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Presence and molecular characteristics of oxazolidinone resistance in staphylococci from household animals in rural China

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Objectives: To investigate the presence and molecular characteristics of oxazolidinone resistance genes *cfr* and *optrA* in staphylococci from household animals in rural China.

Methods: Various samples were collected from household animals in 12 rural villages. Staphylococcal isolates showing florfenicol MICs ≥10 mg/L were identified and screened for the presence of *cfr* and/or *optrA*. PCR-positive isolates were characterized by antimicrobial susceptibility testing, S1 nuclease PFGE and Southern blotting. WGS data were analysed to identify the core-genome phylogenetic profile of each isolate as well as the genetic environment of *cfr* and/or *optrA*.

Results: Nine *optrA*-positive (seven *Staphylococcus sciuri* and two *Staphylococcus simulans*) and 10 *cfr*-positive staphylococci were identified from eight and five villages, respectively. The gene *optrA* was chromosomally encoded in all nine isolates, whereas *cfr* was located on a plasmid in one *S. sciuri* and three *Staphylococcus saprophyticus* and in the chromosomal DNA of single *Staphylococcus cohnii* and *Staphylococcus lentus* isolates and two *S. sciuri* isolates. The remaining two *cfr*-carrying *Staphylococcus haemolyticus* isolates were indistinguishable by PFGE. Most *optrA*- or *cfr*-carrying staphylococci also harboured phenicol, tetracycline and/or macrolide-lincosamide-streptogramin B resistance genes. Genetic environment analysis showed that, for the first time, *optrA* was associated with transposon Tn*6261*, while *cfr* was adjacent to both a *tnp* (transposase) gene and a Tn*558* transposon.

Conclusions: The current study reveals for the first time the wide distribution of oxazolidinone resistance genes *optrA* and *cfr* in household animals in rural areas of China and is the first identification of *optrA* in *S. simulans* isolates.

Introduction

Oxazolidinones are some of the few remaining options for antimicrobial treatment of infections caused by MDR Gram-positive pathogens, including staphylococci.[1,2] The emergence and dissemination of transferable oxazolidinone resistance genes, including the resistance gene *cfr*, has severely curtailed the effectiveness of these compounds.[3–5] Since its initial identification in a bovine *Staphylococcus sciuri* isolate in 2000,[6] *cfr* has rapidly disseminated amongst both human and animal bacteria of various genera, including *Staphylococcus*,[6] *Bacillus*,[7] *Enterococcus*,[8] *Macrococcus*,[9] *Jeotgalicoccus*,[9] *Streptococcus*,[10] *Proteus*[11] and *Escherichia*[12] and meanwhile represents a serious threat to public health.

Of even more concern, a novel transferable oxazolidinone resistance gene, *optrA*, was identified in 2015.[13] The gene *optrA* confers cross-resistance to phenicols and oxazolidinones, including the recently approved expanded-spectrum oxazolidinone tedizolid.[13] The emergence and dissemination of this gene may pose an even greater threat to human health. Compared with the ubiquity of *cfr*, surveillance studies indicated that the presence of *optrA* is limited to a few species of the genus *Enterococcus* and only one species of *Staphylococcus*, namely *S. sciuri*.[14,15]

Livestock is widely recognized as a reservoir of antimicrobial resistance. Worldwide reports of *cfr*- and *optrA*-mediated oxazolidinone resistance amongst bacterial isolates of animal origin have largely focused on animals from intensive commercial farms.[14–19] In contrast, there is little information currently available regarding the presence of oxazolidinone-resistant bacterial strains in the backyard farming sector in rural areas, which accounts for a large

proportion of livestock production in China.[20] Moreover, backyard farming—in comparison with commercial farming—often allows even closer contact between humans and their animals, thereby allowing more likely spread of bacteria, including resistant bacteria, by direct contact. To investigate the dissemination of *cfr* and *optrA* among staphylococci in this poorly studied farming system, we collected samples from pigs, chickens, dogs and cats from households in 12 villages in a rural area of Shandong Province in China.

Materials and methods

Sample collection, bacterial isolation and identification

In July 2015, a total of 753 households in 12 villages in a rural area of Shandong Province in China were visited and 404 pig ear swab samples, 114 chicken throat swab samples, 69 dog nasal swab samples and 14 cat nasal swab samples were collected from 325 of the 753 households (Table S1 and Figure S1, available as Supplementary data at *JAC* Online). The study design, village selection and household selection have been described previously.[21] For sample collection, the ESwab collection kit (Copan, Brescia, Italy) was used according to the manufacturer's instructions. Staphylococcal isolates with florfenicol MICs ≥10 mg/L, tentatively considered florfenicol resistant, were isolated as described previously[22] and identified to the species level using MALDI-TOF MS (Bruker Daltonik GmbH, Bremen, Germany) and 16S rRNA gene sequencing. Florfenicol-resistant staphylococcal isolates were screened for the presence of *cfr*, *cfr*(B), *cfr*(C) and *optrA* using previously described PCR assays.[13,16,23,24]

Antimicrobial susceptibility testing and determination of the location of optrA and cfr

Antimicrobial susceptibility testing of *cfr*- and/or *optrA*-positive staphylococci was performed using the broth microdilution method with two-fold dilutions of linezolid, tedizolid, florfenicol, clindamycin, tiamulin, virginiamycin, cefoxitin, gentamicin, tetracycline, ciprofloxacin, erythromycin, vancomycin and tigecycline in Mueller–Hinton broth (Oxoid, Basingstoke, UK). Susceptibility was determined according to the recommendations given in CLSI documents VET01S and M100-S26.[25,26] *Staphylococcus aureus* ATCC 29213 served as the quality control strain. S1 nuclease PFGE and Southern blotting were performed to identify the locations of *optrA* or *cfr* within each isolate.[8]

WGS, assembly and analysis of the flanking regions of optrA and cfr

All *optrA*- and *cfr*-positive staphylococci were subjected to WGS. A KAPA Hyper Prep Kit (Kapa Biosystems, Boston, MA, US) was used for library construction and 150 bp paired-end reads with a minimum of 200-fold coverage for each isolate were obtained following sequencing using the Illumina HiSeq X Ten System (Annoroad Genomics Co., Beijing, China). A draft assembly of the cleaned reads was generated using CLC Genomics Workbench 9 (CLC Bio, Aarhus, Denmark) with de Bruijn graphs. All contigs were searched for *optrA* and *cfr* using stand-alone BLAST analysis. Mutations in domain V of the 23S rRNA or in the genes coding for 50S ribosomal proteins L3, L4 and L22 were investigated by searching against the corresponding sequences of linezolid-susceptible staphylococcal strains deposited in GenBank, including *Staphylococcus saprophyticus* ATCC 15305 (GenBank accession number NC_007350.1), *S. sciuri* FDAARGOS_285 (NZ_CP014016), *Staphylococcus cohnii* SNUDS-2 (CP019597.1), *Staphylococcus haemolyticus* JCSC1435 (NC_007168.1) and *Staphylococcus simulans* FDAARGOS_124 (NZ_CP014016). The putative coding sequences of the genes flanking *optrA* or *cfr* were obtained using PATRIC.[27] Detection of resistance-associated genes was conducted using stand-alone BLAST analysis against ResFinder.[28] Based on draft genomes, core-genome SNP-based phylogenetic trees of *optrA*- and *cfr*-positive staphylococci belonging to the same species were constructed using Parsnp in the Harvest package[29] with the default parameter settings and then visualized using iTOL.[30]

Results

Sample collection, species identification and detection of optrA and cfr

From the 601 swabs, 103 staphylococcal isolates with florfenicol MICs ≥10 mg/L were identified (Table S1). *S. haemolyticus* (34/103, 33.0%) was the predominant species amongst the isolates, followed by *S. sciuri* (24/103, 23.3%), *S. simulans* (18/103, 17.5%), *S. saprophyticus* (9/103, 8.7%), *Staphylococcus xylosus* (6/103, 5.8%), *S. aureus* (5/103, 4.9%) and *S. cohnii* (3/103, 2.9%). We also identified one isolate each (1/103, 1.0%) of *Staphylococcus chromogenes*, *Staphylococcus hyicus*, *Staphylococcus epidermidis* and *Staphylococcus lentus*. Among these, 10 (9.7%) and 9 (8.7%) isolates were positive for *cfr* and *optrA*, respectively (Table 1). Coexistence of *cfr* and *optrA* was not observed and all isolates were negative for *cfr*(B) and *cfr*(C). The 10 *cfr*-positive staphylococci were identified from porcine samples from five villages and included 3 *S. saprophyticus*, 3 *S. sciuri*, 2 *S. haemolyticus*, 1 *S. cohnii* and 1 *S. lentus*. The *optrA*-positive staphylococci were isolated from samples collected from eight different villages, with seven of the nine *optrA*-positive isolates derived from porcine samples. The remaining two isolates were from one dog and one cat sample. Most of the *optrA*-carrying staphylococci were *S. sciuri* (7/9, 77.8%), and the remaining two isolates were identified as *S. simulans* and were recovered from porcine samples from the same village. Overall, the staphylococci positive for either *cfr* or *optrA* genes were isolated from samples obtained from 11 of the 12 villages. None of the chicken samples contained *cfr*- or *optrA*-positive staphylococci.

Antimicrobial susceptibility testing

Overall, 8 of the 10 *cfr*-positive staphylococci exhibited the $PhLOPS_A$ (resistance to phenicols, lincosamides, oxazolidinones, pleuromutilins and streptogramin A) phenotype, with the remaining two *S. haemolyticus* isolates (DY29, JY20) showing susceptibility to linezolid (MIC 0.5–2 mg/L) (Table 1). Most of the *cfr*-positive staphylococcal isolates also exhibited resistance to cefoxitin (10/10, 100%), gentamicin (8/10, 80%), tetracycline (8/10, 80%) and erythromycin (5/10, 50%), and all isolates were susceptible to vancomycin (Table 1). It is noteworthy that two *cfr*-positive staphylococcal isolates, *S. sciuri* GY53 and *S. cohnii* GY02, showed resistance to tedizolid (MIC 2 mg/L) and one of them (GY02) also showed resistance to tigecycline (MIC 1 mg/L) (Table 1).

All *optrA*-positive staphylococci exhibited high levels of florfenicol resistance (MIC 64–128 mg/L), whereas only three of the nine *optrA*-positive isolates (BY05, IY19, IY37) demonstrated resistance to linezolid (MIC 8 mg/L) (Table 1). None of the *optrA*-positive isolates exhibited tedizolid resistance, with the tedizolid MIC ranging from 0.25 to 1 mg/L (Table 1). Like the *cfr*-positive isolates, most of the *optrA*-positive staphylococci also exhibited resistance to cefoxitin (9/9, 100%), tetracycline (7/9, 77.8%) and erythromycin (6/9, 66.7%), but all isolates were susceptible to vancomycin and tigecycline (Table 1). There were noticeable differences in gentamicin

Table 1. Phenotypic and genotypic characteristics of *cfr*/*optrA*-positive staphylococcal isolates investigated in this study

Isolate[a]	Village	Species	*cfr*/*optrA* gene location	OptrA variants[d]	PhLOPS$_A$ agent MICs (mg/L)[e]						Other resistance	
					FFC	CLI	TIA	VIR[f]	LZD	TZD	phenotype	genes[g]
CY01	C	*S. saprophyticus*	*cfr*-P	—	**32**	**64**	**32**	**16**	**8**	1	FOX, TET	*aacA-aphD*, *aadD*, *str*, *ble*
CY04	C	*S. saprophyticus*	*cfr*-P	—	**128**	**128**	**32**	**16**	**8**	0.5	FOX, TET	*aacA-aphD*, *aadD*, *str*, *tet*(K), *ble*
DY29	D	*S. haemolyticus*	*cfr*-NA	—	**32**	**8**	**32**	**16**	2	0.5	FOX, GEN, ERY	*fexA*, *blaZ*, *mecA*, *aacA-aphD*, *aadD*, *aadE*, *spc*, *str*, *erm*(A), *erm*(B), *erm*(C), *mph*(C), *dfrG*, *tet*(K), *tet*(S), *fosB*
GY02	G	*S. cohnii*	*cfr*-C	—	**128**	**128**	**64**	**16**	**8**	**2**	FOX, GEN, TET, CIP, ERY, TGC	*fexA*, *aacA-aphD*, *erm*(B), *erm*(C), *mph*(C), *tet*(L), *msr*(A)
GY27	G	*S. sciuri*	*cfr*-C	—	**128**	**128**	**32**	**16**	**8**	1	FOX, GEN, TET	*fexA*, *aacA-aphD*, *aadD*, *str*, *dfrG*, *tet*(K), *ble*
GY35	G	*S. lentus*	*cfr*-C	—	**64**	**128**	**32**	**16**	**8**	0.25	FOX, GEN, TET, CIP, ERY	*fexA*, *cat*$_{pC223}$, *mecA*, *aacA-aphD*, *aadD*, *aadE*, *spc*, *str*, *erm*(A), *erm*(B), *erm*(C), *mph*(C), *dfrG*, *tet*(K), *tet*(S), *fosB*
GY48	G	*S. saprophyticus*	*cfr*-P	—	**128**	**128**	**32**	**16**	**8**	1	FOX, GEN, TET, ERY	*fexA*, *cat*$_{pC223}$, *aacA-aphD*, *str*, *erm*(B), *dfrG*, *tet*(K)
GY53	G	*S. sciuri*	*cfr*-C	—	**128**	**128**	**32**	**16**	**8**	**2**	FOX, GEN	*fexA*, *mecA*, *aacA-aphD*, *aadD*, *aadE*, *spc*, *str*, *erm*(A), *erm*(C), *mph*(C), *dfrG*, *tet*(K), *tet*(S), *fosB*
HY16	H	*S. sciuri*	*cfr*-P	—	**128**	**128**	**128**	**16**	**8**	0.25	FOX, GEN, TET, CIP, ERY	*fexA*, *mecA*, *aacA-aphD*, *aadD*, *aadE*, *erm*(B), *dfrG*, *tet*(K), *tet*(M), *ble*
JY20	J	*S. haemolyticus*	*cfr*-NA	—	**16**	**4**	**32**	**8**	0.5	0.5	FOX, GEN, TET, CIP	*fexA*, *cat*$_{pC223}$, *blaZ*, *aacA-aphD*, *aadE*, *dfrG*, *tet*(K), *tet*(L), *tet*(M), *lnu*(B)
BY05	B	*S. sciuri*	*optrA*-C	EYDD	**64**	0.5	**32**	**2**	**8**	1	FOX, TET	*fexA*, *tet*(L), *tet*(M), *lnu*(A), *mdlB-1*, *mdlB-2*
CY35	C	*S. sciuri*	*optrA*-C	EYDD	**128**	**128**	16	**2**	2	0.5	FOX, TET, CIP, ERY	*fexA*, *aacA-aphD*, *ant(6)-Ia*, *erm*(B), *erm*(C), *dfrG*, *tet*(L), *tet*(M), *mdlB-1*, *mdlB-2*
DY31	D	*S. sciuri*	*optrA*-C	EYDDK	**64**	**128**	16	**2**	2	0.125	FOX, TET, ERY	*fexA*, *aadD*, *erm*(B), *tet*(K), *mdlB-1*, *mdlB-2*
EY13	E	*S. sciuri*	*optrA*-C	—	**64**	0.5	16	**2**	2	0.25	FOX, TET	*fexA*, *tet*(K), *lnu*(A), *mdlB-1*, *mdlB-2*
IY19	I	*S. simulans*	*optrA*-C	EYDD	**128**	**128**	0.25	**2**	**8**	1	FOX, ERY	*fexA*, *erm*(A), *erm*(C), *dfrG*, *lnu*(A)
IY37	I	*S. simulans*	*optrA*-C	EYDD	**128**	**128**	0.25	**1**	**8**	1	FOX, ERY	*fexA*, *blaZ*, *erm*(A), *erm*(C), *dfrG*, *lnu*(A)
A22[b]	A	*S. sciuri*	*optrA*-C	EYDD	**64**	1	8	**1**	2	0.5	FOX, GEN, TET, CIP, ERY	*fexA*, *mecA*, *aacA-aphD*, *aadE*, *erm*(B), *erm*(C), *tet*(K), *tet*(S), *mdlB-1*, *mdlB-2*
F08[c]	F	*S. sciuri*	*optrA*-C	EYDD	**64**	0.5	**32**	**2**	2	0.25	FOX, TET	*fexA*, *tet*(K), *lnu*(A), *mdlB-1*, *mdlB-2*
G07[b]	G	*S. sciuri*	*optrA*-C	EDD	**64**	0.5	**32**	**2**	4	0.25	FOX, TET, ERY	*fexA*, *erm*(Y), *mph*(C), *dfrG*, *tet*(L), *msr*(A), *mdlB-1*, *mdlB-2*

NA, not available; P, plasmid; C, chromosome; FFC, florfenicol; CLI, clindamycin; TIA, tiamulin; VIR, virginiamycin; LZD, linezolid; TZD, tedizolid; GEN, gentamicin; TET, tetracycline; ERY, erythromycin; CIP, ciprofloxacin; FOX, cefoxitin; RIF, rifampicin; TGC, tigecycline.

[a]Isolates are of pig origin unless indicated.

[b]Isolate of dog origin.

[c]Isolate of cat origin.

[d]EYDD, Lys3Glu, Asn12Tyr, Tyr176Asp, Gly393Asp; EYDDK, Lys3Glu, Asn12Tyr, Tyr176Asp, Gly393Asp, Glu583Lys; EDD, Lys3Glu, Tyr176Asp, Gly393Asp.

[e]MICs at or above the resistance breakpoints are indicated in bold.

[f]Virginiamycin resistance breakpoint tentatively set at MIC $\geq$1 mg/L.

[g]Acquired antimicrobial resistance genes.

resistance between the *cfr*-positive and *optrA*-positive staphylococci, with the majority (8/10, 80%) of the former showing resistance to gentamicin and the majority of the latter (8/9, 88.9%) being susceptible to gentamicin.

Antimicrobial resistance gene profiles

Almost all of the 19 staphylococcal isolates carried the phenicol resistance gene *fexA* (17/19, 89.5%) and tetracycline resistance genes of different *tet* gene classes (16/19, 84.2%). Various additional resistance genes were identified, including genes of different *erm* gene classes, *msr*(A), *mph*(C), *lnu*(A), *lnu*(B), *aacA-aphD*, *aadD*, *aadE*, *str*, *spc*, *ant(6)-Ia*, *fosB*, *dfrG*, cat_{pC223}, *mdlB-1*, *mdlB-2*, *blaZ* and *mecA* (Table 1). BLAST analysis demonstrated that none of the isolates contained oxazolidinone resistance-mediating mutations in domain V of the 23S rRNA gene or in the genes coding for 50S ribosomal proteins L3, L4 and L22.

Comparison of the deduced OptrA amino acid sequences of the nine isolates with the original OptrA protein from *Enterococcus faecalis* E349 (designated the WT) revealed three OptrA variants. Substitutions at positions 3 (Lys3Glu), 12 (Asn12Tyr), 176 (Tyr176Asp) and 393 (Gly393Asp) were identified in four of the *S. sciuri* isolates and the two *S. simulans* isolates. Substitutions at positions 3 (Lys3Glu), 12 (Asn12Tyr), 176 (Tyr176Asp), 393 (Gly393Asp) and 583 (Glu583Lys) were identified in *S. sciuri* DY31. Substitutions at positions 3 (Lys3Glu), 176 (Tyr176Asp) and 393 (Gly393Asp) were identified in *S. sciuri* G07. The deduced OptrA amino acid sequence in *S. sciuri* EY13 is coincident with that of the WT.[31]

Localization of optrA and cfr genes

S1-PFGE and Southern blotting analyses revealed that *cfr* was located in the chromosomal DNA of four isolates and on plasmids in another four isolates, whereas the location could not be determined in the two *S. haemolyticus* isolates (DY29, JY20). The gene *optrA* was identified in the chromosomal DNA of all nine *optrA*-positive isolates (Figure S2 and Table 1).

Core-genome phylogenetic analysis

Draft genomes of *S. sciuri* ($n = 10$; average genomic length: 2 877 411 bp; average contig number: 163) and *S. saprophyticus* ($n = 3$; average genomic length: 2 891 247 bp; average contig number: 275) were aligned to the genome of *S. sciuri* strain SUNDS-18 and *S. saprophyticus* strain ATCC 15305 as references, respectively. In addition, the sequences of one international isolate of each species, retrieved from the GenBank database, was included. A total of 41 890 and 7067 core-genome SNPs were identified and then used to build core-genome SNP-based phylogenetic trees of *S. sciuri* and *S. saprophyticus*, respectively (Figure S3). The majority of the 10 *S. sciuri* isolates were genetically unrelated. However, a single porcine isolate (EY13) recovered from village E was closely related to an isolate (F08) from a cat of the neighbouring village F (Figure S3A). In addition, *S. saprophyticus* isolates CY01 and CY04 recovered from different households in the same village C were closely related, although all *S. saprophyticus* isolates (CY01, CY04, GY48) were genetically distant from isolates deposited in GenBank, including *S. saprophyticus* strains ATCC 15305 and FDAARGOS 336 (Figure S3B).

Genetic environment of optrA

The lengths of the nine *optrA*-carrying fragments ranged from 90 059 to 1 316 181 bp and showed three genomic backbone profiles designated types I ($n = 5$), II ($n = 2$) and III ($n = 2$) (Figure 1a). As observed previously,[14] the *fexA*-carrying transposon Tn*558* and the putative transcriptional regulator gene *araC* were conserved in all three genomic backbone profiles. Type I and type III *optrA*-carrying fragments exhibited a high degree of similarity to the corresponding regions of *S. sciuri* S49-1 (GenBank accession no. KX447572) and *S. sciuri* strain Wo22-7 (GenBank accession no. KX982170.1), respectively. The type II *optrA*-carrying fragment was specific to the two *S. simulans* isolates. Unlike types I and III, within which *optrA* was adjacent to the ABC multidrug transporter genes *mdlB-1* and *mdlB-2*, the MLS_B resistance gene *erm*(A) was detected in the 3201 bp region downstream of *optrA* in the type II backbone profile. This 3201 bp region exhibited 100% amino acid identity to the corresponding region of transposon Tn*6261* (GenBank accession no. KU354267.1) from *E. faecalis*.

Genetic environment of cfr

The *cfr*-carrying fragments of sufficient length for analysis were obtained from 5 of the 10 *cfr*-positive isolates after WGS. The lengths of the fragments ranged from 6892 to 21 488 bp. Again, the fragments could be categorized into three genomic backbone profiles, designated types I ($n = 2$), II ($n = 2$) and III ($n = 1$) (Figure 1b). Type II and III *cfr*-carrying fragments exhibited a high degree of similarity to the corresponding regions of plasmid pWo28-3 (GenBank accession no. KT601170.1) from *S. sciuri* and plasmid pHNCR35 (GenBank accession no. KF861983.1) from *S. simulans* DKCR35, respectively. The downstream region of *cfr* in type I fragments was composed of a resolvase gene (*res*), a transposon gene (*tnp*) and a Tn*558* transposon.

Nucleotide sequence accession numbers

The nucleotide sequences of *cfr*- or *optrA*-carrying contigs in *S. simulans* IY19, *S. sciuri* BY05, *S. sciuri* G07, *S. saprophyticus* CY01, *S. cohnii* GY02 and *S. sciuri* GY27 have been deposited in GenBank under accession numbers MF805730 to MF805735.

Discussion

Almost half of China's large population lives in rural areas,[32] where a traditional household backyard farming system is common.[20] The paucity of antimicrobial resistance reports from these areas has encouraged a perception that rural household animals are not a primary source of antimicrobial resistance. To the best of our knowledge, the current study represents the first attempt to assess the presence of oxazolidinone resistance amongst household animals in rural areas of China. We detected *cfr*- and *optrA*-positive staphylococci isolated from household animals from almost all of the tested villages (11/12). The carriage rates of *optrA* (9/103, 8.7%) and *cfr* (10/103, 9.7%) in staphylococci in this rural area were even higher than rates reported from a slaughterhouse[14] (*optrA*, 6.9%) and a commercial farm[22] (*cfr*, 3.8%),

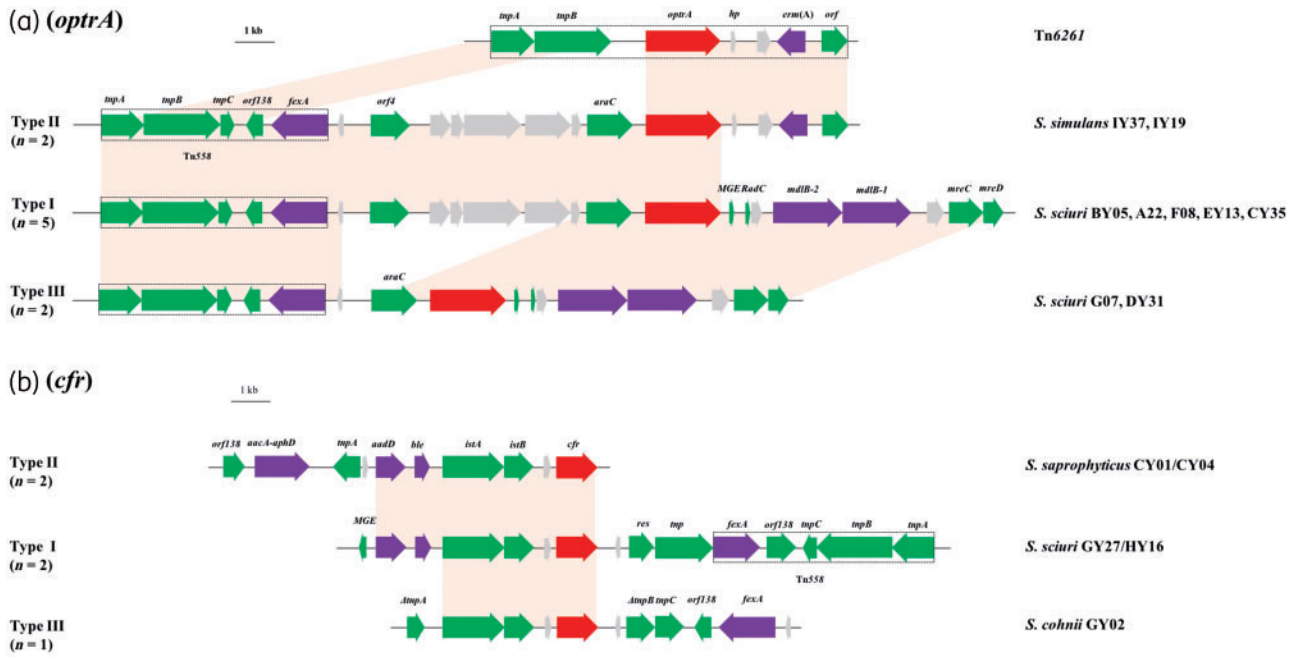

Figure 1. Schematic representation and comparison of the genetic environment of *optrA*-flanking regions (a) and *cfr*-flanking regions (b). Arrows indicate the directions of the different genes shown in different colours. Regions of ≥99.0% nucleotide sequence identity are indicated by shading. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

indicating that these genes occur more often in staphylococci from the household backyard farming sector in rural China than assumed. The fact that two porcine *S. simulans* isolates contained *optrA* is of particular concern, as it indicates the possibility that the horizontal transfer of *optrA* to *Staphylococcus* species other than *S. sciuri* is possible. To the best of our knowledge, this is the first time that *optrA* has been found in *S. simulans*.

Compared with the high MIC values of $PhLOPS_A$ antimicrobial agents for eight *cfr*-positive staphylococci isolates, the two *S. haemolyticus* isolates (DY29, JY20) proved to be susceptible to linezolid and also exhibited low MIC values of florfenicol, clindamycin and virginiamycin, which is presumably due to an impaired function of *cfr*, as the failure of the *cfr* gene conferring $PhLOPS_A$ phenotype was reported in *E. faecalis*.[33] Only three of nine *optrA*-positive staphylococci exhibited linezolid resistance phenotype and none exhibited tedizolid resistance, which is in accordance with previous reports that OptrA conferred either resistance to or elevated MICs of oxazolidinones and phenicols.[13,14]

As has been reported previously,[34,35] staphylococcal isolates in the present study simultaneously carried several resistance genes that conferred the same resistance phenotype, such as the *tet*(K), *tet*(M), *tet*(S) and *tet*(L) genes for tetracycline resistance and the *erm*(A), *erm*(B), *erm*(C) and *erm*(Y) genes for macrolide-lincosamide-streptogramin B resistance. The phenicol resistance gene *fexA* was detected as part of Tn*558* transposons in almost all (17/19, 89.5%) of the *optrA*- and *cfr*-positive staphylococcal isolates, despite the fact that *optrA* and *cfr* also confer phenicol resistance. Oxazolidinones are not approved for veterinary use in China, whereas tetracyclines, macrolides and florfenicol are estimated to be among the most used antimicrobial agents both in swine and poultry production in China.[36,37] Therefore, the prevalence of *optrA* and *cfr* in staphylococci of household animal origin in the current study may be explained by co-selection, which leads to the persistence of resistance genes in the absence of direct selective pressure. In particular, the regions flanking *optrA* and *cfr* harbour multiple antimicrobial resistance genes, including *fexA*, *erm*(A), *mdlB-1* and *mdlB-2* in the regions flanking *optrA*, and *fexA*, *aacA-aphD*, *aadD* and *ble* in the regions flanking *cfr*. Selection for any of these resistance genes could also result in co-selection for *optrA* and/or *cfr*. Therefore, any steps taken to control *optrA*- or *cfr*-mediated oxazolidinone resistance should not only rely on the ban or restriction of use of the antimicrobial agents to which these genes confer resistance, as this would be unlikely to result in a decrease in *cfr* and/or *optrA* genes.

With regard to the complete resistance profiles of the isolates, the WGS-based approach used in the current study also provided a discriminatory genetic method for epidemiological investigation of the isolates. The close genetic relationship between some of the isolates, as revealed by the core-genome phylogenetic analysis, demonstrated the possibility of transmission of this pathogen between different households within the same village or even between different villages, providing information that can be used to guide a rational design of effective control measures.

To the best of our knowledge, the current study revealed for the first time the wide dissemination of *optrA* and *cfr* genes amongst *Staphylococcus* isolates from household animals in rural areas of China and represents the first report of *optrA* in *S. simulans* isolates. Furthermore, we identified genetic evidence of the co-selection of *optrA*, *cfr* and a number of other antimicrobial resistance genes. In the light of the key role of oxazolidinones in human medicine, further surveillance and investigation of transferable oxazolidinone resistance genes is needed to maintain the therapeutic efficacy

of these drugs and should consequently be made a public health priority.

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Transparency declarations

None to declare.

Supplementary data

Table S1 and Figures S1 to S3 are available as Supplementary data at *JAC* Online.

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