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 Tn558 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 McBay, 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 daptoycin. 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 (Kebergen and Schwarz, 2006). Other genes that encoded resistance to phenicols (fexA), lincomycin [Inu(A), (F)], oxazolidinones (aptrA), pleuromutilins (vg4AV), 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 (Kebergen 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.

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1http://saureus.mlst.net/
2http://www.spaserver.ridom.de/
3https://blast.ncbi.nlm.nih.gov/Blast.cgi
Statistical Analyses
Statistical significance for the comparison of prevalence data and proportions was determined using a $\chi^2$ test. $P < 0.05$ was considered to be statistically 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 sulfamethoxazole/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 sulfamethoxazole/trimethoprim (60 vs. 40.7%) and rifampicin (30 vs. 19.4%) (Figure 1). In addition, the proportion of isolates with increased linezolid MIC ($\geq 2$ $\mu$g/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. SI-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 $\sim 50$ kb plasmids. The 9,880 bp
cfr-containing regions comprised a truncated \textit{tnpA} (\textit{DeltatnpA}), \textit{istA}, \textit{istB}, \textit{cfr}, \textit{tnpB}, \textit{tnpC}, \textit{orf138}, and \textit{fexA}. This was a Tn558 variant with a 5’ deletion of \textit{tnpB} by insertion of the IS2158 element (\textit{istA-B}) and \textit{cfr} in the same orientation. Type II was similar to that in plasmid pHNCR35 (KF861983), pSS-02 (JX827253), pHK01 (KC820816), and pSA737 (KCN06006). Type II was found in two electrotransformants among which the complete nucleotide sequences of 7,057-bp circular plasmids harboring \textit{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) (\textit{rep-Deltapre/mob-cfr-pre/mob-ermC}) (Figure 4).

**DISCUSSION**

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

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**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 |
|---|---|---|---|---|---|---|---|
| 5ZX1 | ST9-t7880 | 2012 | Pig | FFC, AMZ, ERY, GLDM, CIP, AMP, CTX, TET, GEN, TYL, RIF, VAL | fexA, ermC | Plasmid (~50) | I |
| 5ZB12 | ST9-t899 | 2011 | Pig | FFC, AMZ, ERY, GLDM, CIP, AMP, CTX, TET, GEN, TYL, RIF, VAL | fexA, ermC, optrA | Plasmid (~50) | I |
| 6ZB3 | ST9-t899 | 2012 | Pig | FFC, AMZ, ERY, GLDM, CIP, AMP, CTX, TET, GEN, TYL, S/T, VAL | fexA, ermC, optrA | Plasmid (~50) | I |
| 2ZX3 | ST9-t899 | 2012 | Pig | FFC, AMZ, ERY, GLDM, CIP, AMP, CTX, TET, GEN, TYL, RIF, VAL | fexA, ermC, optrA | Plasmid (~50) | I |
| 5ZB14 | ST9-t899 | 2012 | Pig | FFC, AMZ, ERY, GLDM, AMP, CTX, TET, GEN, CIP, S/T, VAL | fexA, ermC, optrA | Plasmid (~50) | I |
| N9 | ST9-t899 | 2012 | Pig | FFC, AMZ, ERY, GLDM, CIP, AMP, CTX, TET, GEN, CIP, S/T, VAL | fexA, ermC, optrA | Plasmid (~50) | I |
| 25FS35 | ST9-t899 | 2016 | Pig | FFC, AMZ, ERY, GLDM, CIP, AMP, CTX, TET, GEN, CIP, S/T, VAL | fexA, ermC, optrA | Plasmid (~50) | I |
| HJB6 | ST9-t899 | 2016 | Pig | FFC, AMZ, ERY, GLDM, CIP, AMP, CTX, TET, GEN, CIP, S/T, VAL | fexA, ermC, optrA | Plasmid (~50) | I |
| YFC28 | ST9-t899 | 2014 | Chicken | FFC, AMZ, ERY, GLDM, TLY, TET, GEN, AMP, CTX, TET, S/T, VAL | fexA, ermC, optrA | Plasmid (~50) | I |
| HB119 | ST9-t899 | 2016 | Chicken | FFC, AMZ, ERY, GLDM, CIP, AMP, CTX, TET, GEN, CIP, S/T, VAL | fexA, ermC, optrA | Plasmid (~50) | I |
| 25FS24 | ST9-t899 | 2016 | Pig | FFC, AMZ, ERY, GLDM, TLY, CTX, TET, GEN, CIP, S/T, VAL | fexA, ermC, optrA | Plasmid (~50) | I |
| 26FS31 | ST9-t899 | 2016 | Chicken | FFC, AMZ, ERY, GLDM, TLY, CTX, TET, GEN, CIP, S/T, VAL | fexA, ermC, optrA | Plasmid (~7.1) | II |
| 6Y2C | ST398-t7829 | 2012 | Duck | AMP, CTX, TET, FFC, GEN, CIP, TLYL, AMZ, ERY, RIF, GLDM, S/T, VAL | fexA, ermC, optrA | ND | |
| 6ZB5 | ST9-t899 | 2012 | Pig | AMP, CTX, TET, FFC, GEN, CIP, TLYL, AMZ, ERY, RIF, GLDM, S/T, VAL | fexA, ermC, optrA | ND | |
| 7SX2 | ST9-t899 | 2012 | Pig | AMP, CTX, TET, FFC, GEN, CIP, TLYL, AMZ, ERY, CDM, S/T, VAL | fexA, ermC, optrA | ND | |
| N4-2 | ST9-t899 | 2012 | Pig | AMP, CTX, TET, FFC, GEN, CIP, TLYL, AMZ, ERY, CDM, S/T, VAL | fexA, ermC, optrA | ND | |
| BA13 | ST9-t899 | 2016 | Human | AMP, CTX, TET, FFC, GEN, CIP, TLYL, AMZ, ERY, CDM, S/T, VAL | fexA, ermC, optrA | ND | |
| 161429 | ST764-t1084 | 2016 | Human | AMP, CTX, TET, FFC, GEN, CIP, TLYL, AMZ, ERY, CDM, S/T, VAL | fexA, ermC, optrA | ND | |
| 161494 | ST764-t1084 | 2016 | Human | AMP, CTX, TET, FFC, GEN, CIP, TLYL, AMZ, ERY, CDM, VAL | fexA, ermC, optrA | ND | |

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

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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
**FIGURE 3** | PFGE fingerprint patterns of Smal-digested total DNA preparations from 20 MRSA strains harboring cfr and 10 cfr-negative 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.

| 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     | VIII | +            |
| 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     | XVIII| + |
| 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     | XXVIII| + |
| G22ZG3   | FS     | pig    | 2012 | ST9-t899     | XXX  | +            |
is 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-Lefèvre et al., 2005), it has become the most prevalent MLST-type in LA-MRSA in the United States and Europe (Armand-Lefèvre 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 ST5 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.

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.
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 Tn558 variant in the large plasmids that co-carried fexA. This suggested that the acquisition of cfr could be involved in IS21-558 mediated recombination. Importantly, the Tn558 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

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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.

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