FOODBORNE PATHOGENS AND DISEASE
Volume 0, Number 0, 2015

DOI: 10.1089/fpd.2014.1891

Original Article

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

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Abstract

Bacteria harboring *cfr*, a multidrug resistance gene, have high prevalence in livestock in China and might be transmitted to humans through direct contact or via contaminated food products. To better understand the epidemiology of *cfr* producers in the food chain, the prevalence and genetic analysis of *Staphylococcus* isolates recovered from pigs, workers, and meat-handling facilities (a slaughterhouse and a hog market in Guangzhou, China) were examined. Twenty (4.5%) *cfr*-positive *Staphylococcus* isolates (18 *Staphylococcus simulans*, 1 *S. cohnii*, and 1 *S. aureus*) were derived from pigs (16/312), the environment (2/52), and workers (2/80). *Sma*I pulsed-field gel electrophoresis of 26 staphylococcal strains (22 *S. simulans* and 4 *S. cohnii*), including previously reported *cfr*-carrying staphylococci of animal food origin, exhibited 19 major pulsed-field gel electrophoresis patterns (A–S). Clonal spread of *cfr*-carrying staphylococci among pigs, workers, and meat products was detected. The genetic contexts of *cfr* in plasmids (pHNKF3, pHNZT2, and pHNCR35) obtained from *S. simulans* of swine or human origin were similar to that of *Staphylococcus* species isolated from human clinics and animal-derived food. The *cfr*-carrying *S. aureus* strain isolated from floor swabs of the hog market was *spa*-type t889 and belonged to the ST9 clonal lineage. In summary, both clonal spread and horizontal transmission via mobile elements contributed to *cfr* dissemination among staphylococcal isolates obtained from different sources. To monitor potential outbreaks of *cfr*-positive bacteria, continued surveillance of this gene in animals at slaughter and in animal-derived food is warranted.

Introduction

Linezolid was the first oxazolidinone antibiotic introduced in 2000 for the clinical treatment of infections caused by Gram-positive pathogens, especially methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (Shaw and Barbachyn, 2011). Resistance to linezolid was first reported in a MRSA isolate from a patient who received linezolid treatment in 2001 (Tsiodras *et al*., 2001). The prevalence of linezolid resistance among staphylococci is relatively low, presumably because mutation-mediated resistance develops slowly and is not transmissible (Shaw and Barbachyn, 2011; Witte and Cuny, 2011). However, the emergence of transferable linezolid resistance mediated by *cfr* is a global concern and poses a significant challenge to the clinical treatment of this infection.

The transferable gene *cfr*, originally identified in the pSCFS1 plasmid from a bovine *S. sciuri* isolate (Schwarz *et al*., 2000), encodes an RNA methyltransferase that modifies the adenosine at position 2503 in 23S rRNA. *Cfr* mediates resistance to oxazolidinones, phenicols, lincosamides, pleuromutilins, and streptogramin A, all of which are widely used for treating human and animal infections (Long *et al*., 2006). To date, *cfr* has been detected in *Staphylococcus*, *Enterococcus*, *Bacillus*, *Macrococcus*, *Jeotgalicoccus*, *Escherichia coli*, *Proteus vulgaris* (Shen *et al*., 2013), and *Streptococcus suis* (Wang *et al*., 2013) of animal origin and is also found globally in human clinical *Staphylococcus* and *Enterococcus* isolates (Shen *et al*., 2013). *Cfr* is widely disseminated among pigs farmed in China (Shen *et al*., 2013) and retail meat in Guangzhou, China has also tested positive for *cfr* (Zeng *et al*., 2014). This indicates that *cfr*-positive bacteria may find their way into humans through direct

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animal–human contact or contaminated meat products. In this study, we investigated the prevalence and dissemination of *cfr* among staphylococcal isolates from pigs, workers, and the environment of a hog market and a slaughterhouse in Guangzhou.

Materials and Methods

Sample collection and cfr *detection*

From March 2011 to May 2011, a total of 569 samples were collected from a hog market and a slaughterhouse in Guangzhou. The samples included 207 pig nasal swab samples collected from the hog market (no more than 5 samples were collected from pigs at the same farm), 179 pig nasal swab samples from 1 slaughterhouse, 110 human nasal swab samples from workers at the hog market and the slaughterhouse, 31 floor swab samples from the hog market, and 42 surface swab samples from pig transportation vehicles.

All samples were cultivated in brain-heart infusion broth for 18–24 h at 37°C. The cultured broth was then streaked onto plates of mannitol salt agar for 18–24 h at 37°C, and 1 isolate per sample was selected for further study. Whole-cell DNA of all isolates was extracted using previously described methods (Louie *et al*., 2002). A previously described polymerase chain reaction (PCR) assay for *cfr* detection was performed (Kehrenberg and Schwarz, 2006). Species identification of all *cfr*-positive strains was performed by sequencing both *gap* and 16S rRNA (Ghebremedhin *et al*., 2008). Other resistance genes, such as *ermA*, *ermB*, *ermC*, *msrA*, *msrB*, *vga*, *vgaAv*, *fexA*, *mecA*, and *lsa*(C), were also detected by PCR (Lina *et al*., 1999; Louie *et al*., 2002; Haroche *et al*., 2003; Kehrenberg and Schwarz, 2006; Malbruny *et al*., 2011).

Molecular typing

Genetic diversity of *cfr*-positive staphylococcal isolates was determined by performing *Sma*I pulsed-field gel electrophoresis (PFGE) according to previously reported protocols (McDougal *et al*., 2003). Seven previously described *cfr*-positive staphylococci, including four *S. simulans* and three *S. cohnii* isolates, collected from pork and chicken meat sold in Guangzhou in 2012, were selected for comparison (Zeng *et al.*, 2014). Comparison of PFGE patterns was performed with BioNumerics software (Applied Maths, Sint-Martens-Latem, Belgium) using the Dice coefficient (1.5% optimization and 1.0% tolerance). Patterns were clustered using the unweighted pair-group method with arithmetic mean. A similarity cut-off of 80% was used to define a PFGE cluster (McDougal *et al.*, 2003). Different PFGE clusters were indicated by capital letters in alphabetical order.

The *cfr*-carrying *S. aureus* strain was characterized by *spa* typing (Shopsin *et al*., 1999) and multilocus sequence typing [MLST] (Enright *et al*., 2000) according to previously described procedures. The *spa* type was assigned using the Ridom web server (http://spa.ridom.de). The allelic profiles of the seven housekeeping gene sequences and sequence types (ST) were obtained from the MLST website database (http://www.mlst.net).

Transformation and hybridization experiments

Plasmid DNA was extracted using the E.Z.N.A. Plasmid DNA Midi Kit (Omega, Norcross, GA) following the manufacturer's instructions. The resulting plasmid DNA was transferred into the RN4220 *S. aureus* recipient strain by electroporation at 2.5 kV using 0.2-cm cuvettes and a Gene Pulser apparatus (Bio-Rad, Munich, Germany). Transformation experiments were performed using two randomly selected *cfr*-positive *S. simulans* strains of swine and human origin with different PFGE patterns. Transformants were selected on brain-heart infusion agar supplemented with 10 μg/mL florfenicol, and the presence of *cfr* was further confirmed by PCR. Southern blot hybridization was performed using plasmid DNA of the original strains to identify the location of *cfr*. Extracted plasmid DNA fragments were transferred to Hybond N+ membranes (Amersham Biosciences, Piscataway, NJ) and hybridized with a non-radioactively labeled *cfr*-specific probe using a DIG High Prime DNA Labeling and Detection Kit (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instruction.

Antimicrobial susceptibility testing

The original *cfr*-positive isolates, recipient strain *S. aureus* RN4220, and transformants were tested for their susceptibility to clindamycin, chloramphenicol, florfenicol, tiamulin, valnemulin, linezolid, vancomycin, oxacillin, tetracycline, gentamicin, ciprofloxacin, and erythromycin using the agar dilution method. In addition, isolates were investigated for resistance to quinupristin/dalfopristin (15 μg) using the agar disc diffusion method. Both susceptibility tests were performed and evaluated using protocols recommended by the VET01-S2 (CLSI, 2013a) and M100-S23 (CLSI, 2013b) of the Clinical and Laboratory Standards Institute (Wayne, PA). *S. aureus* reference strains ATCC 29213 and ATCC 25923 were used for quality control.

Genetic environment of the cfr *gene*

To identify the sequences surrounding *cfr*, partial nucleotide sequences of *cfr*-carrying plasmids were determined using an inverse PCR strategy and PCR mapping using primers corresponding to the sequences of the *cfr*-carrying plasmids pHK01 (GenBank accession number KC820816), pSS-01(accession no. JQ041372), and pSS-03 (accession no. JF834911; Supplementary Table S1; Supplementary Data are available online at www.liebertpub.com/fpd). Inverse PCR was performed using the primers cfrIF (5′-ATAGTGAGG AACGCAGCAAAT-3′) and cfrIR (5′-TCCAATGTCGCC TGTAGCA-3′) with sequences located within the *cfr* gene under the following conditions: initial cycle of 94°C for 2 min, followed by 30 cycles of 30 s at 94°C, and 10 min at 68°C, with a final extension of 10 min at 72°C. Using BLAST (http://blast.ncbi.nlm.nih.gov/Blast), DNA sequences were compared to those deposited in GenBank. Sequences were annotated using the Vector NTI program (Invitrogen, Carlsbad, CA).

Nucleotide sequence accession number

The nucleotide sequences of a 7320-bp fragment of the pHNKF3 plasmid, a 9880-bp segment of pHNCR35, and a 5086-bp segment of pHNZT2 were deposited in GenBank under the accession nos. KF861984, KF861983, and KF861985, respectively.

Results

Identification of the cfr *gene in* Staphylococcus *isolates*

From a total of 569 samples, 444 *Staphylococcus* isolates (165 from hog market pigs, 147 from slaughterhouse pigs, 80 from workers, 29 from surface swabs from pig transport vehicles, and 23 from hog market floor swabs) were recovered. Among them, *cfr* was present in 20 (4.5%) *Staphylococcus* isolates obtained from pigs ($n = 16$), the environment ($n = 2$), and workers ($n = 2$; Table 1). *S. simulans* ($n = 18$) was the predominant species isolated. One isolate each of *S. cohnii* from pig and *S. aureus* from hog market floor swabs were also isolated (Table 2).

Molecular typing

PFGE analysis of the 26 *cfr*-positive staphylococci (22 *S. simulans* and 4 *S. cohnii*) obtained from pigs, the environment, humans, and animal food products revealed that the 22 *S. simulans* isolates from diverse sources exhibited 15 different PFGE patterns (A–O), whereas the 4 *S. cohnii* isolates of swine and animal food origin exhibited 4 different PFGE patterns (P–S; Fig. 1; Supplementary Fig. S1). Thus, *cfr* dissemination might not be due to the spread of a specific *Staphylococcus* clone. However, some isolates obtained from different sources had similar PFGE patterns (Fig. 1). Interestingly, one porcine isolate and one strain of human origin showed two similar patterns (C1 and C2) with a difference of a single band (Fig. 1). In addition, one pork isolate (TLD22) and three pig isolates showed three similar patterns (B1, B2, and B3). The *cfr*-positive *S. aureus* strain BKED was shown to belong to *spa*-type t889 and clonal lineage ST9.

Localization of cfr

Southern blot hybridization indicated that *cfr* was harbored in plasmid DNA (17 isolates) and chromosomal DNA (3 isolates). In addition, plasmid DNA was obtained from *S. simulans* DKCR35 of human origin and from *S. simulans* AKZT2 of swine origin and then successfully transferred to *S. aureus* RN4220. Plasmids obtained from resultant transformants were designated as pHNCR35 (human origin) and pHNZT2 (swine origin).

Table 1. Detection of *cfr* in Staphylococci Isolates from Different Sources

Source	*No. of samples*	*No. of staphylococci strains*	*Isolates carrying* cfr, *no. (%)*
Pigs	386	312	16 (5.1)
Pig from the hog market	207	165	12 (7.3)
Pig from the slaughterhouse	179	147	4 (2.7)
Workers	110	80	2 (2.5)
Environment	73	52	2 (3.8)
Surface swabs from pig transportation vehicles	42	29	0
Floor swabs from the hog market	31	23	2 (8.7)

Antimicrobial resistance and resistance determinants

Antimicrobial susceptibility testing showed that all 20 *cfr*-positive isolates were resistant to clindamycin, tetracycline, and erythromycin and showed elevated minimum inhibitory concentrations for chloramphenicol, florfenicol, tiamulin, and valnemulin; however, isolates were susceptible to vancomycin. Of the 20 *cfr*-positive isolates, 3 were considered resistant and 10 were shown to have intermediate resistance to quinupristin/dalfopristin. Additionally, nine (45%), 14 (70%), nine (45%), and 14 (70%) isolates exhibited resistance to linezolid, gentamicin, oxacillin, and ciprofloxacin, respectively.

The two transformants (*S. aureus* RN4220:pHNCR35 and RN4220:pHNZT2) exhibited resistance to clindamycin, chloramphenicol, and linezolid and showed elevated minimal inhibitory concentrations of florfenicol, tiamulin, and valnemulin compared with the *S. aureus* recipient strain RN4220. For the assay using quinupristin/dalfopristin discs, the growth inhibition zone diameters of transformants were 7–8 mm smaller than those of the recipient RN4220. This finding indicated *cfr* prevalence and expression in transformants (Table 2). In addition, the two transformants were resistant to erythromycin, but susceptible to tetracycline, ciprofloxacin, and oxacillin. Transformants carrying the plasmid pHNZT2 also showed resistance to gentamicin.

The 20 *cfr*-positive isolates and the transformant RN4220: pHNZT2 carried *fexA*. In addition, all of the 20 *cfr*-positive isolates and the 2 transformants carried 1–3 erythromycin resistance genes, *ermA*, *ermB*, and *ermC* (Table 2). Five strains harbored *vga* or *vgaAv*. All of the nine oxacillin-resistant isolates harbored *mecA* and were therefore considered to be methicillin-resistant coagulase-negative staphylococci.

Genetic environment of cfr *in the plasmids pHNKF3, pHNCR35, and pHNZT2*

The genetic structure surrounding *cfr* was assessed using inverse PCR combined with PCR mapping. A 7.3-kb *cfr*-containing fragment of plasmid pHNKF3 carried by *S. simulans* BKF3 was sequenced. Sequencing results showed that a Tn*4001*-like transposon, including the aminoglycoside resistance gene *aacA-aphD* flanked by an incomplete IS*256* and IS*256*-like element, was upstream of *cfr*. In addition, another IS*256*-like element was present in the downstream region of *cfr*. This 7.3-kb *cfr*-containing fragment showed 99% identity to plasmid pSS-01 (accession no. JQ041372) obtained from an *S. cohnii* strain recovered from a pig in Shandong, China, and plasmid p7LC (accession no. JX910899) obtained from a human *S. epidermidis* isolate from the United States (Fig. 2).

In plasmid pHNCR35 from human *S. simulans*, a 9880-bp region encompassing *cfr* was sequenced, and a Tn*558* variant was identified. The segment containing an IS*21-558* insertion and *cfr* was integrated into the Tn*558* element, replacing parts of transposase genes *tnpA* and *tnpB*. This 9.9-kb region containing *cfr* on plasmid pHNCR35 was identical to the corresponding region of plasmids pHK01 (accession no. KC820816) found in one *S. cohnii* strain from a Chinese patient, pSA737 (accession no. KC206006) from clinical linezolid-resistant *S. aureus* in the United States, pHNTLD18 (accession no. KF751702) from the TLD18 *S. equorum* strain of animal food origin in Guangzhou, China, pSS-02

Table 2. Characteristics of *cfr*-Carrying Isolates and Their Transformants

Strain	Staphylococcal species	Other resistance genes	Location of *cfr*[a]	MIC (μg/mL)[b]							Other resistance patterns[d]
				CLI	CHL	FFC	TIA	VAL	LZD	Q–D[c] (cm)	
BKED	*S. aureus*	*ermA*, *ermB*, *ermC*, *fexA*	C	>128	32	>256	>128	16	4	1.5	TET, GEN, CIP, ERY
DKG4	*S. cohnii*	*ermC*, *fexA*	P	>128	256	>256	>128	32	8	2	TET, GEN, CIP, ERY
BKC3	*S. simulans*	*ermB, mecA, fexA*	C	>128	16	8	16	4	0.5	2	OXA, TET, GEN, CIP, ERY
CKGD	*S. simulans*	*ermA, ermB, ermC, mecA, vga, fexA*	P	>128	256	>256	>128	8	4	2.2	OXA, TET, GEN, CIP, ERY
AKA1	*S. simulans*	*ermA, ermB, ermC, mecA, fexA*	P	>128	16	>256	32	32	2	2	OXA, TET, ERY
DKZ2	*S. simulans*	*ermA, ermC, fexA*	P	>128	256	>256	>128	128	8	1.7	TET, CIP, ERY
BKF3	*S. simulans*	*ermA, ermB, ermC, fexA*	P	>128	>256	>256	>128	128	0.5	1.6	TET, GEN, CIP, ERY
BKJ6	*S. simulans*	*ermC, mecA, vgaAv, fexA*	P	>128	256	>256	>128	>128	2	1.5	OXA, TET, GEN, ERY
DKCR35	*S. simulans*	*ermA, ermC, mecA, vgaAv, fexA*	P	>128	32	64	128	16	8	1.8	OXA, TET, CIP, ERY
CKJ4	*S. simulans*	*ermC, fexA*	P	>128	128	>256	64	4	8	1.7	TET, GEN, CIP, ERY
AKA5	*S. simulans*	*ermC, fexA*	P	>128	256	>256	64	4	1	2	TET, GEN, CIP, ERY
DKTR1	*S. simulans*	*ermA, fexA*	C	>128	32	32	128	8	2	2.6	TET, ERY
AKZT2	*S. simulans*	*ermA, ermC, vga, fexA*	P	>128	>256	>256	>128	>128	8	1.7	TET, GEN, ERY
BKJ3	*S. simulans*	*ermA, fexA*	P	>128	256	>256	>128	4	1	1.7	TET, CIP, ERY
BKF4	*S. simulans*	*ermB, mecA, fexA*	P	>128	32	16	>128	4	4	1.7	OXA, TET, GEN, CIP ERY
AKD2	*S. simulans*	*ermC, fexA*	P	>128	256	>256	64	2	8	2	TET, GEN, CIP, ERY
DKC4	*S. simulans*	*ermA, ermC, mecA, vga, fexA*	P	>128	32	64	>128	4	1	1.8	OXA TET, CIP, ERY
CKZ8	*S. simulans*	*ermC, mecA, fexA*	P	>128	256	>256	>128	128	8	1.6	OXA, TET, GEN, ERY
DKG2	*S. simulans*	*ermC*, *mecA*, *fexA*	P	>128	32	>256	128	16	8	1.7	OXA, TET, GEN, CIP, ERY
AKZ10	*S. simulans*	*ermA, fexA*	P	>128	256	>256	128	4	16	1.2	TET, GEN, ERY
Recipient RN4220	*S. aureus*			0.125	2	4	2	0.06	1	2.6	
RN4220: pHNZT2	*S. aureus*	*ermA*, *ermC*	P	>128	>256	>256	>128	128	8	1.8	GEN, ERY
RN4220: pHNCR35	*S. aureus*	*ermA*, *ermC*, *fexA*	P	>128	32	64	128	16	8	1.9	ERY

[a]C, chromosome; P, plasmid.
[b]MIC, minimum inhibitory concentration; CLI, clindamycin; CHL, chloramphenicol; FFC, florfenicol; TIA, tiamulin; VAL, valmulin; VAN, vancomycin; LZD, linezolid.
[c]Q-D, quinupristin/dalfopristin. The antimicrobial susceptibility of quinupristin/dalfopristin was determined by the agar disk-diffusion method.
[d]OXA, oxacillin; TET, tetracycline; GEN, gentamycin; CIP, ciprofloxacin; ERY, erythromycin. All the isolates are susceptible to vancomycin.

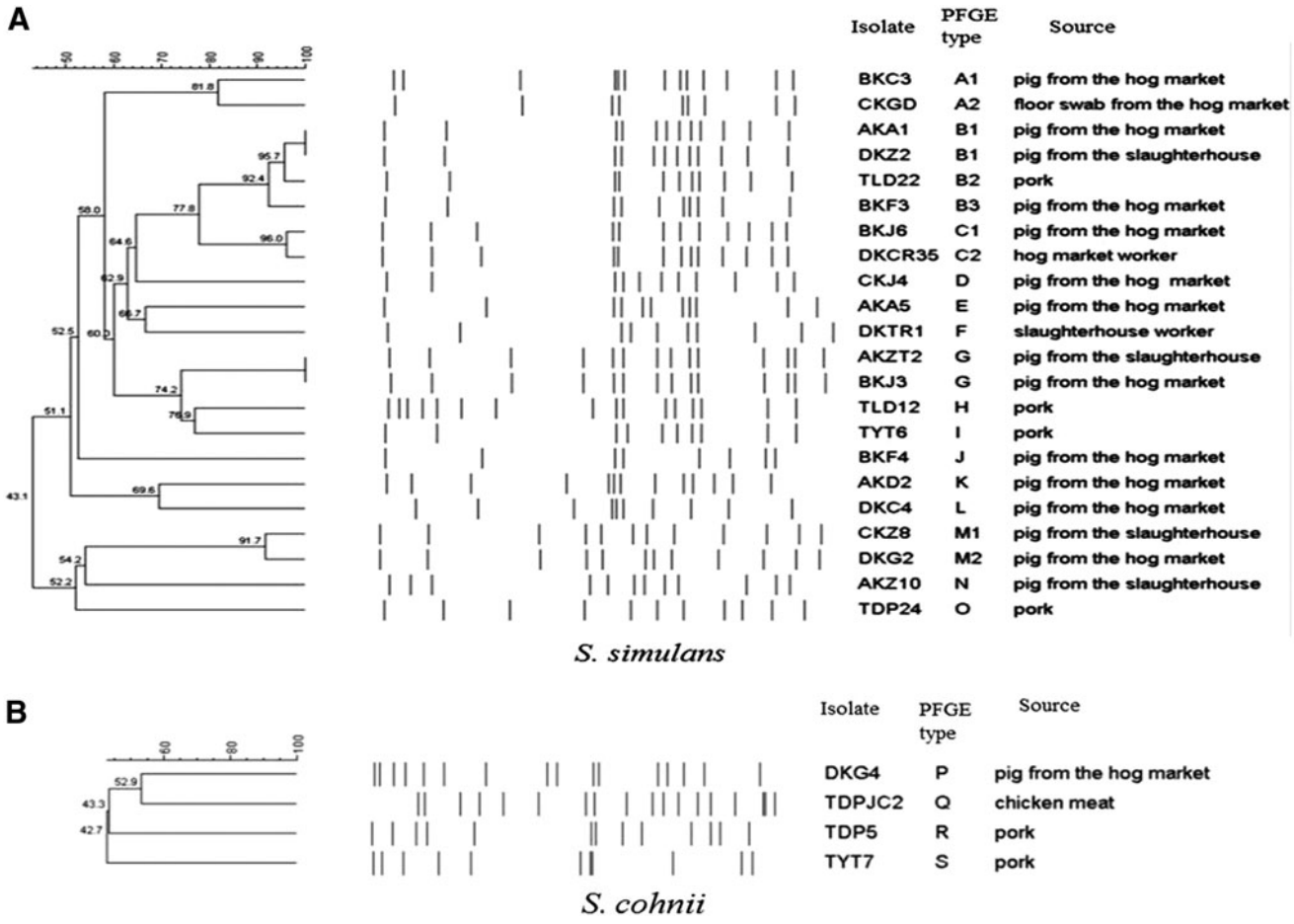

FIG. 1. Dendogram of the pulsed-field gel electrophoresis profile of *Sma*I-digested genomic DNA of 25 *cfr*-positive staphylococcal strains recovered from pigs, environment, workers, and retail meat. **(A)** *Staphylococcus simulans*; **(B)** *S. cohnii*.

(accession no. JX827253) carried by two *S. cohnii* and one *S. haemolyticus* isolates from swine (Wang *et al*., 2012), and a clinical linezolid-resistant *S. haemolyticus* strain from China (Fig. 2).

A 5086-bp segment carrying *cfr* of the plasmid pHNZT2 was also obtained and sequenced to discover an incomplete *rep,* Δ*pre/mob* upstream of *cfr* and a partial *pre/mob* downstream of *cfr*. This segment was completely identical to the corresponding region of plasmids pSS-03 (accession no. JQ219851) carried by an *S. cohnii* strain of swine origin, pMSA16 (accession no. JQ246438) extracted from a bovine MRSA strain, and pHNLKJC2 (accession no. KF751701) from an *S. sciuri* isolate from animal food in Guangzhou (Fig. 2).

Discussion

The distribution *cfr* among staphylococcal isolates simultaneously collected from animals at slaughter, the surrounding environment, and people in close contact with them has not been previously studied. We screened 444 staphylococcal isolates collected from pigs, workers, and the environment in a hog market and a slaughterhouse to assess *cfr* prevalence. The data indicated that *cfr* was present in 5.1%, 3.8%, and 2.5% of staphylococcal isolates obtained from pigs, the environment, and workers, respectively. These data may provide lower estimates for *cfr* prevalence in pigs at slaughter because the staphylococcal isolates used in this study were randomly selected without any antimicrobial selection. A previous study showed that 22.1% of porcine staphylococcal isolates (screened with selective media containing 10 μg/mL florfenicol) obtained from swine farms in China harbored *cfr* (Wang *et al*., 2012). The high prevalence of Cfr-producing *Staphylococcus* among pigs may be responsible for the high *cfr* prevalence (18.6%) in retail meat samples as reported in our previous study (Zeng, 2014).

The majority of the *cfr*-positive staphylococcal isolates were clonally unrelated. However, the high similarity among PFGE profiles of *S. simulans* isolates obtained from different sources indicated that clonal spread of *cfr*-carrying *S. simulans* had occurred among pigs, humans, the environment, and animal-derived food. The *cfr* flanking sequences in plasmids from porcine or human *S. simulans* strains characterized in this study were highly homologous to those that were previously obtained from humans, animals, and foods, indicating that horizontal transmission mediated by mobile genetic elements had occurred and may be the primary *cfr* dissemination mechanism among staphylococcal isolates. The transmission of *cfr*-carrying plasmids between staphylococci from pigs and humans has already occurred (Cui *et al.*, 2013). A previous study indicated that ingestion of contaminated

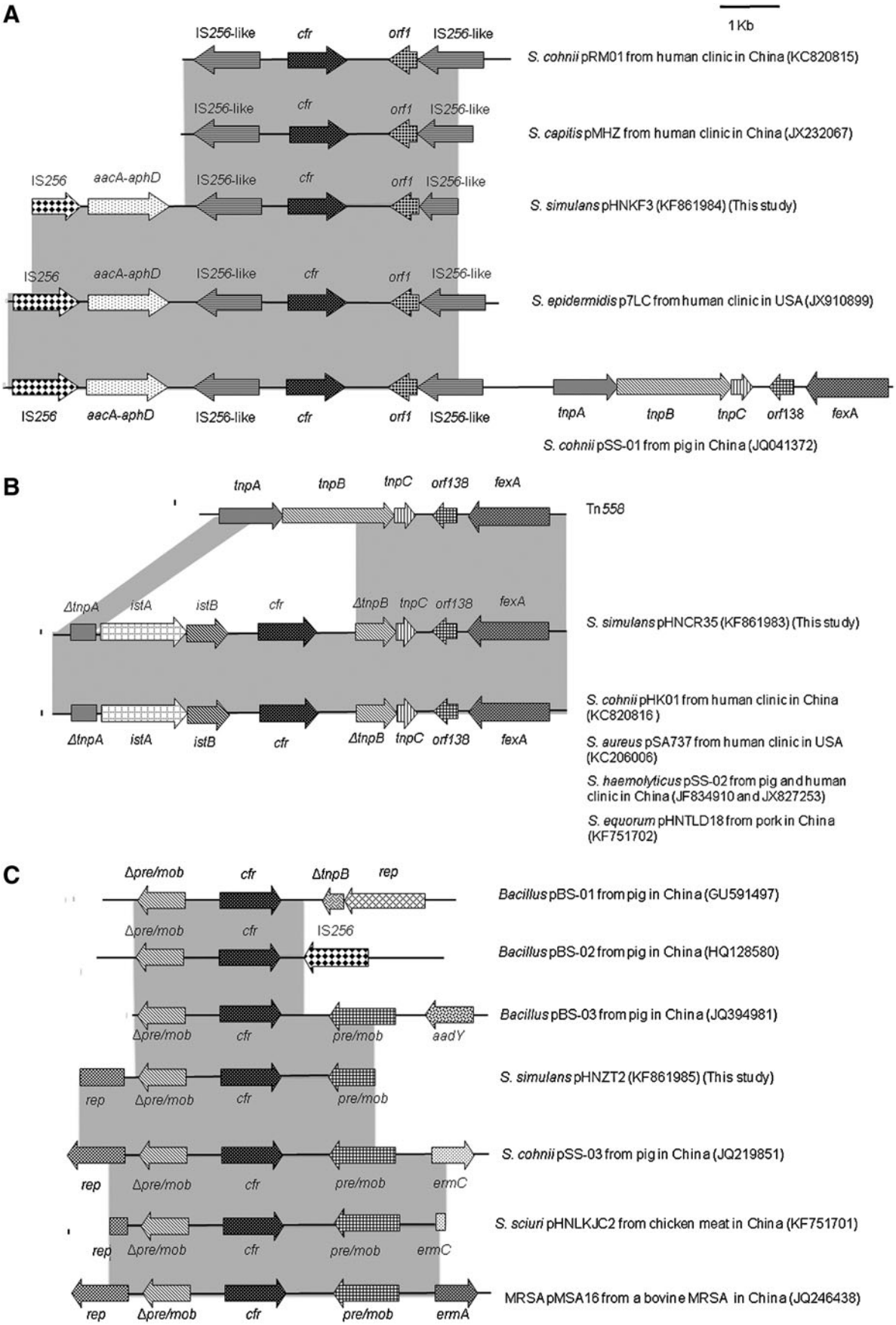

FIG. 2. Genetic environment of *cfr* in plasmids (pHNKF3, pHNCR35, and pHNZT2) and their structural comparison with other similar plasmids. The arrows indicate the positions and directions of the transcription of the genes. Regions of >98% homology are shaded in gray. Δ indicates a truncated gene. 1-kb distance scale is displayed in the upper right corner of Fig. 2A. **(A)** pHNKF3; **(B)** pHNCR35; **(C)** pHNZT2.

meat might result in the dissemination of resistant bacteria between food, animals, and humans (Leverstein-van Hall, 2011). The emergence and dissemination of *cfr*-carrying staphylococcal strains and plasmids among pigs at slaughter, retail meat, and the environment is worrisome and requires continued vigilance, given the possibility that *cfr* can be transferred from animals to humans through the food chain and via fomites and potentially induce further human-to-human spread in the community (Geenen *et al*., 2010).

The *S. aureus* strain BKED belonged to *spa*-type t889 and was assigned to the MLST-type ST9. ST9 is frequently associated with pigs. Although ST9 is a minor *S. aureus* MLST in Europe, Canada, and the United States, it is identified as the predominant sequence type in China (Cui *et al*., 2009; Boost *et al*., 2012). To date, among all *cfr*-positive *S. aureus* isolated so far, only one porcine methicillin-susceptible *S. aureus* ST9 collected from Germany and one bovine MRSA ST9 collected from China have been reported (Shen *et al*., 2013). Previous studies indicate that there is a significant risk of colonization by *S. aureus* ST9 in pig-handlers (Cui *et al*., 2009). Several cases of human infections with MRSA ST9 have been reported in Miami, FL (Chung *et al*., 2004) and Guangzhou, China (Liu *et al*., 2009). The ST9 *S. aureus* strain analyzed in this study might have been colonized in pigs. Although the origin of this isolate remains unclear, the transfer of *cfr*-carrying *S. aureus* ST9 from swine to humans through a contaminated environment and further spread among humans cannot be precluded since the most common route of *S. aureus* transmission from animals to humans is through direct contact with animals and their surrounding environment (Geenen *et al*., 2010).

Our findings indicated that *cfr* dissemination among staphylococcal isolates obtained from different sources occurred via both clonal spread of *cfr*-positive strains and through horizontal transfer mediated by mobile elements. These data indicate the potential for *cfr* transfer from animals to humans through food or fomites. Thus, continuous surveillance of *cfr* dissemination in staphylococcal isolates obtained from animals and food is needed to safeguard public health.

Acknowledgments

This study was supported in part by the National Key Basic Research Program of China (No. 2013CB127200), the Program for Changjiang Scholars and Innovative Research Team in University (IRT13063), the Guangdong Natural Science Foundation (No. S2012030006590), the Foundation for the Author of Guangdong Excellent Doctoral Dissertation (No. sybzzxm201225), and the fund for Training of PhD Students from the Ministry of Education of China (201044041100).

Disclosure Statement

No competing financial interests exist.

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