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Characterization of the Multi-Drug Resistance Gene *cfr* in Methicillin-Resistant *Staphylococcus aureus* (MRSA) Strains Isolated From Animals and Humans in China

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We investigated *cfr*-positive and -negative MRSA strains isolated from animals and humans in different geographical areas of China, from 2011 to 2016. Twenty *cfr*-positive strains (15.6%) were identified from 128 MRSA strains including 17 from food animals and three from humans. The resistance rates and prevalence of the tested antibiotic resistance genes (ARGs) in the *cfr*-positive MRSA isolates were higher than that in the *cfr*-negative MRSA isolates. All *cfr*-positive MRSA isolates were co-carrying *fexA* and *ermC*, and had significantly higher *optrA* incidence rate vs. the *cfr*-negative isolates ($P < 0.05$). In addition, multilocus sequence typing (MLST) assays showed that ST9 and *spa*-type t899 were the most prevalent ST and *spa* types in the study strains. However, all of the 20 *cfr*-positive and 10 randomly selected *cfr*-negative MRSA isolates were clonally unrelated as determined by pulsed-field gel electrophoresis (PFGE) analyses. Importantly, the *cfr* gene was successfully transferred to a recipient *Staphylococcus aureus* strain RN4220 from 13 of the 20 *cfr*-positive MRSA isolates by electroporation. Among these 13 *cfr*-positive MRSA isolates, two different genetic contexts surrounding *cfr* were determined and each was associated with one type of *cfr*-carrying plasmids. Of note, the predominant genetic context of *cfr* was found to be a Tn*558* variant and locate on large plasmids (∼50 kb) co-harboring *fexA* in 11 of the 13 MRSA isolates. Furthermore, the *cfr* gene was also identified on small plasmids (∼ 7.1 kb) that co-carried *ermC* in two of the 13 MRSA isolates. Our results demonstrated a high occurrence of multi-drug resistance in *cfr*-positive MRSA isolates, and the spread of *cfr* might be attributed to horizontal dissemination of similar *cfr*-carrying transposons and plasmids.

Keywords: *cfr*, MRSA, multi-drug resistance, plasmid, food animals

INTRODUCTION

The chloramphenicol–florfenicol resistance (*cfr*) gene encodes a methyltransferase that modifies position A-2503 in bacterial 23S rRNA and confers resistance to five classes of antibiotics (phenicols, lincosamides, oxazolidinones, pleuromutilins, and streptogramin A) (Long et al., 2006; Morales et al., 2010). These antibiotics have been widely used for the treatment of infections in human and animal (Inkster et al., 2017; Li J. et al., 2017). Since the first identification of the *cfr* gene in *Staphylococcus sciuri* isolates in 2000, it has been subsequently found in *Enterococcus* spp., *Bacillus* spp., *Streptococcus suis*, *Proteus vulgaris*, and *Escherichia coli* (Long et al., 2006; Wang et al., 2011, 2012a,b). In China, most *cfr*-positive isolates were derived from domestic animals (mainly pigs). In addition, plasmids and insertion sequences were implicated in *cfr* gene dissemination between species and genera (Shen et al., 2013).

Methicillin-resistant *Staphylococcus aureus* (MRSA) can cause a wide range of infections, including skin and soft-tissue infections as well as endocarditis and respiratory tract infections (Marshall and McBryde, 2014; Rodvold and McConeghy, 2014). Hospital-acquired MRSA (HA-MRSA) and community-acquired MRSA (CA-MRSA) are the primary origins for infections in humans (Woodford and Livermore, 2009). However, livestock-acquired MRSA (LA-MRSA) have been identified in pigs, ducks, poultry, and rats (Voss et al., 2005; Wulf et al., 2006; de Neeling et al., 2007; van de Giessen et al., 2009). Importantly, LA-MRSA containing the plasmid-borne *cfr* gene has been identified in infections of farmers suggesting zoonotic transmission (Wulf et al., 2006; Cui et al., 2009).

In this study, we investigated the epidemiological characteristics and dissemination of the *cfr* gene in clinical MRSA isolates from animal and human sources. We compared the phenotypic and genotypic profiles of *cfr*-positive MRSA strains with *cfr*-negative MRSA strains.

MATERIALS AND METHODS

In total, 128 MRSA strains were isolated from pigs, chickens, and ducks in 10 different regions of China and from clinical patients in two different hospitals in Guangzhou, China during 2011–2016. All MRSA isolates were confirmed by MALDI-TOF/MS system (Shimadzu-Biotech, Japan), multiplex PCR amplification, and DNA sequencing of the *mecA* gene.

Minimum inhibitory concentrations (MIC) were determined using a standard agar dilution method -CLSI M100-S28 and VET01-A4/VET01-S2. The tested antibiotics were phenicols (florfenicol), lincosamides (clindamycin), oxazolidinones (linezolid), pleuromutilins (valnemulin), β-lactams (ampicillin and cefotaxime), macrolides (tylosin, azithromycin, and erythromycin) and ciprofloxacin, gentamycin, tetracycline, rifampicin, trimethoprim-sulfamethoxazole, vancomycin, and daptomycin. The MIC breakpoints of each antibiotic against MRSA were used as recommended by the current CLSI guidance (Clinical and Laboratory Standards Institute [CLSI], 2013, 2018). *S. aureus* ATCC 29213 was used as a quality control strain.

Detection of Resistance Genes

The presence of the *cfr* gene in the MRSA strains was determined with PCR as described previously (Kehrenberg and Schwarz, 2006). Other genes that encoded resistance to phenicols (*fexA*), lincomycin [*lnu*(A), (F)], oxazolidinones (*optrA*), pleuromutilins (*vgaAV)*, macrolide–lincosamide–streptogramin B (*ermA-C*), macrolides (*ereA-B*), tetracycline [*tet*(A), (C), (L), (M), and (K)]. and aminoglycosides [*aac(3′)-Ia, aac(3′)-IIc*, *aadA1*, *aadB*, *aph(3′)-II*, *aph(3′)-IV*, *aph(4′)-Ia*, and *aac(6′)-Ib*] were identified by PCR using gene-specific primers (**Supplementary Table S1**).

Molecular Typing

Genetic diversity of *cfr*-positive and -negative MRSA isolates was determined by *SmaI* pulsed-field gel electrophoresis (PFGE) (Tenover et al., 1995). Comparison of PFGE patterns was performed with BioNumerics software (Applied Maths, Sint-Martens-Latem, Belgium). Dendrograms were generated using Dice similarity coefficient and analogical values to categorize identical PFGE types cut-offs were fixed at 100%. Further determinations of clonality were performed by multilocus sequence typing (MLST) and *spa* typing as described previously.[1,2] *Salmonella enterica* serotype Braenderup H9812 DNA was used as a molecular size marker (Tenover et al., 1995).

Transformation of *cfr* Gene and Determination of *cfr* Location

Plasmid DNA from *cfr*-positive MRSA strains was extracted using a Qiagen Prep Plasmid Midi Kit (Qiagen, Hilden, Germany) and transferred into a recipient *S. aureus* strain RN4220 by electroporation using Gene Pulser apparatus (Bio-Rad, Hercules, CA, United States). Electrotransformants were selected on brain heart infusion (BHI) agar containing 8 μg/mL of florfenicol. The presence of *cfr* was further confirmed by PCR (Kehrenberg et al., 2009). To determine the location of *cfr* gene, DNA was separated by PFGE after treatment with *S1* nuclease (Takara, Dalian, China) and plasmids carrying *cfr* were identified by Southern blot hybridization using a digoxigenin-labeled *cfr* probe (Roche, Mannheim, Germany) according to the manufacturer's instruction.

Genetic Environment of *cfr* Gene

The genetic environment surrounding *cfr* was determined by PCR mapping, inverse PCR, and sequencing (Wang et al., 2015). The primers used to determine the regions upstream and downstream of *cfr* gene and reference sequences containing the *cfr* gene used for PCR mapping are listed in **Supplementary Table S2**. The obtained DNA sequences were analyzed using BLAST,[3] and then compared to those deposited in GenBank.

[1]http://saureus.mlst.net/

[2]http://www.spaserver.ridom.de/

[3]https://blast.ncbi.nlm.nih.gov/Blast.cgi

Statistical Analyses

Statistical significance for the comparison of prevalence data and proportions was determined using a χ^2 test. $P < 0.05$ was consideredto be statistical significant.

RESULTS

Antimicrobial Susceptibility and Presence of Resistance Genes

We demonstrated that >80% of the 128 MRSA isolates were resistant to all tested antimicrobial agents with the exception of sulfamethozaxole/trimethoprim (43.8%), rifampicin (21.1%), linezolid (1.56%), vancomycin (0%), and daptomycin (0%) (**Figure 1**). Importantly, resistance rates in *cfr*-positive strains were higher than in *cfr*-negative strains for sulfamethozaxole/trimethoprim (60 vs. 40.7%) and rifampicin (30 vs. 19.4%) (**Figure 1**). In addition, the proportion of isolates with increased linezolid MIC (≥2 μg/mL) was significantly higher in the *cfr*-positive MRSA vs. the *cfr*-negative MRSA (40 vs. 6.5%, $P < 0.001$) (**Supplementary Table S3**).

In addition, 20 of the 128 MRSA strains (15.6%) harbored the *cfr* gene, and included 13 isolates from pigs (10.2%), three from chickens (2.3%), one from duck (0.8%), and three from humans (2.3%). Interestingly, all of the *cfr*-positive MRSA strains also carried the *fexA*, *ermC*, *ereA*, and *aadA1* genes (**Table 1**). In addition, the prevalence of all the other tested antibiotic resistance genes (ARGs) was higher in the *cfr*-positive MRSA isolates than in *cfr*-negative MRSA isolates, especially for the *optrA*, *ereB*, *aac (3′)-IIc*, and *aph (3′)-IV* genes ($P < 0.05$; **Figure 2**).

Molecular Typing

The 128 MRSA strains contained eight ST types and seven *spa* types, and ST9 (82.0%, 105/128) and *spa* type t899 (80.5%, 103/128) were predominated. In the *cfr*-positive MRSA isolates, three ST types and four *spa* types were observed, and ST9 (85%, 17/20) and *spa* type t899 (75%, 15/20) were also the most prevalent of these types. We observed 12 different profiles using a combination of MLST and *spa* typing in the 128 MRSA isolates. ST9-t899 (78.9%, 101/128) and ST764-t1084 (6.3%, 8/128) were the most and second most ST-*spa* types, respectively (**Supplementary Table S4**).

We also found 20 different PFGE profiles in the *cfr*-positive and 10 *cfr*-negative MRSA isolates (**Figure 3**). PFGE analysis suggested that MRSA isolates in the current study were epidemiologically unrelated clones.

Transfer of *cfr* and Plasmids Analyses

The *cfr* gene from 13 of the 20 *cfr*-positive MRSA isolates were successfully transferred to a recipient strain (*S. aureus* RN4220) and showed 4- to 64-fold increases in the MICs of florfenicol as compared with the recipient strain lacking the *cfr* gene. In addition, *cfr* gene transfenerated strains were resistant to erythromycin, azithromycin, and clindamycin. Co-transfer of *cfr* with *fexA* and *ermC* genes were found in eight of 13 electrotransformants. *S1*-PFGE and Southern blot hybridizations revealed that the *cfr* genes were located on plasmids with sizes of 50 kb ($n = 11$) or 7.1 kb ($n = 2$) (**Table 1**).

Genetic Environment of *cfr* Gene

The genomic structure surrounding *cfr* in the 13 *cfr*-carrying electrotransformants showed two different genetic contexts. Type I was the most common structure observed in 11 of 13 among which the *cfr* gene was located on ∼ 50 kb plasmids. The 9,880 bp

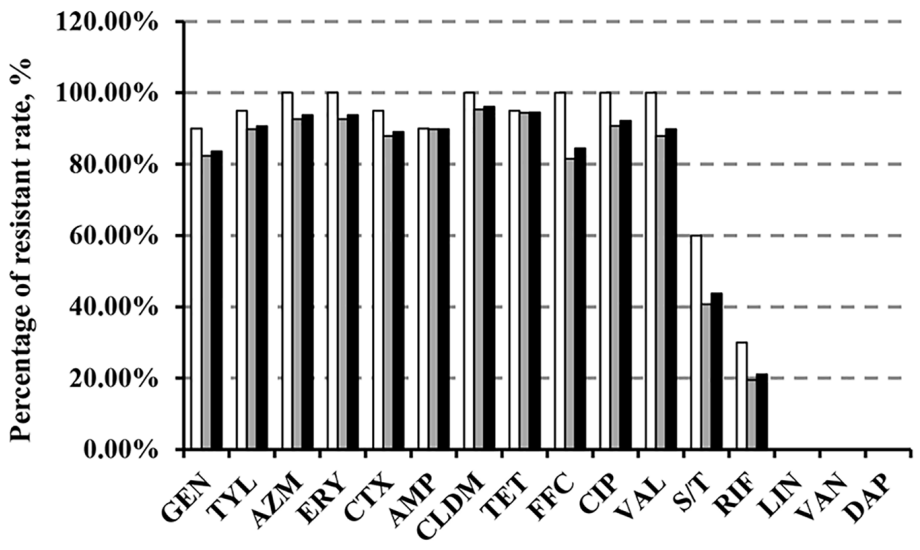


FIGURE 1 | Antibiotic resistance in MRSA strains from animals and humans. GEN, gentamicin; TYL, tylosin; AZM, azithromycin; ERY, erythromycin; CTX, cefotaxime; AMP, ampicillin; CLDM, clindamycin; TET, tetracycline; FFC, florfenicol; CIP, ciprofloxacin; VAL, valnemulin; S/T, sulfamethoxazole/trimethoprim; RIF, rifampicin; LIN, linezolid; VAN, vancomycin; DAP, daptomycin.

TABLE 1 | Background information and characteristics of *cfr*-positive MRSA.

Strains[a]	ST-*spa*	Year	Source	Resistance profile[b]	Other resistance genes[c]	*cfr* location (size, kb)	*cfr* genetic environmental types
5ZX13	ST9-t7880	2012	Pig	FFC, AMZ, ERY, CLDM, CIP, AMP, CTX, TET, GEN, TYL, RIF, VAL	*fexA*, *ermC* *optrA*, *ermA*	Plasmid (~50)	I
5ZB12	ST9-t899	2011	Pig	FFC, AMZ, ERY, CLDM, CIP, TYL, AMP, CTX, TET, GEN, S/T, VAL	*fexA*, *ermC*, *optrA*	Plasmid (~50)	I
6ZB3	ST9-t899	2012	Pig	FFC, AMZ, ERY, CLDM, CIP, AMP, CTX, TET, GEN, TYL, S/T, VAL	*fexA*, *ermC*, *optrA*	Plasmid (~50)	I
2ZG3	ST9-t899	2012	Pig	FFC, AMZ, ERY, CLDM, CIP, TYL, AMP, CTX, TET, GEN, TIG, S/T, VAL	*fexA*, *ermC*, *ermA*	Plasmid (~50)	I
2ZX3	ST9-t899	2012	Pig	FFC, AMZ, ERY, CLDM, CIP, AMP, CTX, TET, GEN, TYL, RIF, VAL	*fexA*, *ermC*, *optrA*, *ermA*	Plasmid (~50)	I
5ZB14	ST9-t899	2012	Pig	FFC, AMZ, ERY, CLDM, TYL, AMP, CTX, TET, GEN, CIP, S/T, VAL	*fexA*, *ermC*, *optrA*	Plasmid (~50)	I
N3	ST9-t899	2012	Pig	FFC, AMZ, ERY, CLDM, CIP, AMP, CTX, TET, TIG, GEN, TYL, VAL	*fexA*, *ermC*, *optrA*, *ermA*	Plasmid (~50)	I
25FS35	ST9-t899	2016	Pig	FFC, AMZ, ERY, CLDM, CIP, AMP, CTX, TET, RIF, TYL, S/T, VAL	*fexA*, *ermC*, *optrA*	Plasmid (~50)	I
HYB6	ST9-t899	2016	Pig	FFC, AMZ, ERY, CLDM, TYL, AMP, TIG,CTX, TET, GEN, CIP, VAL	*fexA*, *ermC*, *optrA*, *ermA*	Plasmid (~50)	I
YFC28	ST9-t899	2014	Chicken	FFC, AMZ, ERY, CLDM, TYL, TET, GEN, AMP, CTX, RIF, CIP, VAL	*fexA*, *ermC*, *optrA*, *ermA*	Plasmid (~50)	I
HB119	ST9-t899	2016	Chicken	FFC, AMZ, ERY, CLDM, AMP, CTX, TET, GEN, CIP, TYL, VAL	*fexA*, *ermC*, *ermA*	Plasmid (~50)	I
25FS24	ST9-t899	2016	Pig	FFC, AMZ, ERY, CLDM, TYL, CTX, TET, RIF, CIP, S/T, VAL	*fexA*, *ermC*, *optrA*	Plasmid (~7.1)	II
26FS31	ST9-t899	2016	Chicken	FFC, AMZ, ERY, CLDM, TYL, CTX, TET, GEN, CIP, BAC, VAL	*fexA*, *ermC*, *optrA*	Plasmid (~7.1)	II
6Y2C	ST398-t7829	2012	Duck	AMP, CTX, TET, FFC, GEN, CIP,TYL, AMZ, ERY, RIF, CLDM, S/T, VAL	*fexA*, *ermC*, *optrA*, *ermA*	ND	
6ZB5	ST9-t899	2012	Pig	AMP, CTX, TET, FFC, GEN, CIP, TYL, TIG, AMZ, ERY, CLDM, VAL	*fexA*, *ermC*, *optrA*, *ermA*	ND	
7SX2	ST9-t899	2012	Pig	AMP, CTX, TET, FFC, GEN, CIP, TYL, AMZ, ERY, CLDM, VAL	*fexA*, *ermC*, *optrA*, *ermA*	ND	
N4-2	ST9-t899	2012	Pig	AMP, CTX, TET, FFC, GEN, CIP, TYL, RIF, AMZ, ERY, CLDM, S/T, VAL	*fexA*, *ermC*, *optrA*, *ermA*	ND	
BA13	ST9-t899	2016	Human	AMP, CTX, TET, FFC, GEN, CIP, TYL, RIF, AMZ, ERY, CLDM, S/T, VAL	*fexA*, *ermC*, *optrA*	ND	
161429	ST764-t1084	2016	Human	AMP, CTX, TET, FFC, GEN, CIP, TYL, AMZ, ERY, CLDM, S/T, VAL	*fexA*, *ermC*, *optrA*, *ermA*	ND	
161494	ST764-t1084	2016	Human	AMP, CTX, TET, FFC, GEN, CIP, TYL, AMZ, ERY, CLDM, VAL	*fexA*, *ermC*, *optrA*, *ermA*	ND	

[a]*Isolates from electrotransformants are underlined.* [b]*Resistance profiles of transferred strains are underlined. AMP, ampicillin; CTX, cefotaxime; TET, tetracycline; FFC, florfenicol; GEN, gentamicin; CIP, ciprofloxacin; TYL, tylosin; AZM, azithromycin; ERY, erythromycin; CLDM, clindamycin; S/T, sulfamethoxazole/trimethoprim; RIF, rifampicin; VAL, valnemulin.* [c]*Genes that were co-transferred with cfr are underlined. ND, not determined.*

cfr-containing regions comprised a truncated *tnpA* (*DeltatnpA*), *istA*, *istB*, *cfr*, *tnpB*, *tnpC*, *orf138*, and *fexA*. This was a Tn*558* variant with a 5′ deletion of *tnpB* by insertion of the IS*21-558* element (*istA-B*) and *cfr* in the same orientation. Type II was similar to that in plasmid pHNCR35 (KF861983), pSS-02 (JX827253), pHK01 (KC820816), and pSA737 (KC206006). Type II was found in two electrotransformants among which the complete nucleotide sequences of 7,057-bp circular plasmids harboring *cfr* (p26FS31 and p25FS24) were obtained. A plasmid comparison based on a BLAST query revealed that p26FS31 and p25FS24 were identical to plasmid pSS-03 (JQ219851) and pHNLKJC2 (KF751701). These plasmids consisted of five open reading frames (ORF) (*rep-Deltapre/mob-cfr-pre/mob-ermC*) (**Figure 4**).

DISCUSSION

In this study, we investigated the prevalence of *cfr* in 128 MRSA strains isolated from animals and humans in China. Our study showed a significantly higher positive rate of *cfr* in LA-MRSA strains from animals (15.17%) than that recently

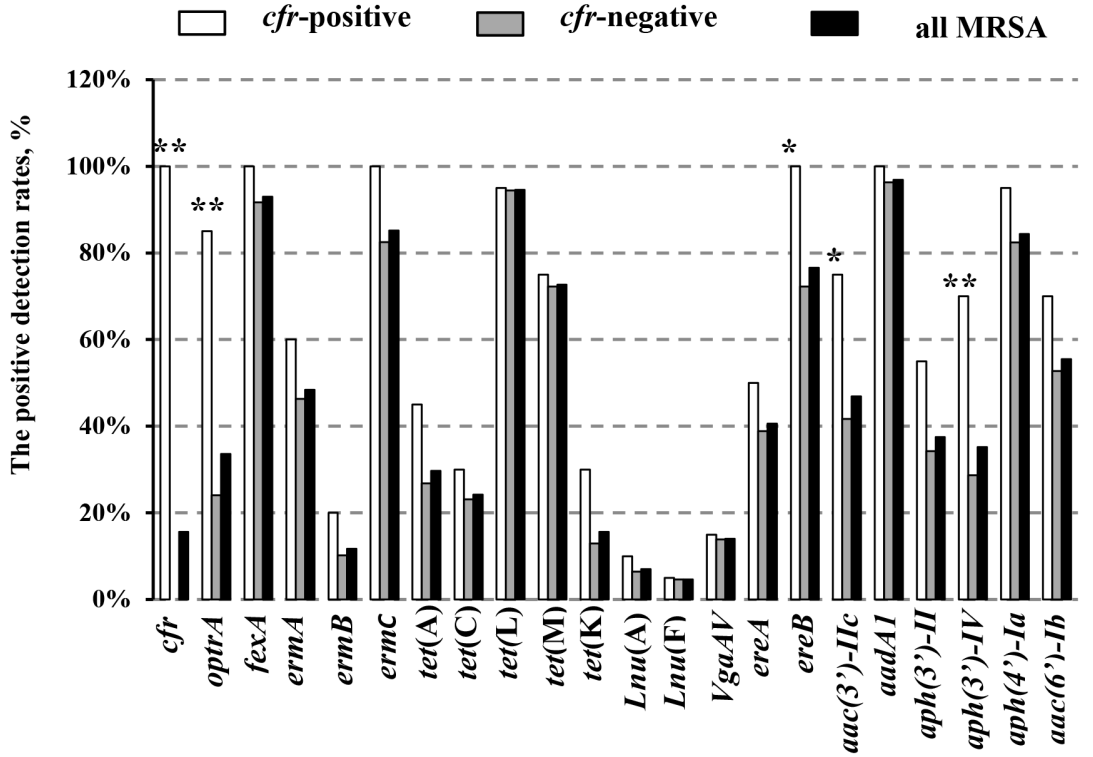
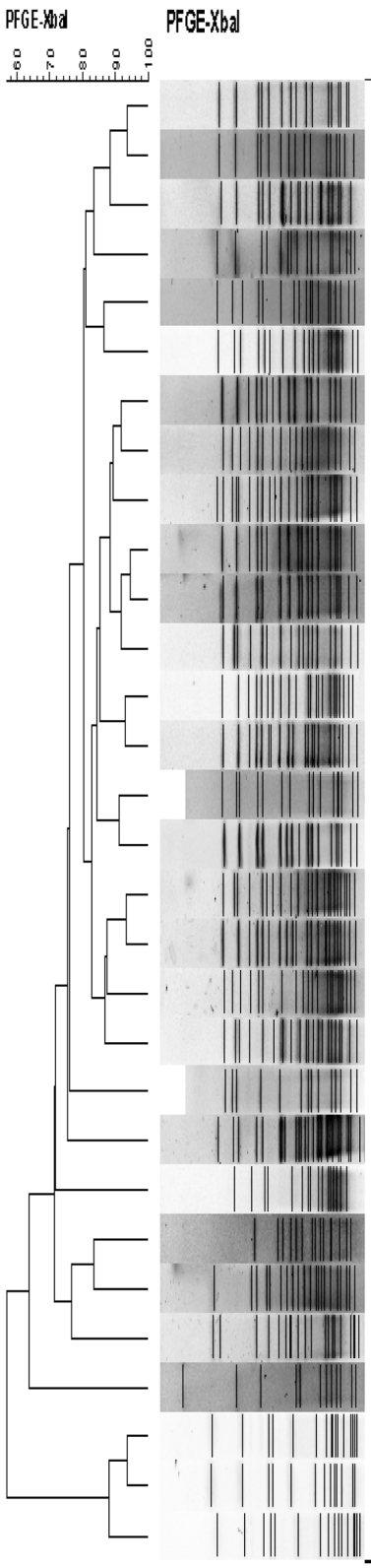


FIGURE 2 | Positive detection rates of genes related to antibiotic resistance in the study MRSA strains. Detection rates between the *cfr*-positive and *cfr*-negative MRSA strains were determined using the χ^2 test. $^*P < 0.05$; $^{**}P < 0.01$ *cfr*-positive MRSA strains vs. *cfr*-negative MRSA strains.

reported in domestic studies (1.11–3.46%) (Li et al., 2015; Li J. et al., 2017). In addition, *cfr* was also present in one MRSA isolate from domestic duck. To the best of our knowledge, this is the first report on the *cfr* gene in MRSA strains from waterfowl. This finding may implicate a recent and rapid dissemination process of *cfr* in MRSA strains from different food animals in China. Moreover, the prevalence of *cfr* in MRSA strains from humans (2.34%) was higher in the current study than that previously reported for clinical patients (0.30%) (Cai et al., 2015), but lower in isolates from a teaching hospital in a different region of China (9.38%) (Tian et al., 2014).

Most of the *cfr*-positive MRSA strains in the current study presented a multidrug-resistant phenotype and harbored diverse ARGs. These observations were similar to the high occurrence of multidrug resistance previously reported in *cfr*-positive MRSA isolates from swine farms and retail meat in China (Zeng et al., 2014; Li J. et al., 2017). In addition, the *cfr* gene has been reported to be associated with oxazolidinone resistance in several studies (Schwarz et al., 2000; Shen et al., 2013), but it only mediated low levels of resistance to this antibiotic class. In the current study, we found that the proportion of MRSA isolates with increased linezolid MICs in the *cfr*-positive MRSA strains was higher than in the *cfr*-negative MRSA strains. Interestingly, we also determined that the majority of *cfr*-positive MRSA isolates harbored *optrA*, which is in agreement with previous reports suggesting that *optrA* and *cfr* coexist (Li et al., 2016; Fan et al., 2017). In addition to *optrA*, our study *cfr*-positive MRSA isolates also co-carried *fexA* and *ermC*, which is also consistent with previous studies (Liu .X et al., 2017). This linked the cotransmission of *fexA* and *ermA-C* with *cfr* gene in diverse plasmids from coagulase-negative *Staphylococci* as well as *Enterobacteriaceae* of different origins (Wang et al., 2012a, 2013; Ye et al., 2015). Moreover, we observed different ratios of *ermA*, *ermB*, and *ermC* in our study strains that may be related to the location of the genes. For instance, *ermA* and *ermB* are primary chromosomal genes, while *ermC* gene is often plasmid-borne (Schwarz et al., 2011; Kadlec et al., 2012). The *ermB* was present in a minority of our strains, while *ermA* and *ermC* were frequent in MRSA strains (Lina et al., 1999; Liu H. et al., 2017). Furthermore, we found that the majority of the *cfr* genes were located on plasmids. Therefore, these factors may have influenced on the high ratio of *ermC* as we observed in the current studies.

Among all the study MRSA isolates, ST9 and t899 were the most prevalent ST and *spa* types, respectively. ST9 was reported as the predominant ST type in *S. aureus* isolates from animals in China (Cui et al., 2009), and sporadically occurred in Canada, England, Germany, and the United States (Mulders et al., 2010; Fessler et al., 2011; Dhup et al., 2015). In other and our current study, ST9 in *S. aureus* isolates were also found in farmers (Fessler et al., 2011; Dhup et al., 2015; Sun et al., 2015). Emergence of the *cfr* gene in the prevalent ST9 MRSA



Strains	Region	Source	Year	ST-Spa typing	PFGE	*cfr*-positive
G161494	GZ	human	2016	ST764-t1084	I	+
G161813	GZ	human	2016	ST764-t1084	II	-
G161429	GZ	human	2016	ST764-t1084	III	+
SD6	SD	chicken	2016	ST9-t899	IV	-
BA13	HY	people	2016	ST9-t899	V	+
PNB91	FS	pig	2011	ST9-t899	VI	-
G22FS18	FS	duck	2016	ST9-t899	VII	-
HYB6	HY	pig	2016	ST9-t899	VIII	+
HB119	QHD	chicken	2016	ST9-t899	VIIII	+
HB113	QHD	chicken	2016	ST9-t899	X	-
S11W	FS	pig	2012	ST9-t899	XI	-
G4ZB8	FS	pig	2012	ST9-t899	XII	-
G2ZX3	FS	pig	2012	ST9-t899	XIII	+
HYM6	HY	pig	2016	ST9-t899	XIV	-
HB127	QHD	chicken	2016	ST9-t899	XV	-
N3	FS	pig	2012	ST9-t899	XVI	+
G5ZB14	FS	pig	2012	ST9-t899	XVII	+
G7SX2	FS	pig	2012	ST9-t899	XVIII	+
G25FS35	FS	pig	2016	ST9-t899	XVIIII	+
G26FS31	FS	chicken	2016	ST9-t899	XX	+
G5ZX13	FS	pig	2012	ST9-t899	XXI	+
N4-2	FS	pig	2012	ST9-t899	XXII	+
AA12	HY	people	2016	ST59-t437	XXIII	-
G6Y2C	FS	duck	2012	ST398-t7829	XXIV	+
G6ZB5	FS	pig	2012	ST764-t1084	XXV	+
G25FS24	FS	pig	2016	ST9-t899	XXVI	+
YFC28	FS	chicken	2014	ST9-t899	XXVII	+
G5ZB12	FS	pig	2011	ST9-t899	XXVIII	+
G6ZB3	FS	pig	2012	ST9-t899	XXVIIII	+
G2ZG3	FS	pig	2012	ST9-t899	XXX	+

FIGURE 3 | PFGE fingerprint patterns of *SmaI*-digested total DNA preparations from 20 MRSA strains harboring *cfr* and 10 *cfr*-negatives MRSA strains. A similarity cutoff of 100% was used to identify a PFGE cluster. Guangzhou (GZ), Qinhuangdao (QHD), Foshan (FS), Shandong (SD), Huadu (HD), and Heyuan (HY). "+", *cfr*-positive; "–" <LIST>*cfr*-negative.

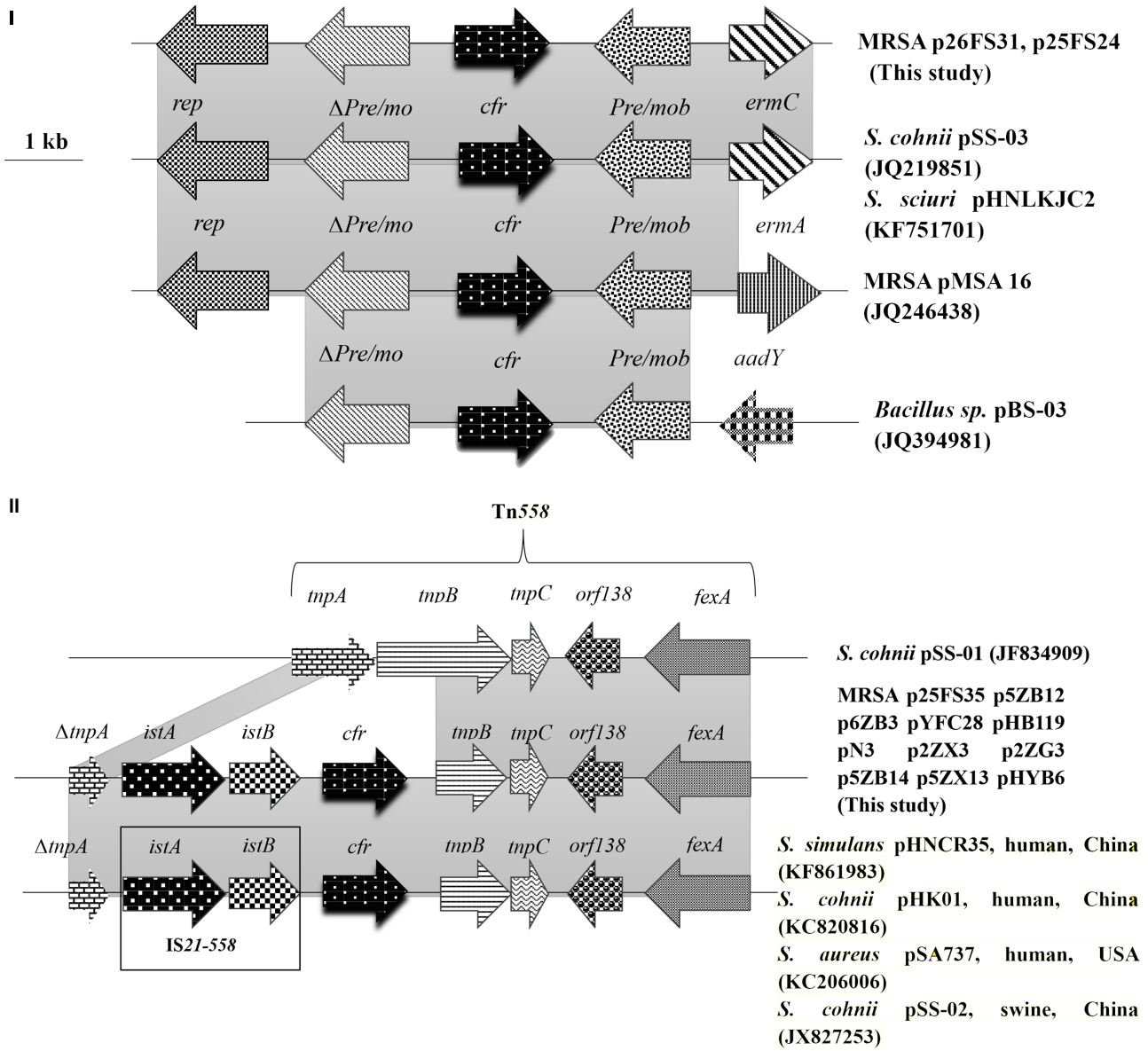


FIGURE 4 | The genetic context surrounding the *cfr* gene in plasmids and their structural comparison with plasmids possessing have >98% similarity. The arrows indicate the positions and directions of the transcription of each gene. Gray shaded regions indicate homology >98%. "*Delta*" represents a truncated gene.

isolates from pigs and pig-handlers would probably extend the potential reservoirs and expand the risk to human health (Ye et al., 2015; Yan et al., 2016). Since the ST398 was first identified in pigs and pig farmers in 2005 (Armand-Lefevre et al., 2005), it has become the most prevalent MLST-type in LA-MRSA in the United States and Europe (Armand-Lefevre et al., 2005; Cuny et al., 2010; Antoci et al., 2013). More importantly, the ST398 LA-MRSA carrying the *cfr* gene has been detected in Korea and other countries (Kadlec et al., 2012; Moon et al., 2015). Despite the wide and rapid dissemination of *cfr* gene in S. *aureus* isolates in China, to date, *cfr* was only identified in ST398 MRSA isolates from pigs (Li W. et al., 2017).

In the current study, we also found a *cfr*-positive ST398 MRSA strain isolated from a duck indicating a possibility of widespread dissemination of the *cfr*-harboring ST398 LA-MRSA clone in China. In addition, all of the three *cfr*-positive MRSA isolates from patients were identified as ST764, the increased prevalent hybrid variant of the ST5 HA-MRSA lineage with the arginine catabolic mobile element (ACME) in China, Japan, and other Asian areas (Otsuka et al., 2012; Nakaminami et al., 2014; Wang et al., 2016). These results indicated that *cfr*-positive MRSA isolates from animals and humans belonged to different ST types and were probably from epidemiologically unrelated MRSA clones.

In the MRSA isolates from food animals, the *cfr* genes were primarily located on two types of transferable plasmids with sizes of ∼ 50 and ∼ 7.1 kb. Two different genetic contexts surrounding *cfr* were found, and each was associated with one type of *cfr*-carrying plasmid. The predominant genetic context of *cfr* was found to be a Tn*558* variant in the large plasmids that co-carried *fexA*. This suggested that the acquisition of *cfr* could be involved in IS*21-558* mediated recombination. Importantly, the Tn*558* variant also occurred in *Bacillus*, *S. sciuri, Staphylococcus simulans*, and MRSA isolates from humans and swine (Wang et al., 2015; Li J. et al., 2017).

In addition, we also found the *cfr* gene on small plasmids that co-carried *ermC* in MRSA isolates from food animals.These small plasmids were also identified in *Staphylococcus* and *Bacillus* species isolates from pigs (Wang et al., 2012c). The high similarity of the genetic environment of *cfr* among diverse MRSA strains and sources indicated that horizontal transmission mediated by plasmids and transposons played a significant role in dissemination of *cfr*.

CONCLUSION

Our studies demonstrated higher antibiotic resistance rates in the *cfr*-positive vs. -negative MRSA isolates. Horizontal transmission mediated by plasmids and transposons likely played an important role in co-dissemination of *cfr* with *fexA* and *ermC*. The transmission of similar *cfr*-carrying transposons and plasmids from diverse bacteria species and origins requires continued investigation.

ETHICS STATEMENT

All procedure of strain isolation from animals was approved by the South China Agriculture University (SCAU) Animal Ethics Committee and conducted in strict accordance with technical guidelines for isolation and identification of animal-origin *Staphylococcus aureus* (DB51/T 2363-2017), as issued by the Quality and Technical Supervision Bureau of China, and in accordance with the SCAU Institutional Animal Care and Use Committee guidelines. The owner of farms from which animal-related samples were taken gave permission for their animals to be used in this study. All strains with human-origin were kindly provided by the Third Affiliated Hospital of Sun Yat-sen University and Guangdong Second Traditional Chinese Medicine Hospital, and the isolation procedure was in accordance with their Institutional Strain Isolation guidelines.

AUTHOR CONTRIBUTIONS

Y-HL and Y-QX designed and organized the study. S-ML did the research. J-HD, F-RL, H-QL, Y-TW, and W-QG did the assisted help. LL, L-XF, X-PL, and JS analyzed the data. S-ML and Y-FZ wrote the paper.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2018.02925/full#supplementary-material

REFERENCES

Antoci, E., Pinzone, M. R., Nunnari, G., Stefani, S., and Cacopardo, B. (2013). Prevalence and molecular characteristics of methicillin-resistant *Staphylococcus aureus* (MRSA) among subjects working on bovine dairy farms. *Infez. Med.* 21, 125–129.

Armand-Lefevre, L., Ruimy, R., and Andremont, A. (2005). Clonal comparison of *Staphylococcus aureus* isolates from healthy pig farmers, human controls, and pigs. *Emerg. Infect. Dis.* 11, 711–714. doi: 10.3201/eid1105.040866

Cai, J. C., Hu, Y. Y., Zhou, H. W., Chen, G. X., and Zhang, R. (2015). Dissemination of the same cfr-carrying plasmid among methicillin-resistant *Staphylococcus aureus* and coagulase-negative staphylococcal isolates in China. *Antimicrob. Agents Chemother.* 59, 3669–3671. doi: 10.1128/AAC.04580-14

Clinical and Laboratory Standards Institute [CLSI] (2013). *Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests Forbacteria Isolated from Animals; Approved Standard. VET01-A4/VET01-S2.* Wayne, PA: CLSI.

Clinical and Laboratory Standards Institute [CLSI] (2018). *Performance Standards for Antimicrobial Susceptibility Testing. CLSI Document M100-S28.* Wayne, PA: CLSI.

Cui, S., Li, J., Hu, C., Jin, S., Li, F., Guo, Y., et al. (2009). Isolation and characterization of methicillin-resistant *Staphylococcus aureus* from swine and workers in China. *J. Antimicrob. Chemother.* 64, 680–683. doi: 10.1093/jac/dkp275

Cuny, C., Friedrich, A., Kozytska, S., Layer, F., Nubel, U., Ohlsen, K., et al. (2010). Emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) in different animal species. *Int. J. Med. Microbiol.* 300, 109–117. doi: 10.1016/j.ijmm.2009.11.002

de Neeling, A. J., Van Den Broek, M. J., Spalburg, E. C., Van Santen-Verheuvel, M. G., Dam-Deisz, W. D., Boshuizen, H. C., et al. (2007). High prevalence of methicillin resistant *Staphylococcus aureus* in pigs. *Vet. Microbiol.* 122, 366–372. doi: 10.1016/j.vetmic.2007.01.027

Dhup, V., Kearns, A. M., Pichon, B., and Foster, H. A. (2015). First report of identification of livestock-associated MRSA ST9 in retail meat in England. *Epidemiol. Infect.* 143, 2989–2992. doi: 10.1017/S0950268815000126

Fan, R., Li, D., Fessler, A. T., Wu, C., Schwarz, S., and Wang, Y. (2017). Distribution of optrA and cfr in florfenicol-resistant *Staphylococcus sciuri* of pig origin. *Vet. Microbiol.* 210, 43–48. doi: 10.1016/j.vetmic.2017.07.030

Fessler, A. T., Kadlec, K., Hassel, M., Hauschild, T., Eidam, C., Ehricht, R., et al. (2011). Characterization of methicillin-resistant *Staphylococcus aureus* isolates from food and food products of poultry origin in Germany. *Appl. Environ. Microbiol.* 77, 7151–7157. doi: 10.1128/AEM.00561-11

Inkster, T., Coia, J., Meunier, D., Doumith, M., Martin, K., Pike, R., et al. (2017). First outbreak of colonization by linezolid- and glycopeptide-resistant *Enterococcus faecium* harbouring the cfr gene in a UK nephrology unit. *J. Hosp. Infect.* 97, 397–402. doi: 10.1016/j.jhin.2017.07.003

Kadlec, K., Fessler, A. T., Hauschild, T., and Schwarz, S. (2012). Novel and uncommon antimicrobial resistance genes in livestock-associated methicillin-resistant *Staphylococcus aureus*. *Clin. Microbiol. Infect.* 18, 745–755. doi: 10.1111/j.1469-0691.2012.03842.x

Kehrenberg, C., Cuny, C., Strommenger, B., Schwarz, S., and Witte, W. (2009). Methicillin-resistant and -susceptible *Staphylococcus aureus* strains of clonal lineages ST398 and ST9 from swine carry the multidrug resistance gene cfr. *Antimicrob. Agents Chemother.* 53, 779–781. doi: 10.1128/AAC.01376-08

Kehrenberg, C., and Schwarz, S. (2006). Distribution of florfenicol resistance genes fexA and cfr among chloramphenicol-resistant staphylococcus isolates. *Antimicrob. Agents Chemother.* 50, 1156–1163. doi: 10.1128/AAC.50.4.1156-1163.2006

Li, D., Wang, Y., Schwarz, S., Cai, J., Fan, R., Li, J., et al. (2016). Co-location of the oxazolidinone resistance genes optrA and cfr on a multiresistance plasmid from *Staphylococcus sciuri*. *J. Antimicrob. Chemother.* 71, 1474–1478. doi: 10.1093/jac/dkw040

Li, D., Wu, C., Wang, Y., Fan, R., Schwarz, S., and Zhang, S. (2015). Identification of multiresistance gene cfr in methicillin-resistant *Staphylococcus aureus* from pigs: plasmid location and integration into a Staphylococcal cassette chromosome mec complex. *Antimicrob. Agents Chemother.* 59, 3641–3644. doi: 10.1128/AAC.00500-15

Li, J., Jiang, N., Ke, Y., Fessler, A. T., Wang, Y., Schwarz, S., et al. (2017). Characterization of pig-associated methicillin-resistant *Staphylococcus aureus*. *Vet. Microbiol.* 201, 183–187. doi: 10.1016/j.vetmic.2017.01.017

Li, W., Liu, J. H., Zhang, X. F., Wang, J., Ma, Z. B., Chen, L., et al. (2017). Emergence of methicillin-resistant *Staphylococcus aureus* ST398 in pigs in China. *Int. J. Antimicrob. Agents* 51, 275–276. doi: 10.1016/j.ijantimicag.2017.10.013

Lina, G., Quaglia, A., Reverdy, M. E., Leclercq, R., Vandenesch, F., and Etienne, J. (1999). Distribution of genes encoding resistance to macrolides, lincosamides, and streptogramins among *Staphylococci*. *Antimicrob. Agents Chemother.* 43, 1062–1066. doi: 10.1128/AAC.43.5.1062

Liu, H., Li, S., Meng, L., Dong, L., Zhao, S., Lan, X., et al. (2017). Prevalence, antimicrobial susceptibility, and molecular characterization of *Staphylococcus aureus* isolated from dairy herds in northern China. *J. Dairy Sci.* 100, 8796–8803. doi: 10.3168/jds.2017-13370

Liu, X., Deng, S., Huang, J., Huang, Y., Zhang, Y., Yan, Q., et al. (2017). Dissemination of macrolides, fusidic acid and mupirocin resistance among *Staphylococcus aureus* clinical isolates. *Oncotarget* 8, 58086–58097. doi: 10.18632/oncotarget.19491

Long, K. S., Poehlsgaard, J., Kehrenberg, C., Schwarz, S., and Vester, B. (2006). The Cfr rRNA methyltransferase confers resistance to phenicols, lincosamides, oxazolidinones, pleuromutilins, and streptogramin A antibiotics. *Antimicrob. Agents Chemother.* 50, 2500–2505. doi: 10.1128/AAC.00131-06

Marshall, C., and McBryde, E. (2014). The role of *Staphylococcus aureus* carriage in the pathogenesis of bloodstream infection. *BMC Res. Notes* 7:428. doi: 10.1186/1756-0500-7-428

Moon, D. C., Tamang, M. D., Nam, H. M., Jeong, J. H., Jang, G. C., Jung, S. C., et al. (2015). Identification of livestock-associated methicillin-resistant *Staphylococcus aureus* isolates in Korea and molecular comparison between isolates from animal carcasses and slaughterhouse workers. *Foodborne Pathog. Dis.* 12, 327–334. doi: 10.1089/fpd.2014.1868

Morales, G., Picazo, J. J., Baos, E., Candel, F. J., Arribi, A., Pelaez, B., et al. (2010). Resistance to linezolid is mediated by the cfr gene in the first report of an outbreak of linezolid-resistant *Staphylococcus aureus*. *Clin. Infect. Dis.* 50, 821–825. doi: 10.1086/650574

Mulders, M. N., Haenen, A. P., Geenen, P. L., Vesseur, P. C., Poldervaart, E. S., Bosch, T., et al. (2010). Prevalence of livestock-associated MRSA in broiler flocks and risk factors for slaughterhouse personnel in the Netherlands. *Epidemiol. Infect.* 138, 743–755. doi: 10.1017/S0950268810000075

Nakaminami, H., Noguchi, N., Ito, A., Ikeda, M., Utsumi, K., Maruyama, H., et al. (2014). Characterization of methicillin-resistant *Staphylococcus aureus* isolated from tertiary care hospitals in Tokyo, Japan. *J. Infect. Chemother.* 20, 512–515. doi: 10.1016/j.jiac.2014.03.006

Otsuka, T., Zaraket, H., Fujii, K., Masuda, Y., Komiyama, K., Ishikawa, Y., et al. (2012). Molecular epidemiology of methicillin-resistant *Staphylococcus aureus* isolated from children in a community with low antimicrobial pressure in Japan. *Jpn. J. Infect. Dis.* 65, 483–488. doi: 10.7883/yoken.65.483

Rodvold, K. A., and McConeghy, K. W. (2014). Methicillin-resistant *Staphylococcus aureus* therapy: past, present, and future. *Clin. Infect. Dis.* 58(Suppl. 1), S20–S27. doi: 10.1093/cid/cit614

Schwarz, S., Fessler, A. T., Hauschild, T., Kehrenberg, C., and Kadlec, K. (2011). Plasmid-mediated resistance to protein biosynthesis inhibitors in *Staphylococci*. *Ann. N. Y. Acad. Sci.* 1241, 82–103. doi: 10.1111/j.1749-6632.2011.06275.x

Schwarz, S., Werckenthin, C., and Kehrenberg, C. (2000). Identification of a plasmid-borne chloramphenicol-florfenicol resistance gene in *Staphylococcus sciuri*. *Antimicrob. Agents Chemother.* 44, 2530–2533. doi: 10.1128/AAC.44.9.2530-2533.2000

Shen, J., Wang, Y., and Schwarz, S. (2013). Presence and dissemination of the multiresistance gene cfr in gram-positive and gram-negative bacteria. *J. Antimicrob. Chemother.* 68, 1697–1706. doi: 10.1093/jac/dkt092

Sun, J., Yang, M., Sreevatsan, S., and Davies, P. R. (2015). Prevalence and characterization of *Staphylococcus aureus* in growing pigs in the USA. *PLoS One* 10:e0143670. doi: 10.1371/journal.pone.0143670

Tenover, F. C., Arbeit, R. D., Goering, R. V., Mickelsen, P. A., Murray, B. E., Persing, D. H., et al. (1995). Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J. Clin. Microbiol.* 33, 2233–2239.

Tian, Y., Li, T., Zhu, Y., Wang, B., Zou, X., and Li, M. (2014). Mechanisms of linezolid resistance in staphylococci and enterococci isolated from two teaching hospitals in Shanghai, China. *BMC Microbiol.* 14:292. doi: 10.1186/s12866-014-0292-5

van de Giessen, A. W., Van Santen-Verheuvel, M. G., Hengeveld, P. D., Bosch, T., Broens, E. M., and Reusken, C. B. (2009). Occurrence of methicillin-resistant *Staphylococcus aureus* in rats living on pig farms. *Prev. Vet. Med.* 91, 270–273. doi: 10.1016/j.prevetmed.2009.05.016

Voss, A., Loeffen, F., Bakker, J., Klaassen, C., and Wulf, M. (2005). Methicillin-resistant *Staphylococcus aureus* in pig farming. *Emerg. Infect. Dis.* 11, 1965–1966. doi: 10.3201/eid1112.050428

Wang, J., Lin, D. C., Guo, X. M., Wei, H. K., Liu, X. Q., Chen, X. J., et al. (2015). Distribution of the multidrug resistance gene cfr in staphylococcus isolates from pigs, workers, and the environment of a hog market and a slaughterhouse in Guangzhou, China. *Foodborne Pathog. Dis.* 12, 598–605. doi: 10.1089/fpd.2014.1891

Wang, R., Wan, H. Y., Shi, G. C., Li, M., Han, L. Z., Jin, X. Y., et al. (2016). Gene typing and antibiotic resistance of methicillin-resistant *Staphylococcus aureus* isolated from lower respiratory tract at two hospitals in Shanghai. *Zhonghua Jie He He Hu Xi Za Zhi* 39, 286–290. doi: 10.3760/cma.j.issn.1001-0939.2016.04.007

Wang, Y., He, T., Schwarz, S., Zhao, Q., Shen, Z., Wu, C., et al. (2013). Multidrug resistance gene cfr in methicillin-resistant coagulase-negative staphylococci from chickens, ducks, and pigs in China. *Int. J. Med. Microbiol.* 303, 84–87. doi: 10.1016/j.ijmm.2012.12.004

Wang, Y., He, T., Schwarz, S., Zhou, D., Shen, Z., Wu, C., et al. (2012a). Detection of the staphylococcal multiresistance gene cfr in *Escherichia coli* of domestic-animal origin. *J. Antimicrob. Chemother.* 67, 1094–1098. doi: 10.1093/jac/dks020

Wang, Y., Wang, Y., Schwarz, S., Shen, Z., Zhou, N., Lin, J., et al. (2012b). Detection of the staphylococcal multiresistance gene cfr in *Macrococcus caseolyticus* and *Jeotgalicoccus pinnipedialis*. *J. Antimicrob. Chemother.* 67, 1824–1827. doi: 10.1093/jac/dks163

Wang, Y., Zhang, W., Wang, J., Wu, C., Shen, Z., Fu, X., et al. (2012c). Distribution of the multidrug resistance gene cfr in *Staphylococcus* species isolates from swine farms in China. *Antimicrob. Agents Chemother.* 56, 1485–1490. doi: 10.1128/AAC.05827-11

Wang, Y., Wang, Y., Wu, C. M., Schwarz, S., Shen, Z., Zhang, W., et al. (2011). Detection of the staphylococcal multiresistance gene cfr in Proteus vulgaris of food animal origin. *J. Antimicrob. Chemother.* 66, 2521–2526. doi: 10.1093/jac/dkr322

Woodford, N., and Livermore, D. M. (2009). Infections caused by gram-positive bacteria: a review of the global challenge. *J. Infect.* 59(Suppl. 1), S4–S16. doi: 10.1016/S0163-4453(09)60003-7

Wulf, M., Van Nes, A., Eikelenboom-Boskamp, A., De Vries, J., Melchers, W., Klaassen, C., et al. (2006). Methicillin-resistant *Staphylococcus aureus* in veterinary doctors and students, the Netherlands. *Emerg. Infect. Dis.* 12, 1939–1941. doi: 10.3201/eid1212.060355

Yan, X., Li, Z., Chlebowicz, M. A., Tao, X., Ni, M., Hu, Y., et al. (2016). Genetic features of livestock-associated *Staphylococcus aureus* ST9 isolates from Chinese pigs that carry the lsa(E) gene for quinupristin/dalfopristin resistance. *Int. J. Med. Microbiol.* 306, 722–729. doi: 10.1016/j.ijmm.2016.08.001

Ye, X., Liu, W., Fan, Y., Wang, X., Zhou, J., Yao, Z., et al. (2015). Frequency-risk and duration-risk relations between occupational livestock contact and methicillin-resistant *Staphylococcus aureus* carriage among workers in Guangdong, China. *Am. J. Infect. Control* 43, 676–681. doi: 10.1016/j.ajic.2015.03.026

Zeng, Z. L., Wei, H. K., Wang, J., Lin, D. C., Liu, X. Q., and Liu, J. H. (2014). High prevalence of cfr-producing *Staphylococcus* species in retail meat in Guangzhou, China. *BMC Microbiol.* 14:151. doi10.1186/1471-2180-14-151

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.