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Presence of the *optrA* Gene in Methicillin-Resistant *Staphylococcus sciuri* of Porcine Origin

Run Fan,[a] Dexi Li,[a] Yang Wang,[a] Tao He,[b] Andrea T. Feßler,[c,d] Stefan Schwarz,[a,c,d] Congming Wu[a]

Beijing Advanced Innovation Center for Food Nutrition and Human Health, College of Veterinary Medicine, China Agricultural University, Beijing, People's Republic of China[a]; Institute of Food Safety and Detection, Jiangsu Academy of Agricultural Sciences, Nanjing, People's Republic of China[b]; Institute of Farm Animal Genetics, Friedrich-Loeffler-Institut (FLI), Neustadt-Mariensee, Germany[c]; Centre for Infection Medicine, Institute of Microbiology and Epizootics, Department of Veterinary Medicine, Freie Universität Berlin, Berlin, Germany[d]

A total of 57 methicillin-resistant *Staphylococcus aureus* (MRSA) isolates and 475 methicillin-resistant coagulase-negative staphylococci (MRCoNS) collected from pigs in the Guangdong province of China in 2014 were investigated for the presence of the novel oxazolidinone-phenicol resistance gene *optrA*. The *optrA* gene was detected in 6.9% ($n = 33$) of the MRCoNS, all of which were *Staphylococcus sciuri* isolates, but in none of the MRSA isolates. Five *optrA*-carrying methicillin-resistant (MR) *S. sciuri* isolates also harbored the multiresistance gene *cfr*. Pulsed-field gel electrophoresis (PFGE) and *dru* typing of the 33 *optrA*-carrying MR *S. sciuri* isolates revealed 25 patterns and 5 sequence types, respectively. S1 nuclease PFGE and Southern blotting confirmed that *optrA* was located in the chromosomal DNAs of 29 isolates, including 1 *cfr*-positive isolate. The remaining four isolates harbored a ~35-kb pWo28-3-like plasmid on which *optrA* and *cfr* were located together with other resistance genes, as confirmed by sequence analysis. Six different types of genetic environments (types I to VI) of the chromosome-borne *optrA* genes were identified; these types had the *optrA* gene and its transcriptional regulator *araC* in common. Tn*558* was found to be associated with *araC-optrA* in types II to VI. The *optrA* gene in types II and III was found in close proximity to the *ccr* gene complex of the respective staphylococcal cassette chromosome *mec* element (SCC*mec*). Since oxazolidinones are last-resort antimicrobial agents for the control of serious infections caused by methicillin-resistant staphylococci in humans, the location of the *optrA* gene close to the *ccr* complex is an alarming observation.

Methicillin-resistant coagulase-negative staphylococci (MRCoNS) and methicillin-resistant *Staphylococcus aureus* (MRSA) play a major role in both health care- and community-associated infections (1, 2). MRCoNS have also been detected in recent years as pathogens in animals (3, 4) as well as in food of animal origin (5) and in non-health care environments (6). Linezolid is one of the few clinically effective drugs for the control of MRSA and MRCoNS infections. Resistance to linezolid in staphylococci is still very rare but has been increasing in recent years (7). Resistance to linezolid in staphylococci is due mainly to mutations in domain V of 23S rRNA or in the genes coding for the ribosomal proteins L3 and L4 (8, 9). However, transferable linezolid resistance due to the *cfr* gene has been known since the year 2000. The *cfr* gene was first discovered in a bovine *Staphylococcus sciuri* strain and was later reported in various *Staphylococcus* species (10–12). Recently, a *cfr* homologue, *cfr*(B), was discovered in *Clostridium difficile* and *Enterococcus faecium* (13, 14). Besides resistance to oxazolidinones, the *cfr* gene also mediates cross-resistance to phenicols, lincosamides, pleuromutilins, and streptogramin A antibiotics (15). Very recently, a novel transferable oxazolidinone resistance gene, *optrA*, has been identified, mainly in enterococci from humans and animals (16–19). In contrast to *cfr*, *optrA* confers cross-resistance only to phenicols and oxazolidinones. This cross-resistance, however, also includes resistance to the expanded-spectrum oxazolidinone tedizolid. Tedizolid shows improved activity against MRSA and MRCoNS, including *cfr*-positive isolates (20). To date, the *optrA* gene has been detected only in a single staphylococcal isolate, namely, a porcine *Staphylococcus sciuri* isolate (21), where it was located on the 60.5-kb nonconjugative multiresistance plasmid pWo28-3. To see how widespread *optrA* is among porcine staphylococci, we investigated MRSA and MRCoNS isolates of pig origin from Guangdong Province, China, for the presence and the location of this gene.

MATERIALS AND METHODS

Bacterial isolation and identification. From June 2013 to June 2014, a total of 910 samples of porcine nasal swabs were collected from three different slaughterhouses (designated A, B, and C). Pigs in these slaughterhouses came from 88 farms in different geographical areas of the Guangdong province of China. The 332 samples in slaughterhouse A originated from 33 farms, the 290 samples in slaughterhouse B from 26 farms, and the 288 samples in slaughterhouse C from 29 farms. MRSA and MRCoNS were isolated and identified as described previously (12, 21). Isolates were screened for the *optrA* gene by use of primers described previously (16). The bacterial species of the *optrA*-positive isolates were identified using matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) (Bruker Daltonik GmbH, Bremen, Germany), and these results were further confirmed by 16S rRNA sequencing.

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Address correspondence to Congming Wu, wucm@cau.edu.cn, or Stefan Schwarz, stefan.schwarz@fli.bund.de.

R.F. and D.L. contributed equally to this article.

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Antimicrobial susceptibility testing and screening for resistance genes/mutations. All *optrA*-positive isolates were tested for susceptibility to 14 antimicrobial agents by broth microdilution according to CLSI recommendations issued in 2013 (22) and 2016 (23). *S. aureus* ATCC 29213 served as a quality control strain. Screening for the *cfr* and *cfr*(B) genes and for mutations accounting for oxazolidinone resistance was performed using PCR assays described previously (8, 13, 14, 24–27).

Molecular analysis and determination of the location of *optrA*. The *optrA*-carrying isolates were subjected to SmaI pulsed-field gel electrophoresis (PFGE) and *dru* typing (http://dru-typing.org) (27, 28). PFGE results were analyzed using BioNumerics (version 5.1; Applied Maths, USA). The definition of a PFGE cluster was based on a similarity cutoff of 80% (29). Different PFGE clusters were listed in alphabetical order. S1 nuclease PFGE (S1-PFGE) and Southern blotting were performed to determine the locations of the *optrA* and *cfr* genes and the sizes of the *optrA*-carrying plasmids (21).

Whole-genome sequencing and analysis. The genetic environments of the *optrA* gene in selected *Staphylococcus* isolates were determined by whole-genome sequencing using the Illumina HiSeq 2500 system, which produced 125-bp paired-end reads (Berry Genomics Company, Beijing, China). A draft assembly of the sequences was conducted using CLC Genomics Workbench, version 5 (CLC bio, Aarhus, Denmark); the assembly algorithm works by using de Bruijn graphs (30). All contigs with an average coverage of >100-fold were searched for the *optrA* gene using BLAST analysis. The regions flanking the *optrA* gene-carrying contig were identified by a primer walking strategy (16). Sequence analysis was conducted using the ORF finder (https://www.ncbi.nlm.nih.gov/orffinder/) and the BLAST functions (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Accession number(s). The *optrA*-carrying sequences in MRCoNS isolates S25-1, MS58-20, S13-1, S031-25, S032-3, MS11-3, and S49-1 were assigned GenBank accession numbers KX447566, KX447567, KX447568, KX447569, KX447570, KX447571, and KX447572, respectively.

RESULTS AND DISCUSSION

Identification of *optrA*-positive isolates. A total of 532 methicillin-resistant staphylococci, including 57 MRSA and 475 MRCoNS isolates, were included in this study. Among these, 33 (6.9%) MRCoNS isolates were positive for the *optrA* gene, and all MRSA isolates were negative. The carriage rates for *optrA* in staphylococci from slaughterhouses A and B were 9.7% (23/237) and 4.2% (10/238), respectively, while none of the isolates from slaughterhouse C was *optrA* positive. Further analysis of the origins of the *optrA*-positive isolates revealed that they were from 12 farms delivering their pigs to slaughterhouse A and 7 farms delivering their pigs to slaughterhouse B. All *optrA*-harboring MRCoNS were identified as *Staphylococcus sciuri*.

MICs and detection of resistance genes. All 33 *optrA*-positive MRCoNS were resistant to oxacillin, tetracycline, and chloramphenicol and also showed high MICs to tiamulin, virginiamycin M1, and florfenicol. Most of these isolates also exhibited resistance to gentamicin (94%), clindamycin (88%), and erythromycin (88%) and had high spectinomycin MICs (76%), but all of them proved to be susceptible to sulfamethoxazole. The linezolid and tedizolid MICs ranged from 1 to 16 mg/liter and 0.125 to 2 mg/liter, respectively. Among the 33 isolates, only 11 were classified on the basis of their MICs as intermediate or resistant to linezolid (MICs, 4 to 16 mg/liter), while only 4 isolates proved to be nonsusceptible to tedizolid (MICs, ≥1 mg/liter). It is noteworthy that only 3 isolates showed cross-resistance to linezolid and tedizolid, while 5 and 22 isolates were borderline susceptible (MIC, 4 mg/liter) or susceptible (MICs, 1 to 2 mg/liter) to linezolid, respectively. None of the isolates had oxazolidinone resistance-mediating mutations in domain V of the 23S rRNA or in the genes coding for ribosomal proteins L3 and L4.

Comparison of the deduced OptrA amino acid sequences of the 33 isolates with the original OptrA protein from *Enterococcus faecalis* E349 (designated the wild type) revealed three OptrA variants, all of which differed from the wild type in at least two amino acid positions. Substitutions at positions 176 (Tyr176Asp) and 393 (Gly393Asp) (DD variant) were identified in 27 isolates; alterations at positions 176 (Tyr176Asp), 247 (Asp247Asn), and 393 (Gly393Asp) (DND variant) were found in 4 isolates; and alterations at positions 176 (Tyr176Asp), 247 (Asp247Asn), 393 (Gly393Asp), and 622 (Ile622Met) (DNDM variant) were identified in 2 isolates. Comparative analysis of isolates with the DD variant showed that their linezolid MICs ranged from 1 to 16 mg/liter and their tedizolid MICs ranged from 0.125 to 2 mg/liter. A similar situation was seen for isolates carrying the DND variant, with linezolid MICs ranging from 1 to 16 mg/liter and tedizolid MICs from 0.125 to 0.5 mg/liter. The two isolates with the DNDM variant exhibited linezolid and tedizolid MICs of 1 and 0.125 mg/liter, respectively, which classified these isolates as susceptible to both linezolid and tedizolid. These observations suggested that at least the dominant substitutions at amino acids 176 (Tyr176Asp) and 393 (Gly393Asp), seen in all 33 isolates, had no obvious impact on the linezolid and tedizolid MICs. It should be noted that the three isolates with the highest linezolid MIC of 16 mg/liter also carried the *cfr* gene.

With regard to resistance genes other than *optrA*, all 33 isolates carried the *mecA* gene and the phenicol resistance gene *fexA*. The novel spectinomycin resistance genes *spd* and *spw* were present either alone or in combination in all 33 isolates. Combined resistance to pleuromutilins, lincosamides, and streptogramin A antibiotics was based on the presence of the *lsa*(E) gene either alone or together with the *vga*(C) gene in all isolates. Thirty-three isolates were tetracycline resistant and carried the *tet*(L) ($n = 12$) or *tet*(K) ($n = 6$) gene (Table 1). The gentamicin resistance gene *aacA-aphD* was detected in 32 isolates. The macrolide-lincosamide-streptogramin B resistance gene *erm*(C) was present either alone or in combination with *erm*(B) or with both *erm*(A) and *erm*(B) in 29 isolates (Table 1). All 33 isolates were negative for *cfr*(B), while 5 of them harbored the multiresistance gene *cfr*. Overlapping PCR assays (31) confirmed that the resistance genes *lnu*(B) and *lsa*(E) were present in a gene cluster that often also contained the spectinomycin resistance gene *spw*. This observation suggests that this cluster not only exists in MRSA (25, 31) but is also widespread in methicillin-resistant *S. sciuri*. While the resistance genes detected in the MRCoNS isolates can usually explain the observation of resistance phenotypes, there were a few cases in which no gene for a specific resistance property could be detected, such as 15 tetracycline-resistant isolates that were negative for the *tet* genes detected in this study.

Molecular typing and gene location. PFGE analysis of the 33 MR *S. sciuri* isolates revealed 25 patterns (Table 1; see also Fig. S1 in the supplemental material), suggesting a high diversity of *optrA*-positive isolates. As an effective tool applied in previous typing of methicillin-resistant staphylococci (24, 28), *dru* typing was also conducted to analyze the 33 MR *S. sciuri* isolates. Among these, five different *dru* types were identified, including the most common types, dt11y ($n = 25$ [75.8%]) and dt10a ($n = 5$ [15.2%]). Single isolates harbored dt13c or the novel *dru* type dt11db or dt10df (Table 1). S1-PFGE and Southern blotting re-

TABLE 1 Characterization of 33 *optrA*-carrying MR *S. sciuri* isolates from pigs in Guangdong

Isolate	Farm	PFGE type	*dru* type[a]	Location of *optrA*[b]	OptrA variant[c]	MIC (mg/liter) of:		Other resistance phenotype[d]	Resistance genes
						LZD[e]	TZD[f]		
S031-12	1	K	dt11y	P	DND	16	0.5	OXA CHL FFC AMC CLI ERY GEN TET SPT TIA VIM	*mecA*, *optrA*, *cfr*, *fexA*, *aacA-aphD*, *tet*(K), *erm*(B), *erm*(C), *vga*(C), *spw*, *lnu*(B), *lsa*(E)
S031-25	1	O	dt11y	C (II)	DD	8	0.5	OXA CHL FFC AMC CLI ERY GEN TET SPT TIA VIM	*mecA*, *optrA*, *fexA*, *aacA-aphD*, *tet*(K), *erm*(A), *erm*(B), *erm*(C), *spd*, *spw*, *lnu*(B), *lsa*(E)
S032-2	2	H	dt11y	C (I)	DD	16	0.125	OXA CHL FFC AMC CLI ERY GEN TET SPT TIA VIM	*mecA*, *optrA*, *cfr*, *fexA*, *aacA-aphD*, *tet*(L), *erm*(B), *erm*(C), *spw*, *lnu*(B), *lsa*(E)
S032-3	2	A	dt10a	C (III)	DD	2	0.25	OXA CHL FFC AMC CLI ERY GEN TET SPT TIA VIM	*mecA*, *optrA*, *fexA*, *aacA-aphD*, *tet*(K), *erm*(B), *erm*(C), *spd*, *spw*, *lnu*(B), *lsa*(E)
S032-5	2	Y2	dt11y	C (III)	DD	2	0.25	OXA CHL FFC AMC CLI ERY GEN TET SPT TIA VIM	*mecA*, *optrA*, *fexA*, *aacA-aphD*, *tet*(L), *erm*(B), *erm*(C), *spw*, *lnu*(B), *lsa*(E)
S13-1	3	J1	dt11y	C (I)	DD	2	0.25	OXA CHL FFC AMC CLI ERY GEN TET SPT TIA VIM	*mecA*, *optrA*, *fexA*, *aacA-aphD*, *tet*(L), *erm*(B), *erm*(C), *spd*, *spw*, *lnu*(B), *vga*(C), *lsa*(E)
S13-2	3	G1	dt11y	P	DD	2	0.125	OXA CHL FFC AMC GEN TET SPT TIA VIM	*mecA*, *optrA*, *cfr*, *fexA*, *aacA-aphD*, *spd*, *spw*, *lnu*(B), *vga*(C), *lsa*(E)
S22-4	4	P	dt11y	C (I)	DND	1	0.125	OXA CHL FFC AMC CLI ERY GEN TET SPT TIA VIM	*mecA*, *optrA*, *fexA*, *aacA-aphD*, *tet*(K), *erm*(B), *erm*(C), *spd*, *spw*, *lnu*(B), *lsa*(E)
S24-1	5	C	dt13c	C (IV)	DND	1	0.125	OXA CHL FFC AMC CLI ERY GEN TET SPT TIA VIM	*mecA*, *optrA*, *fexA*, *aacA-aphD*, *erm*(B), *erm*(C), *spd*, *spw*, *lnu*(B), *vgaC*, *lsa*(E)
S25-1	6	X	dt10df*	C (IV)	DD	1	0.125	OXA CHL FFC AMC CLI ERY GEN TET SPT TIA VIM	*mecA*, *optrA*, *fexA*, *aacA-aphD*, *tet*(L), *erm*(B), *erm*(C), *spd*, *spw*, *lnu*(B), *lsa*(E)
S28-1	7	V1	dt11y	C (IV)	DD	4	0.25	OXA CHL FFC AMC GEN TET SPT TIA VIM	*mecA*, *optrA*, *fexA*, *aacA-aphD*, *spd*, *spw*, *lnu*(B), *vga*(C), *lsa*(E)
S28-2	7	E	dt11db*	P	DD	16	2	OXA CHL FFC AMC CLI ERY GEN TET SPT TIA VIM	*mecA*, *optrA*, *cfr*, *fexA*, *aacA-aphD*, *erm*(C), *spd*, *spw*, *lnu*(B), *vga*(C), *lsa*(E)
S29-21	8	V3	dt11y	C (IV)	DD	2	0.5	OXA CHL FFC AMC GEN TET SPT TIA VIM	*mecA*, *optrA*, *fexA*, *aacA-aphD*, *spd*, *spw*, *lnu*(B), *lsa*(E)
S29-5	8	W	dt10a	P	DD	8	2	OXA CHL FFC AMC CLI ERY GEN TET SPT TIA VIM	*mecA*, *optrA*, *cfr*, *fexA*, *aacA-aphD*, *tet*(L), *erm*(C), *spd*, *spw*, *lnu*(B), *lsa*(E)
S29-6	8	N	dt10a	C (IV)	DD	2	0.5	OXA CHL FFC AMC CLI ERY GEN TET SPT TIA VIM	*mecA*, *optrA*, *fexA*, *aacA-aphD*, *tet*(L), *erm*(B), *erm*(C), *spd*, *spw*, *lnu*(B), *lsa*(E)
S33-14	9	Y2	dt11y	C (I)	DD	2	0.5	OXA CHL FFC AMC CLI ERY GEN TET SPT TIA VIM	*mecA*, *optrA*, *fexA*, *aacA-aphD*, *erm*(C), *spd*, *spw*, *lnu*(B), *vga*(C), *lsa*(E)
S33-15	9	F	dt11y	C (I)	DD	1	0.125	OXA CHL FFC AMC CLI ERY GEN TET SPT TIA VIM	*mecA*, *optrA*, *fexA*, *aacA-aphD*, *tet*(L), *erm*(B), *erm*(C), *spd*, *spw*, *lnu*(B), *lsa*(E)
S33-18	9	L	dt11y	C (I)	DD	4	0.25	OXA CHL FFC AMC CLI ERY GEN TET TIA VIM	*mecA*, *optrA*, *fexA*, *aacA-aphD*, *tet*(L), *erm*(B), *erm*(C), *spd*, *lnu*(B), *lsa*(E)
S33-2	9	J2	dt11y	C (I)	DD	2	0.25	OXA CHL FFC AMC CLI ERY GEN TET SPT TIA VIM	*mecA*, *optrA*, *fexA*, *aacA-aphD*, *tet*(L), *erm*(B), *erm*(C), *spd*, *spw*, *lnu*(B), *vga*(C), *lsa*(E)
S35-13	10	S1	dt11y	C (IV)	DD	4	0.25	OXA CHL FFC AMC CLI ERY GEN TET SPT TIA VIM	*mecA*, *optrA*, *fexA*, *aacA-aphD*, *erm*(B), *erm*(C), *spd*, *spw*, *lnu*(B), *lsa*(E)
S35-16	10	V2	dt11y	C (I)	DD	2	1	OXA CHL FFC AMC GEN TET SPT TIA VIM	*mecA*, *optrA*, *fexA*, *aacA-aphD*, *spd*, *lnu*(B), *lsa*(E)
S39-1	11	I	dt11y	C (IV)	DD	1	0.25	OXA CHL FFC AMC CLI ERY TET SPT TIA VIM	*mecA*, *optrA*, *fexA*, *erm*(C), *spd*, *spw*, *lnu*(B), *lsa*(E)
S49-1	12	S2	dt11y	C (VI)	DND	2	0.25	OXA CHL FFC AMC CLI ERY GEN TET TIA VIM	*mecA*, *optrA*, *fexA*, *aacA-aphD*, *erm*(B), *erm*(C), *spw*, *lnu*(B), *vga*(C), *lsa*(E)
MS11-3	13	T	dt10at	C (IV)	DD	8	0.5	OXA CHL FFC AMC CLI ERY GEN TET SPT TIA VIM	*mecA*, *optrA*, *fexA*, *aacA-aphD*, *tet*(K), *erm*(B), *erm*(C), *spd*, *spw*, *lnu*(B), *vga*(C), *lsa*(E)
MS22-9	14	V4	dt11y	C (I)	DD	1	0.125	OXA CHL FFC AMC CLI ERY GEN TET SPT TIA VIM	*mecA*, *optrA*, *fexA*, *aacA-aphD*, *tet*(L), *erm*(B), *erm*(C), *spd*, *spw*, *lnu*(B), *lsa*(E)

MS25-5	15	M	dt11y	C (I)	DD	2	0.25	OXA CHL FFC AMC CLI ERY GEN TET SPT TIA VIM	*mecA*, *optrA*, *fexA*, *aacA-aphD*, *tet*(L), *erm*(B), *erm*(C), *spw*, *lnu*(B), *vga*(C), *lsa*(E)
MS35-4	16	G2	dt11y	C (I)	DD	2	0.25	OXA CHL FFC AMC CLI ERY GEN TET SPT TIA VIM	*mecA*, *optrA*, *fexA*, *aacA-aphD*, *erm*(B), *erm*(C), *spd*, *lnu*(B), *lsa*(E)
MS35-12	16	R	dt11y	C (I)	DD	2	0.25	OXA CHL FFC AMC CLI ERY GEN TET SPT TIA VIM	*mecA*, *optrA*, *fexA*, *aacA-aphD*, *erm*(B), *erm*(C), *spd*, *spw*, *lnu*(B), *vga*(C), *lsa*(E)
MS37-7	17	U	dt10t	C (IV)	DD	1	0.25	OXA CHL FFC AMC CLI ERY GEN TET SPT TIA VIM	*mecA*, *optrA*, *fexA*, *aacA-aphD*, *tet*(L), *erm*(B), *erm*(C), *spw*, *lnu*(B), *vga*(C), *lsa*(E)
MS38-4	18	B	dt11y	C (IV)	DD	4	1	OXA CHL FFC AMC CLI ERY GEN TET SPT TIA VIM	*mecA*, *optrA*, *fexA*, *aacA-aphD*, *erm*(A), *erm*(B), *erm*(C), *spd*, *lnu*(B), *lsa*(E)
MS58-2	19	Q	dt11y	C (V)	DNDM	1	0.125	OXA CHL FFC AMC CLI ERY GEN TET SPT TIA VIM	*mecA*, *optrA*, *fexA*, *aacA-aphD*, *erm*(B), *erm*(C), *spd*, *lnu*(B), *lsa*(E)
MS58-20	19	P	dt11y	C (V)	DNDM	1	0.125	OXA CHL FFC AMC CLI ERY GEN TET TIA VIM	*mecA*, *optrA*, *fexA*, *aacA-aphD*, *erm*(B), *erm*(C), *spd*, *lnu*(B), *lsa*(E)
MS58-23	19	D	dt11y	C (V)	DD	4	0.25	OXA CHL FFC AMC CLI ERY GEN TET SPT TIA VIM	*mecA*, *optrA*, *fexA*, *aacA-aphD*, *tet*(K), *erm*(A), *erm*(B), *erm*(C), *spd*, *lnu*(B), *lsa*(E)

[a] New *dru* types (dt11db, 5a-2d-4a-0-3c-5b-3a-2g-4b-4e; dt10df, 5a-3ac-4a-1b-2d-5b-3a-3b-4e-3e) are indicated by asterisks.
[b] P, plasmid; C, chromosome. A Roman numeral in parentheses indicates the type of the genetic environment of *optrA* that was detected. See Fig. 1.
[c] DD, Tyr176Asp Gly393Asp; DND, Tyr176Asp Asp247Asn Gly393Asp; DNDM, Tyr176Asp Asp247Asn Gly393Asp Ile622Met.
[d] OXA, oxacillin; CHL, chloramphenicol; FFC, florfenicol; AMC, amoxicillin-clavulanic acid; CLI, clindamycin; ERY, erythromycin; GEN, gentamicin; TET, tetracycline; SPT, spectinomycin; TIA, tiamulin; VIM, virginiamycin M1.
[e] LZD, linezolid.
[f] TZD, tedizolid.

vealed that *optrA* was located in the chromosomal DNA of 29 isolates and on a plasmid in the remaining 4 isolates. The *cfr* gene was colocated on the *optrA*-carrying plasmid in four isolates and was found in the chromosomal DNA of one isolate.

Genetic environment of *optrA* on plasmids. The four MR *S. sciuri* isolates with plasmid-borne *optrA* exhibited different PFGE patterns, suggesting that these are epidemiologically unrelated isolates. However, all of them harbored a plasmid of ca. 35 kb. A 17,612-bp contig including the *optrA-cfr-aadD-ble-aacA-aphD-fexA* resistance gene cluster on each of the four plasmids was indistinguishable from the corresponding region of the 60.5-kb plasmid pWo28-3 (GenBank accession no. KT601170) (16), suggesting that the plasmids found in the four MR *S. sciuri* isolates are likely to have developed from plasmid pWo28-3 and seem to be disseminated in MR *S. sciuri* isolates from the Guangdong area.

Genetic environment of *optrA* in chromosomal DNA. Six types of *optrA*-carrying fragments (types I to VI), with sizes ranging from 3,455 bp to 28,291 bp, were obtained by whole-genome sequencing of the 29 *S. sciuri* isolates with chromosomal *optrA* and were analyzed. Among these, the type I ($n = 12$) and type IV ($n = 10$) genetic environments were the most prevalent (Fig. 1). Type I carried the shortest contig, in which only the putative transcriptional regulator gene *araC* was identified, located immediately upstream of the *optrA* gene. This *araC-optrA* core region was also conserved in the other five types. Another commonly identified element was the *fexA*-carrying transposon Tn*558*, which was located upstream of *araC-optrA* in type II to VI genetic environments. Whether or not this transposon plays a role in the dissemination of *optrA* in *S. sciuri* remains to be elucidated.

An interesting observation is that a *ccr* complex with the *ccrA-ccrB* genes, the products of which exhibited 96.9% and 98.3% amino acid identity to the corresponding proteins of MR *S. aureus* Gv88 (GenBank accession no. CP012018), was located downstream of *optrA-araC* in type II genetic environments and upstream of *optrA-araC* in type III genetic environments. An *orfX* gene, which is considered the integration site of staphylococcal cassette chromosome *mec* elements (SCC*mec*) in the chromosomal DNA of staphylococci, was detected, and its product exhibited 96.6% amino acid identity to that of *S. aureus* Gv88 (32). Comparative analysis of the *optrA*-carrying fragments of the 10 isolates harboring type IV genetic environments revealed that an *araC-optrA*-Tn*558* segment was apparently inserted into the corresponding chromosomal region of *S. sciuri* DSM 20345 (BioProject record no. PRJNA283171) (Fig. 1). This region is characterized by the presence of the *mdlB-1* and *mdlB-2* genes, coding for a multidrug ABC transporter, and an *hp* gene, coding for a hypothetical protein, in the upstream part. Downstream of the inserted segment, two *hp* genes, as well as the *dct*, *folC*, and *valS* genes, coding for a C_4-dicarboxylate ABC transporter permease, a folylpolyglutamate synthase, and a valine-tRNA ligase, respectively, were identified. In the three isolates representing the type V genetic environment, the same insertion site was used, but the integrated segment was larger than that in type IV. A segment of 8,018 bp comprising three genes, one gene coding for a hypothetical protein and the *mob* and *top* genes, coding for a relaxase/mobilization protein and topoisomerase, respectively, was inserted between the *optrA-araC* genes and transposon Tn*558* (Fig. 1). In the type VI genetic environment, a similar situation was observed. Again, the same insertion site was used, but the fragment between *optrA-araC* and Tn*558* was 6,533 bp long and included five genes

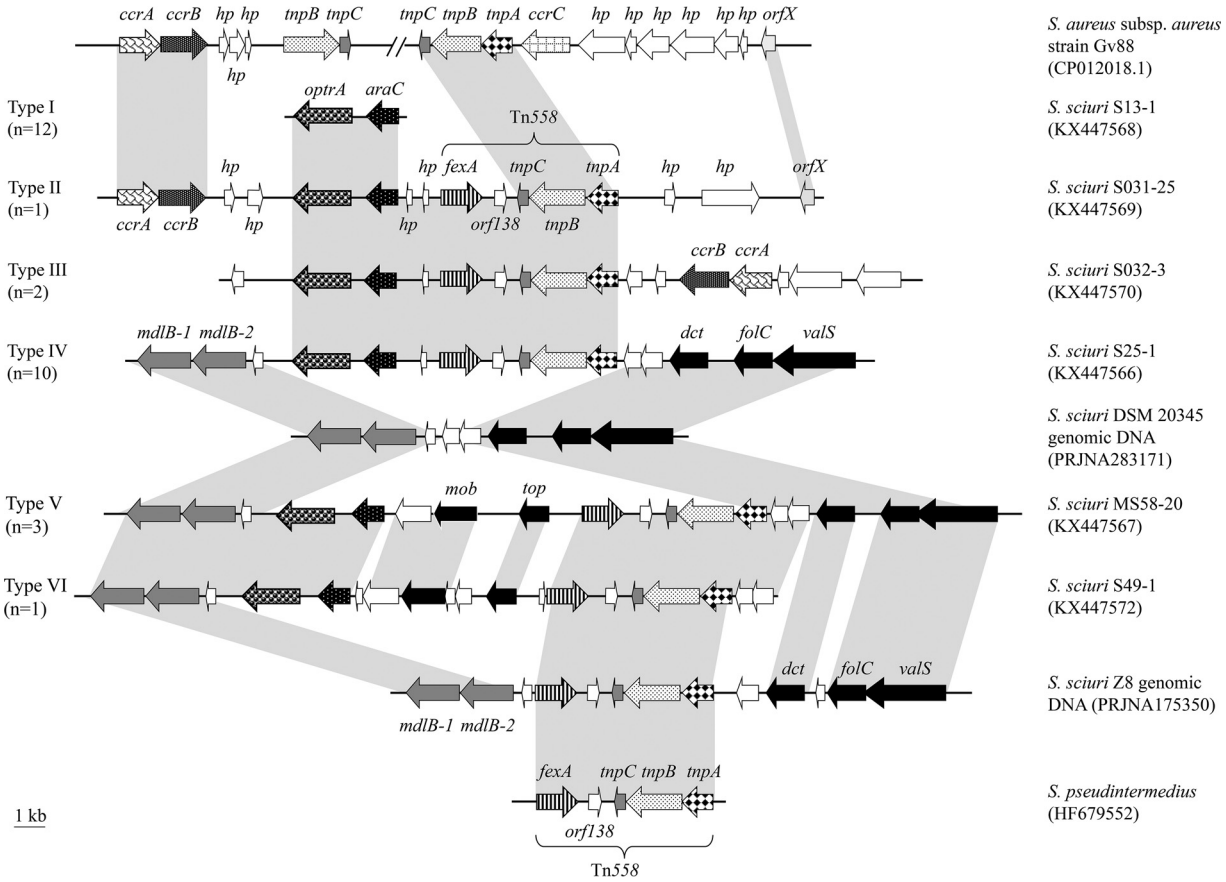

FIG 1 Schematic presentation of the genetic environments of *optrA*-flanking regions in the chromosomal DNAs of the 29 MR *S. sciuri* isolates investigated in this study in comparison to the corresponding chromosomal regions of other *S. aureus* or *S. sciuri* isolates. Shaded areas represent regions of >99% nucleotide sequence identity. Arrows indicate the positions and orientations of the genes. Open arrows represent genes coding for hypothetical proteins.

coding for hypothetical proteins in addition to the *mob* and *top* genes. It should be noted that a complete Tn*558* was found to be integrated into the same site in the chromosomal DNA of *S. sciuri* Z8 (BioProject record no. PRJNA175350). As such, the finding of *optrA-araC* in connection with Tn*558* as well as other genes might be due to several independent integration events.

In conclusion, this is the first large-scale study on the surveillance of the *optrA* gene in staphylococci of animal origin. Given that all *optrA* carriers were *S. sciuri* isolates, it is tempting to speculate about a specific role that this species may play in the dissemination of *optrA*. Further work is needed to better understand why the same OptrA variant is associated with different linezolid and tedizolid MICs and which other factors may have a positive or a negative impact on the expression of the *optrA* gene. Since *S. sciuri* isolates are known to occur in samples from various human, animal, and environmental sources, members of this species may act as a reservoir for virulence and resistance genes, including *optrA* (33). Further investigations are needed to better understand the role of *S. sciuri* in the spread of *optrA*, and surveillance of the transmission and spread of *optrA*-positive staphylococci is urgently needed.

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