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Phenotypic and molecular characteristics of *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* in slaughterhouse pig-related workers and control workers in Guangdong Province, China

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SUMMARY

Pig farmers and veterinarians have high prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) due to the occupational livestock exposure, while few reported this association on slaughterhouse workers. We conducted this cross-sectional study to explore the phenotypic and molecular characteristics of *S. aureus* and MRSA in slaughterhouse pig-related workers and control workers in Guangdong Province, China. Participants were interviewed and provided two nasal swabs. Swabs were tested for *S. aureus*, and isolates were further tested for antimicrobial susceptibility, virulence genes and multi-locus sequence typing. Compared with control workers, pig-related workers have significantly higher prevalence of MRSA carriage (adjusted odd ratio (aOR) 3·70, 95% CI 1·63–8·40). The proportions of MRSA resistant to clindamycin, erythromycin, tetracycline or chloromycetin were significantly higher in pig-related workers than in control workers. The predominant phenotypes of *S. aureus* were resistant to penicillin, clindamycin, erythromycin and tetracycline. Three MRSA CC9 isolates with livestock-associated characteristics (resistance to tetracycline and absence of immune evasion cluster (IEC) genes) were detected in pig-related workers but not in control workers. For human-associated CCs (CC7, CC59, CC6, and CC188), there was no significant difference in IEC profile or antimicrobial resistance between the groups. These findings reveal that there may be a potential risk for livestock-to-human transmission of LA-MRSA and human-to-human transmission of human-associated MRSA.

Key words: Methicillin-resistant, *S. aureus*, multidrug-resistant, *S. aureus*, livestock, human.

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) originated in *Staphylococcus aureus* from acquiring the *mecA* gene, and got the resistance to methicillin and other *β*-lactam antimicrobials [1]. MRSA can cause several diseases, including minor skin and soft tissue infections and severe invasive diseases [2]. MRSA infections are difficult to treat, particularly if the isolates have acquired multiple antimicrobial resistance elements and become multidrug-resistant *S. aureus* (MDRSA) isolates [3]. MRSA isolates were generally classified into hospital-associated MRSA and community-associated MRSA based on epidemiological characteristics. Recently, a new kind of MRSA was found in animals such as pigs, bovine, and poultry, and was referred to as livestock-associated

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MRSA (LA-MRSA) [4–6]. In European countries and North America, the widest spread of LA-MRSA is clonal complex 398 (CC398), while CC9 is predominant in most Asian countries [7].

Of note, more and more studies reported the detection of LA-MRSA not only in animals, but also in humans [8–10]. LA-MRSA could be transmitted to humans especially those workers with occupational livestock exposure such as pig-related farmers and veterinarians. Previous studies mainly focused on pig farmers and indicated that the prevalence of MRSA carriage is positively associated with occupational pig exposure [8, 9]. Such occupational exposure may occur at slaughterhouses and retail markets as well as the farm levels. Although some studies have identified LA-MRSA in slaughterhouse workers in Europe [11, 12], there is still few to explore the potential relationship between phenotypic and molecular characteristics to reveal livestock-associated *S. aureus* in slaughterhouse workers. Therefore, we conducted this study to investigate the prevalence, antimicrobial susceptibility and molecular characteristics of *S. aureus* and MRSA isolates from slaughterhouse pig-related workers and control workers with no occupational livestock exposure, and tested the hypothesis that pig-related workers may have a higher prevalence of livestock-associated *S. aureus* (including MRSA) carriage than control workers due to occupational pig exposure.

METHODS

Ethics statement

The study was approved by the Ethics Committee of Guangdong Pharmaceutical University, and was performed in accordance with the approved guidelines. The survey was qualified as involving no risks to participants. Before participating, all participants signed an informed consent form.

Study design and population

A cross-sectional study was conducted in Guangdong Province of China between November 2013 and November 2014. Four cities from 21 cities in Guangdong Province were randomly sampled to enroll representative participants. In each city, slaughterhouses were selected to enroll about 100 pig-related workers with occupational exposure to pigs. Pig butchers were also included in pig-related workers because butchers go to slaughterhouse to kill pigs at 1–5 AM in the morning and go to the meat markets to sell pork during the day. At the same time, factories unrelated to livestock (i.e. hardware factory or biscuit factory) in each city were sampled to enroll about 200 control workers with no occupational livestock exposure. After obtaining informed consent, two nasal swabs were taken from each participant and a face-to-face questionnaire was administered to collect influencing factors of *S. aureus* carriage, including sex, age (15–30, 31–40, and 41–60 years), education (elementary school, junior high school, senior high school, and above), personal monthly income (⩽¥1000, ¥1001–2000, ¥2001–3000, or ⩾¥3001), occupational pig exposure (yes or no), antimicrobial use in the last month (yes or no), and visit to medical facilities in the last month (yes or no).

Bacterial isolation and identification

Swabs were stored in 5 ml of enrichment broth containing 1% tryptone, 7·5% NaCl, 1% mannitol, and 0·25% yeast extract at 4 °C during transportation and incubated at 35 ± 1 °C for 24 h. Then a loopful of the broth was plated on mannitol salt agar and incubated at 35 ± 1 °C for 24–48 h. Suspected colonies were subcultured to 5% sheep blood agar plates and incubated at 35 ± 1 °C overnight. Initial identification of *S. aureus* was based on Gram staining, morphology, catalase test, DNase test, and tube coagulase test.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed by the disk diffusion method of the Clinical and Laboratory Standards Institute guidelines [13]. The following antimicrobial disks were used: cefoxitin (30 μg), penicillin (10 units), clindamycin (2 μg), tetracycline (30 μg), erythromycin (15 μg), trimethoprim–sulfamethoxazole (25 μg), chloromycetin (30 μg), ciprofloxacin (5 μg), rifampin (5 μg), nitrofurantoin (300 μg), gentamicin (10 μg), and linezolid (30 μg). The reference strain *S. aureus* ATCC 25923 was used for quality control and *S. aureus* ATCC 29213 was used for positive control. *S. aureus* isolates were classified as MDRSA if they were non-susceptible to ⩾3 classes of antimicrobials or were MRSA isolates [14].

Molecular characterization

All *S. aureus* isolates were further tested through polymerase chain reaction (PCR) assays for the carriage of 16S rRNA, *nuc*, and *mecA* genes [15]. Multi-locus

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Table 1. *Study population characteristics by participant category*

Characteristics	Total ($n = 1190$)	Pig-related worker ($n = 335$)	Control worker ($n = 855$)	χ^2	*P-value*
Gender					
Male	593 (49·8)	282 (84·2)	311 (36·4)	220·03	<0·001
Female	597 (50·2)	53 (15·8)	544 (63·6)		
Age (years)					
15–30	344 (28·9)	73 (21·8)	271 (31·7)	11·58	0·003
31–40	336 (28·2)	106 (31·6)	230 (26·9)		
41–60	510 (42·9)	156 (46·6)	354 (41·4)		
Education					
Elementary school	142 (11·9)	44 (13·1)	98 (11·4)	2·17	0·339
Junior high school	746 (62·7)	199 (59·4)	547 (64·0)		
Senior high school and above	302 (25·4)	92 (27·5)	210 (24·6)		
Personal monthly income (¥)					
⩽1000	121 (10·2)	45 (13·4)	76 (8·9)	16·65	<0·001
1001–2000	367 (30·8)	77 (23·0)	290 (33·9)		
2001–3000	512 (43·0)	160 (47·8)	352 (41·2)		
⩾3001	190 (16·0)	53 (15·8)	137 (16·0)		
Visit to medical facilities in the last month					
Yes	429 (36·1)	119 (35·5)	310 (36·3)	0·06	0·812
No	761 (63·9)	216 (64·5)	545 (63·7)		
Antimicrobial use in the last month					
Yes	510 (42·9)	145 (43·3)	365 (42·7)	0·03	0·852
No	680 (57·1)	190 (56·7)	490 (57·3)		
Prevalence					
S. aureus	140 (11·8)	43 (12·8)	97 (11·3)	0·52	0·473
MDRSA	60 (5·0)	23 (6·9)	37 (4·3)	3·24	0·072
MRSA	33 (2·8)	17 (5·1)	16 (1·9)	9·16	0·003

Note. Values are number of participants (proportion of participants surveyed) or as otherwise indicated.
MDRSA, multidrug-resistant *S. aureus*; MRSA, methicillin-resistant *S. aureus*.

sequence typing (MLST) was conducted for all *S. aureus* isolates as previously described [16]. The sequence type (ST) was assigned by comparison with the MLST database (http://www.mlst.net/), and CCs were determined using the eBURST algorithm. The presence of Panton–Valentine leukocidin (*pvl*) and immune evasion cluster (IEC) genes (including *scn, chp, sak*, and *sea*) were determined as previously described [15, 17].

Data analysis

All data were entered in duplicate into an EpiData (version 3.1 database) (the EpiData Association, Odense Denmark). Categorical variables were compared by Pearson's chi-squared (χ^2) test or Fisher exact test when appropriate. The relations between pig exposure and *S. aureus* (including MRSA and MDRSA) carriage were examined using univariable and multivariable logistic regression models. All multivariable models were adjusted for sex, age, education, personal monthly income, antimicrobial use in the last month, and visit to medical facilities in the last month. We defined a two-sided *P*-value of ⩽0·05 as being of statistical significance. All statistical analyses were conducted using STATA version 14·0 (StataCorp LP, College Station, TX, USA).

RESULTS

Study population

In total, 1190 workers voluntarily participated in this study. The participants consisted of 335 pig-related workers (including 178 slaughterhouse workers and 157 butchers) and 855 control workers. There were statistically significant differences between groups with regard to sex, age, and personal monthly income ($P < 0·05$, Table 1). Pig-related workers were more males than control workers, and on average, pig-related workers were older than control workers (mean = 39·3 *vs.* 36·4 years of age; *t*-test, $t' = -4·45$, $P < 0·001$). There was no significant difference in human distribution according to visit to medical facilities in the last month ($P = 0·812$) or antimicrobial use in the last month ($P = 0·852$).

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Table 2. *Group differences in phenotypic and molecular characteristics of S. aureus and MRSA carriage among study participants, in Guangdong, China*

Characteristics of isolates	*S. aureus*				MRSA			
	Total	Pig-related worker	Control worker	*P*-value	Total	Pig-related worker	Control worker	*P*-value
No. of isolates	140	43	97		33	17	16	
Resistance pattern								
FOX-R	33 (23·6)	17 (39·5)	16 (16·5)	0·003	33 (23·6)	17 (39·5)	16 (16·5)	0·003
PEN-R	125 (89·3)	39 (90·7)	86 (88·7)	0·719	31 (22·1)	17 (39·5)	14 (14·4)	0·001
CLI-R	62 (44·3)	20 (46·5)	42 (43·3)	0·724	23 (16·4)	12 (27·9)	11 (11·3)	0·015
ERY-R	51 (36·4)	17 (39·5)	34 (35·1)	0·611	21 (15·0)	11 (25·6)	10 (10·3)	0·020
TET-R	53 (37·9)	22 (51·2)	31 (32·0)	0·031	17 (12·1)	10 (23·3)	7 (7·2)	0·007
CHL-R	16 (11·4)	6 (14·0)	10 (10·3)	0·736	7 (5·0)	5 (11·6)	2 (2·1)	0·048
CIP-R	10 (7·1)	4 (9·3)	6 (6·2)	0·761	7 (5·0)	3 (7·0)	4 (4·1)	0·769
SXT-R	10 (7·1)	7 (16·3)	3 (3·1)	0·015	5 (3·6)	4 (9·3)	1 (1·0)	0·053
GEN-R	5 (3·6)	3 (7·0)	2 (2·1)	0·341	4 (2·9)	3 (7·0)	1 (1·0)	0·162
RIF-R	4 (2·9)	1 (2·3)	3 (3·1)	1·000	2 (1·4)	0 (0·0)	2 (2·1)	1·000
NIT-R	4 (2·9)	3 (7·0)	1 (1·0)	0·162	2 (1·4)	2 (4·7)	0 (0·0)	0·093
LZD-R	2 (1·4)	1 (2·3)	1 (1·0)	0·522	1 (0·7)	1 (2·3)	0 (0·0)	0·307
IEC genes								
scn-negative	46 (32·9)	11 (25·6)	35 (36·1)	0·222	13 (9·3)	5 (11·6)	8 (8·25)	0·749
chp-negative	89 (63·6)	26 (60·5)	63 (65·0)	0·611	19 (13·6)	9 (20·9)	10 (10·3)	0·091
sak-negative	49 (35·0)	14 (32·6)	35 (36·1)	0·687	16 (11·4)	9 (20·9)	7 (7·2)	0·039
sea-negative	122 (87·1)	39 (90·7)	83 (85·6)	0·403	30 (21·4)	17 (39·5)	13 (13·4)	<0·001

Note. Values are number of *S. aureus* or MRSA isolates with specific phenotype or genotype (proportion of these isolates among all *S. aureus* isolates) or as otherwise indicated.
MRSA, methicillin-resistant *S. aureus*; R, resistance; FOX, Cefoxitin; PEN, Penicillin; CLI, Clindamycin; ERY, Erythromycin; TET, Tetracycline; CHL, Chloromycetin; CIP, Ciprofloxacin; SXT, Trimethoprim–sulfamethoxazole; RIF, Rifampin; GEN, Gentamicin; NIT, Nitrofurantoin; LZD, Linezolid.

Group differences in prevalence of *S. aureus*, MDRSA, and MRSA

The overall prevalence of *S. aureus* nasal carriage among the study population was 11·8% (140/1190), with similar prevalences between pig-related workers (12·8%, 43/335) and control workers (11·3%, 97/855) ($P = 0·473$, Table 1). As for MDRSA, the overall prevalence was 5·0% (60/1190, including 6·9% for pig-related workers and 4·3% for controls) and there was no significant difference between the groups ($P = 0·072$). The overall prevalence of MRSA was 2·8% (33/1190, including 5·1% for pig-related workers and 1·9% for controls) and was significantly higher in pig-related workers than in control workers ($P = 0·003$).

Relation of pig exposure with *S. aureus*, MDRSA, and MRSA carriage

After adjusting for gender, age, education, personal monthly income, visit to medical facilities and antimicrobial use, pig-related workers had significantly higher rates of MRSA carriage (adjusted odd ratio (aOR) 3·70, 95% CI 1·63–8·40) than control workers, whereas no significant difference was found in *S. aureus* (aOR 1·40, 95% CI 0·90–2·17) or MDRSA (aOR 1·72, 95% CI 0·93–3·18) carriage.

Group differences in phenotypic characteristics

Antimicrobial susceptibility testing of 140 *S. aureus* isolates revealed that penicillin resistance (89·3%) was the most common phenotype, followed by clindamycin (44·3%), tetracycline (37·9%), erythromycin (36·4%), and cefoxitin (23·6%), while these isolates showed slight resistance to the rest of antimicrobials (Table 2). Notably, among 140 *S. aureus* isolates, the proportions of tetracycline-resistant *S. aureus* and trimethoprim–sulfamethoxazole-resistant *S. aureus* were higher in pig-related workers than in control workers (51·2% *vs.* 32·0%, $P = 0·031$ and 16·3% *vs.* 3·1%, $P = 0·015$, respectively, Table 2). Additionally, the proportions of clindamycin-resistant MRSA, erythromycin-

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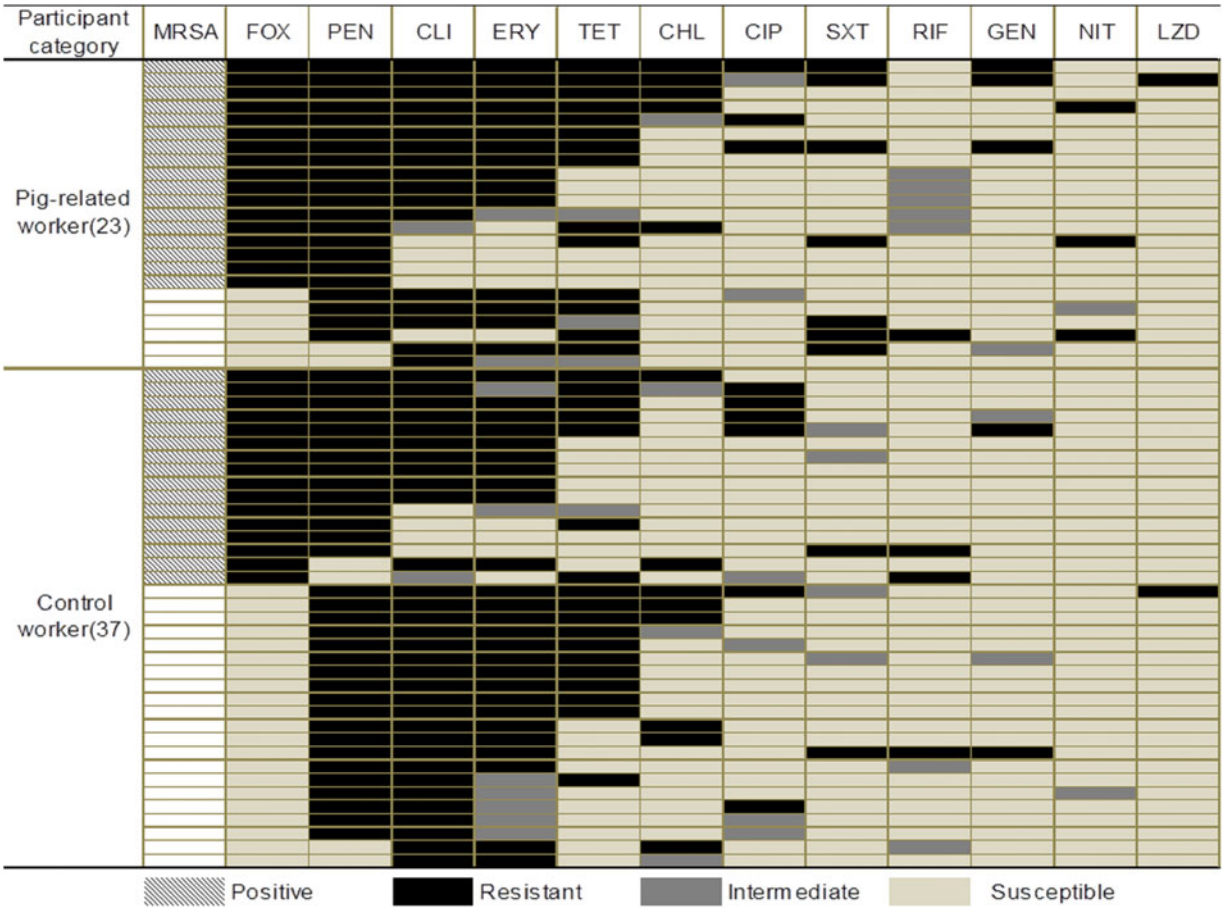

Fig. 1. Antimicrobial resistance profile of MDRSA isolates from the pig-related workers and control workers. Note. FOX, Cefoxitin; PEN, Penicillin; CLI, Clindamycin; ERY, Erythromycin; TET, Tetracycline; CHL, Chloromycetin; CIP, Ciprofloxacin; SXT, Trimethoprim–sulfamethoxazole; RIF, Rifampin; GEN, Gentamicin; NIT, Nitrofurantoin; LZD, Linezolid.

resistant MRSA, tetracycline-resistant MRSA and chloromycetin-resistant MRSA were significantly higher in pig-related workers than in control workers (Table 2).

Among 60 MDRSA isolates from the pig-related workers and control workers, the dominant multidrug-resistant pattern was similar and mainly non-susceptible to clindamycin, erythromycin and tetracycline (Fig. 1). For MRSA isolates, multidrug-resistant pattern was also similar in the two groups (Fig. 1).

Group differences in molecular characteristics

Diversity of CC types was observed among 140 *S. aureus* isolates, the predominant CC types in pig-related workers were CC7 (nine isolates), CC59 (six isolates), CC6 (six isolates), and CC188 (four isolates), followed by CC9 (three isolates), CC15 (three isolates), CC45 (three isolates), and CC1 (two isolates). Similarly, CC7 (24 isolates), CC59 (13 isolates), CC6 (12 isolates), and CC188 (11 isolates) were common among *S. aureus* isolates from control workers (Table S1).

The *pvl* gene was only found in three *S. aureus* isolates from pig-related workers and was absent from all control workers. As to IEC genes, there were no significant differences in IEC(*scn, chp, sak, sea*)-negative *S. aureus* proportions between two groups (Table 2), while pig-related workers showed greater proportions of *sak*-negative MRSA and *sea*-negative MRSA carriage as compared with the control workers ($P = 0{\cdot}039$ and $P < 0{\cdot}001$, respectively, Table 2).

Overlap in phenotypic and molecular characteristics of livestock-associated CC9

For *S. aureus* CC9, presence of the livestock-associated characteristics (tetracycline resistance and IEC-negative) was more prevalent in pig-related workers than in control workers (7·0% *vs.* 0·0%, $P = 0{\cdot}028$).

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All CC9 isolates observed in pig-related workers were MRSA and also resistant to tetracycline, clindamycin, erythromycin, trimethoprim–sulfamethoxazole and gentamicin, whereas the CC9 strain isolated from a control worker was resistant only to penicillin (Table S1). Moreover, all CC9 isolates from pig-related workers were IEC (*scn, chp, sak*, and *sea*)-negative, while the CC9 from control workers carried the IEC genes (*scn, chp*, and *sak*).

Similar in phenotypic and molecular characteristics of human-associated CCs

For *S. aureus* CC7, there was no significant difference in IEC profile between two groups ($P = 0{\cdot}344$ for *scn;* $P = 0{\cdot}784$ for *chp;* $P = 0{\cdot}175$ for *sak;* $P = 0{\cdot}718$ for *sea*) and in antimicrobial resistance between two groups ($P = 0{\cdot}722$ for cefoxitin; $P = 0{\cdot}924$ for penicillin; $P = 0{\cdot}149$ for clindamycin; $P = 0{\cdot}344$ for erythromycin; $P = 0{\cdot}176$ for tetracycline; $P = 1{\cdot}000$ for chloromycetin and gentamicin; $P = 0{\cdot}533$ for ciprofloxacin; $P = 0{\cdot}586$ for trimethoprim–sulfamethoxazole; $P = 0{\cdot}522$ for rifampin; $P = 0{\cdot}093$ for nitrofurantoin; all CC7 isolates were susceptible to linezolid).

As to *S. aureus* CC59, there was similar in IEC profile between two groups ($P = 1{\cdot}000$ for *scn* and *chp;* $P = 0{\cdot}538$ for *sak;* $P = 0{\cdot}930$ for *sea*) and in antimicrobial resistance between two groups ($P = 0{\cdot}133$ for cefoxitin; $P = 0{\cdot}570$ for penicillin; $P = 0{\cdot}554$ for clindamycin; $P = 0{\cdot}550$ for erythromycin; $P = 0{\cdot}058$ for tetracycline; $P = 0{\cdot}701$ for chloromycetin; $P = 0{\cdot}307$ for ciprofloxacin and nitrofurantoin; all CC59 isolates were susceptible to trimethoprim–sulfamethoxazole, gentamicin, rifampin, and linezolid).

Similarly, CC6 *S. aureus* exhibited similar characteristics in the IEC profile between two groups ($P = 0{\cdot}169$ for *scn;* $P = 0{\cdot}762$ for *chp;* $P = 0{\cdot}643$ for *sak;* $P = 0{\cdot}701$ for *sea*) and in antimicrobial resistance between two groups ($P = 1{\cdot}000$ for cefoxitin and rifampin; $P = 0{\cdot}776$ for penicillin; $P = 0{\cdot}371$ for clindamycin; $P = 0{\cdot}223$ for erythromycin and tetracycline; $P = 0{\cdot}522$ for ciprofloxacin and trimethoprim–sulfamethoxazole; all CC6 isolates were susceptible to chloromycetin, gentamicin, nitrofurantoin, and linezolid).

CC188 *S. aureus* also showed similar characteristics in IEC profile between two groups ($P = 0{\cdot}553$ for *scn;* $P = 1{\cdot}000$ for *chp, sak*, and *sea*) and in antimicrobial resistance between two groups ($P = 0{\cdot}307$ for cefoxitin; $P = 1{\cdot}000$ for penicillin, tetracycline, chloromycetin, and nitrofurantoin; all CC188 isolates were susceptible to clindamycin, erythromycin, trimethoprim–sulfamethoxazole, ciprofloxacin, rifampin, gentamicin, and linezolid).

DISCUSSION

This observational study showed that human MRSA carriage is associated with occupational pig exposure. The most striking finding from this study was that the overlap of livestock-associated characteristics (tetracycline-resistant and IEC-negative) was observed only in MRSA CC9 isolated from pig-related workers and human-associated CCs had similar IEC profile and antimicrobial resistance in both pig-related and control workers.

The prevalence of MRSA carriage in pig-related workers (5·1%) in this study was comparable with data found in slaughterhouse workers and butchers in the Netherlands (5·6%) [11], Korea (6·9%) [18], Hong Kong (5·6%) [19], and the USA (3·6%) [20], but lower than prevalence among pig farm workers (farm workers in Germany [8], 86%; veterinarians in Germany [8], 45%; swine workers in America [9], 45%). Additionally, our study indicates that the prevalence of MRSA carriage is substantially higher in pig-related workers than in control workers and the prevalence is positively associated with occupational pig exposure after adjusting for other covariates, which is consistent with findings from previous studies [21, 22]. Notably, our recent study also has revealed that there are significant frequency-risk and duration-risk relations between occupational livestock (pig) exposure and MRSA carriage, suggesting the probability of LA-MRSA spread via animal contact, a scenario demonstrated for LA-MRSA transmission in Europe and Asia [23].

Since substantial amount of antimicrobials are used in livestock farming for growth promotion, prophylactic or therapeutic, increased resistance to multiple antimicrobials among MRSA isolates has become a matter of concern. A recent study conducted in Taiwan pig farms reported that multidrug resistance was prevalent, with >80% porcine MRSA isolates resistant to erythromycin, ciprofloxacin, gentamicin, tetracycline and clindamycin [24]. Another study in China observed that all MRSA from pig and pig farmers were resistant to cefoxitin, ciprofloxacin, clindamycin and tetracycline [25]. In Korea slaughterhouse, porcine MRSA were resistant to tetracycline, erythromycin, clindamycin and ciprofloxacin [18]. In our study, the predominant phenotype of *S. aureus*

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isolates from pig-related workers were resistant to penicillin, clindamycin, tetracycline and erythromycin, and this finding is in agreement with previous studies on pigs and related workers [18, 24, 25]. Note that tetracycline antimicrobials are commonly used in food animal production in Asian livestock farming, and high rates of resistance to tetracycline among MRSA isolates in pig and related workers have been reported [18, 24, 25]. Consistent with knowledge about the use of tetracycline in China, our study indicates that the proportion of tetracycline-resistant *S. aureus* (including MRSA) is significantly higher in pig-related workers than in control workers. These observations highlight the need for further surveillance data (including prevalence and antimicrobial susceptibility) to better understand the epidemiology and transmission of LA-MRSA between animals and humans.

Recently some studies have attempted to differentiate livestock-associated *S. aureus* from human-associated *S. aureus* based on the phenotypic and molecular characteristics [26, 27]. MRSA CC9 is usually isolated from pig and related workers in most Asian countries [7], which has been referred to as the molecular markers of livestock-association. In addition, there is increasing evidence of universal tetracycline resistance among MRSA isolates from livestock, related workers and environmental samples [24, 25, 28], indicating that tetracycline resistance may be an important phenotypic marker for livestock-associated *S. aureus*. In this study, all *S. aureus* CC9 found in pig-related workers were MRSA and resistant to tetracycline, while the isolate from control workers was MSSA and susceptible to tetracycline. Additionally, recent evidence has revealed that the bacteriophage-encoded IEC genes are associated with human specificity and less common in livestock-associated *S. aureus* [29, 30], suggesting that these characteristics (IEC-negative) may be useful to define livestock-associated *S. aureus*. Consistent with previous studies, we found that all MRSA CC9 from pig-related workers were absent of all IEC genes, but the *S. aureus* CC9 from control workers carried the IEC genes (*scn, chp*, and *sak*).

Our latest study on humans with no occupational livestock exposure revealed a potential relation between *S. aureus* CCs and IEC genes and antibiotic resistance patterns in defining livestock-associated *S. aureus* in humans [31]. Now, we build on previous literature to compare the overlap of livestock-associated characteristics of *S. aureus* in pig-related workers with those in control workers. We found that the overlap of livestock-associated characteristics was only observed in MRSA CC9 from pig-related workers, indicating a potential risk for livestock-to-human LA-MRSA transmission by occupational pig exposure. Note that CC7, CC59, CC6, and CC188 were the predominant CC types among *S. aureus* isolates from hospitalized patients [32, 33] and healthy workers in this study, indicating that these CC types probably belonged to human association. We found there was no significant difference in IEC profile or antimicrobial resistance for human-associated CCs between the groups, revealing the potential human-to-human transmission of human-associated MRSA. Future studies should direct more attention to exploring the exact transmission routes and distinguishing livestock-associated *S. aureus* from human-associated *S. aureus* according to the specific phenotypic and molecular characteristics.

This study contributed additionally to the literature by comparing the phenotypic and molecular characteristics of *S. aureus* in pig-related workers with control workers to explore the potential transmission routes of MRSA. However, this study also has potential limitations that cannot be ignored. Firstly, we identified MRSA from *S. aureus* through the detection of *mecA* gene and didn't detect the novel *mecA* homolog *mecC* gene, which should be improved in the further study. Secondly, although MRSA CC9 isolates were only detected in pig-related workers, we cannot draw a causal conclusion but only an association between occupational pig exposure and MRSA CC9 carriage due to the cross-sectional design. Results from this study need to be confirmed in a longitudinal study.

In conclusion, this study reports a detection of LA-MRSA CC9 isolates in pig slaughterhouse workers in China, and a significant overlap of livestock-associated characteristics occurring in LA-MRSA CC9 from pig-related workers, but not in isolates from control workers. In addition to CC9, we also found typical human-associated CCs, which have the similar IEC profile and antimicrobial resistance in both pig-related and control workers. This suggests that there may be a potential risk for livestock-to-human transmission of LA-MRSA and human-to-human transmission of human-associated MRSA.

SUPPLEMENTARY MATERIAL

The supplementary material for this article can be found at https://doi.org/10.1017/S0950268817000085

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DECLARATION OF INTEREST

None.

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