LA-MRSA, non- LA-MRSA, LA-MDRSA, and non-LA-MDRSA carriage in humans, which provides sufficient epidemiological evidence for revealing the high transmission risk of LA-SA and the low transmission risk of non-LA-SA by occupational pig exposure. These findings point out the urgent need for developing effective measures to prevent and cut *S. aureus* spread in the farming environment.

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Disclosure

The authors report no conflicts of interest in this work.

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