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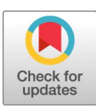

Mobile Oxazolidinone Resistance Genes in Gram-Positive and Gram-Negative Bacteria

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SUMMARY Seven mobile oxazolidinone resistance genes, including *cfr*, *cfr*(B), *cfr*(C), *cfr*(D), *cfr*(E), *optrA*, and *poxtA*, have been identified to date. The *cfr* genes code for 23S rRNA methylases, which confer a multiresistance phenotype that includes resistance to phenicols, lincosamides, oxazolidinones, pleuromutilins, and streptogramin A compounds. The *optrA* and *poxtA* genes code for ABC-F proteins that protect the bacterial ribosomes from the inhibitory effects of oxazolidinones. The *optrA* gene confers resistance to oxazolidinones and phenicols, while the *poxtA* gene confers elevated MICs or resistance to oxazolidinones, phenicols, and tetracycline. These oxazolidinone resistance genes are most frequently found on plasmids, but they are also located on transposons, integrative and conjugative elements (ICEs), genomic islands, and prophages. In these mobile genetic elements (MGEs), insertion sequences (IS) most often flanked the *cfr*, *optrA*, and *poxtA* genes and were able to generate translocatable units (TUs) that comprise the oxazolidinone resistance genes and occasionally also other genes. MGEs and TUs play an important role in the dissemination of oxazolidinone resistance genes across strain, species, and genus boundaries. Most frequently, these MGEs also harbor genes that mediate resistance not only to antimicrobial agents of other classes, but also to metals and biocides. Direct selection pressure by the use of antimicrobial agents to which the oxazolidinone resistance genes confer resistance, but also indirect selection pressure by the use of antimicrobial agents, metals, or biocides (the respective resistance genes against which are colocated on *cfr*-, *optrA*-, or *poxtA*-carrying MGEs) may play a role in the coselection and persistence of oxazolidinone resistance genes.

KEYWORDS oxazolidinones, *cfr*, *optrA*, *poxtA*, horizontal transfer, plasmid, transposon, genomic island, integrative and conjugative element, prophage, mobile genetic element

INTRODUCTION

In 1987, scientists at E. I. DuPont de Nemours & Co. described a new class of synthetic antibacterial agents, the oxazolidinones (1). These are heterocyclic molecules with an oxygen and a nitrogen in a five-membered ring bridged with a carbonyl group. These

agents are active *in vitro*, mainly against streptococci, enterococci, and staphylococci (1). Their *in vivo* activity against these bacteria has been confirmed in the respective animal models (1). Although the lead substance, DuP 721, showed promising antibacterial activities, serious toxicity problems were noted, which finally led not only to the dropping of the development of DuP 721 as a potential antimicrobial agent, but also to the cessation of the entire work on oxazolidinones by DuPont. Scientists at Pharmacia & Upjohn, however, believed that oxazolidinones might represent valuable antimicrobial agents for the therapy of infections caused by the aforementioned Gram-positive pathogens. They developed analogue molecules, including the two novel oxazolidinones U-100592 and U-100766, and tested them for their antimicrobial activity and toxicity in clinical trials (2). Linezolid (formerly known as U-100766) showed a remarkable spectrum of activity, as it proved to be active against vancomycin-resistant *Enterococcus faecalis* and *Enterococcus faecium* (3–7), methicillin-resistant *Staphylococcus aureus* (3–8), and penicillin-resistant *Streptococcus pneumoniae* (3–6, 9), and thereby represented a most valuable agent against multiresistant Gram-positive pathogens. Linezolid also showed modest activity against several Gram-negative bacteria, including *Moraxella catarrhalis*, *Bordetella pertussis*, and *Pasteurella multocida* (3, 10–12). Moreover, linezolid also displayed modest activity against several Gram-positive and Gram-negative anaerobic bacteria, including *Clostridioides difficile*, *Clostridium perfringens*, *Bacteroides fragilis*, *Peptostreptococcus* spp., and *Fusobacterium* spp., as well against *Prevotella* spp. (3, 13–16). Finally, linezolid also showed substantial activity against *Mycobacterium tuberculosis*, the *Mycobacterium avium* complex, and some rapidly growing mycobacteria (3, 17, 18). Oxazolidinones act in a mainly bacteriostatic manner against staphylococci and enterococci, as confirmed by time-kill experiments (3, 8, 19). However, bactericidal activity has been observed not only against *S. pneumoniae*, but also against *Streptococcus pyogenes*, *C. perfringens*, and *B. fragilis* (3).

Linezolid was the first oxazolidinone approved exclusively for human use in April 2000 under the trade name Zyvox (https://www.accessdata.fda.gov/drugsatfda_docs/label/2010/021130s022lbl.pdf). According to the product label, linezolid is approved for the treatment of (i) uncomplicated skin and skin structure infections, (ii) complicated skin and skin structure infections, including diabetic foot infections, without concomitant osteomyelitis, (iii) nosocomial pneumonia, and (iv) community-acquired pneumonia, including concurrent bacteremia. Linezolid is also one of the few treatment options for infections caused by vancomycin-resistant enterococci, such as *E. faecalis* and *E. faecium*, including cases with concurrent bacteremia. In June 2014, the expanded-spectrum oxazolidinone tedizolid was approved, also only for use in humans, under the trade name Sivextro (https://www.accessdata.fda.gov/drugsatfda_docs/label/2014/205435s000lbl.pdf). Tedizolid is indicated for the treatment of acute bacterial skin and skin structure infections caused by susceptible isolates of *S. aureus* (including methicillin-resistant [MRSA] and methicillin-susceptible [MSSA] isolates), *S. pyogenes*, *Streptococcus agalactiae*, the *Streptococcus anginosus* group, and *E. faecalis*. Although not approved for veterinary use, linezolid and tedizolid may be used in non-food-producing animals, such as dogs and cats, via the Animal Medicinal Drug Use Clarification Act of 1994 (AMDUCA) in the United States or via similar cascade regulations in other countries. This extralabel use by veterinarians requires proof—usually via an antibiogram—that there is no other antimicrobial agent approved for veterinary use that is efficacious against the causative bacterial pathogen in this specific case. Due to the high treatment costs, oxazolidinones have rarely been used to treat dogs and cats (20). The future will show whether this will change, since linezolid has recently become a generic drug.

MODE OF ACTION OF OXAZOLIDINONES

The oxazolidinones act by a novel mode of action different from those of all other antimicrobial agents. Several studies have shown that oxazolidinones inhibit bacterial protein biosynthesis. However, oxazolidinones inhibit neither the elongation reaction nor the binding of either *N*-formylmethionyl-tRNA or mRNA to the 30S ribosomal

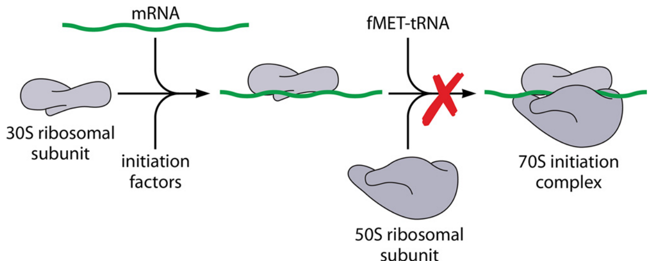

FIG 1 Schematic presentation of the mode of action of oxazolidinones. Oxazolidinones inhibit protein biosynthesis by preventing the formation of a functional 70S initiation complex composed of the 30S ribosomal subunit, initiation factors, mRNA, formylmethionyl-tRNA (fMET-tRNA), and the 50S ribosomal subunit. (Based on data from reference 26.)

subunit. Oxazolidinones also do not prevent the formation of a binary complex between the initiation factor IF2 and *N*-formylmethionyl-tRNA. Instead, oxazolidinones bind to the 23S portion of the 50S subunit and prevent it from forming a complex with the 30S ribosomal subunit, *N*-formylmethionine-tRNA, GTP, mRNA, and the initiation factors IF1 to IF3. Thus, a functional 70S initiation complex cannot be formed and mRNA cannot be translated (Fig. 1). Oxazolidinones thus inhibit bacterial protein biosynthesis at a very early stage, i.e., before it has started (19, 21–24). This mode of action is different from those of other protein biosynthesis inhibitors, such as aminoglycosides (inhibition of translation and misreading), chloramphenicol (blocking of peptide bond formation by inhibition of the peptidyl transferase), macrolides (inhibition of peptide chain elongation and blocking of the assembly of the 50S subunit), streptogramins (inhibition of peptide chain elongation), and tetracyclines (interfering with the binding of incoming aminoacyl-tRNA to the A-site) (25, 26). Due to this novel mode of action and the observation that linezolid is active against Gram-positive pathogens that carry a wide range of resistance genes that code for either modifying enzymes, efflux mechanisms, or proteins that modify/protect the target sites of other antimicrobial agents, cross-resistance between the oxazolidinones and other antimicrobial agents has not been expected (27).

MECHANISMS OF OXAZOLIDINONE RESISTANCE

Zurenko and colleagues investigated the ability of the reference strain *S. aureus* ATCC 29213 to generate spontaneous linezolid-resistant mutants in the presence of 2-, 4-, and 8-fold the MIC. No linezolid-resistant colonies were detected at any of these concentrations, which corresponded to a spontaneous mutation frequency of *S. aureus* ATCC 29213 of less than 8×10^{-11} (3). Similar experiments with four strains each of MRSA, MSSA, and also methicillin-resistant and methicillin-susceptible *Staphylococcus epidermidis* were conducted by Kaatz and Seo (8). They found that the development of spontaneous linezolid resistance at 2-fold the MIC was for all 12 isolates below the detection limit, i.e., less than 1×10^{-9} (8). *In vitro* selection experiments with 10 clinical vancomycin-resistant enterococci (four *E. faecalis*, five *E. faecium*, and one *Enterococcus gallinarum* isolate) and the reference strain *E. faecalis* ATCC 29212 yielded resistant mutants with distinctly elevated linezolid MICs that had single-point mutations at various positions in the central loop of domain V of the 23S rRNA, including C2610G, G2576U, G2513U, G2512U, and G2505A (28). This locus has previously been shown to be the preferential area for oxazolidinone resistance-mediating mutations (29, 30). Studies on *E. faecalis* also showed that recombination proficiency has an impact on the frequency and the locus of mutations conferring linezolid resistance (31) (Table 1).

The first clinical linezolid-resistant *S. aureus* isolate was identified in a patient who was treated with linezolid. Further analysis showed that this isolate had the aforementioned G2576U mutation in the 23S rRNA (32). A linezolid-resistant *E. faecium* isolate that also exhibited the G2576U mutation, was obtained from a patient without prior

TABLE 1 Mechanisms of oxazolidinone resistance

Gene	Resistance mechanism(s)	Associated resistance phenotype[b]	Location(s)[f]	Reference(s)
23S rRNA	Point mutation	LZD	Chromosomal	32–35, 39, 40
rplC	Point mutation, deletion, insertion	LZD[c]	Chromosomal	34–40
rplD	Point mutation, deletion, insertion	LZD[c]	Chromosomal	34–42
cfr	rRNA methylase	PHE, LIN, LZD, PLM, STA	Plasmid and chromosomal	45, 46
cfr(B)	rRNA methylase	PHE, LIN, LZD, PLM, STA	Plasmid and chromosomal	48, 49, 53
cfr(C)	rRNA methylase[a]	PHE, LIN, LZD, PLM, STA	Plasmid and chromosomal	50
cfr(D)	rRNA methylase[a]	PHE, LIN, LZD, PLM, STA	Plasmid and chromosomal	51
cfr(E)	rRNA methylase[a]	(PHE, LIN, LZD, PLM, STA)[d]	Chromosomal	52
optrA	ABC-F protein	PHE, LZD, TZD[e]	Plasmid and chromosomal	54
poxtA	ABC-F protein	PHE, LZD[c], TET[c]	Plasmid and chromosomal	56

[a]Assumed based on structural comparisons.
[b]LIN, lincosamides; LZD, linezolid; PHE, phenicols; PLM, pleuromutilins; STA, streptogramin A; TET, tetracycline; TZD, tedizolid. Depending on the bacterium in which this gene was detected, the resistance phenotype conferred by the gene may overlap with intrinsic resistance properties of the host bacterium.
[c]Confers only a minor increase in the respective MICs.
[d]To be confirmed.
[e]Certain OptrA proteins are known to confer elevated MICs to LZD and TZD.
[f]"Chromosomal" includes chromosomally integrated transposons, integrative and conjugative elements (ICEs), and prophages; "plasmid" also includes transposons integrated into plasmids.

exposure to oxazolidinones in 2001 as well (33). Over the following years, numerous strains with linezolid resistance-mediating mutations in the 23S rRNA have been described. Reviews by Long and Vester (34) and by Stefani et al. (35) summarize the linezolid resistance-mediating mutations seen in the 23S rRNA, including the bacteria in which they have been found. In addition, information concerning whether the respective mutations originated from *in vitro* selection experiments or were detected in clinical isolates was also provided (34). Among all mutations listed, G2576U is most widespread (34, 35).

Besides mutations in the 23S rRNA, linezolid resistance may also be due to modifications in the genes coding for the ribosomal proteins L3 (*rplC*) and L4 (*rplD*) (Table 1). These modifications include point mutations that result in single or multiple amino acid exchanges and deletions of variable length, but also insertions (34–38). Mutations in the L3 and L4 proteins were often found together with modifications in the 23S rRNA (34–36, 39, 40). Studies on *S. aureus* mutants that carried only modifications in the *rplC* or *rplD* genes (36) suggested that these modifications only slightly increased the linezolid MICs, whereas high linezolid MICs were mainly due to 23S rRNA modifications (36). An intermediate status to linezolid, combined with macrolide and chloramphenicol resistance, was found to be due to 6-bp deletions in the *rplD* gene of *S. pneumoniae* (41). A mutation in the *rplD* gene that led to the amino acid exchange G71D was also described in *C. perfringens* (42). Although mutations in the *rplV* gene coding for the ribosomal protein L22 have been observed in coagulase-negative staphylococci (CoNS) (43, 44), they most likely do not play a role in linezolid resistance. All of the aforementioned modifications in the 23S rRNA and in the *rplC* and *rplD* genes were chromosomally fixed and not horizontally transferable. Thus, bacteria carrying these mutations were disseminated by clonal expansion or developed *de novo* from susceptible strains.

This situation changed in 2000, when the first transferable oxazolidinone resistance gene, *cfr*, was identified in a bovine *Staphylococcus sciuri* isolate from Germany (45). This plasmid-borne gene was initially described as a novel chloramphenicol-florfenicol resistance gene. It took another 5 years until the mechanism of resistance was identified (46). The Cfr protein represents an RNA methyltransferase of the radical *S*-adenosylmethionine (SAM) superfamily, which targets the adenine residue at position 2503 in the 23S rRNA and thereby causes resistance (46). This adenine residue, however, is located exactly in the overlapping binding area for five chemically distinct classes of antimicrobial agents that inhibit bacterial protein biosynthesis, namely the phenicols, lincosamides, oxazolidinones, pleuromutilins, and streptogramin A antibiotics (47). The additional methylation at A2503 prevents the binding of the aforementioned

antimicrobial agents to the ribosome, thereby conferring resistance to all of them. The corresponding multiresistance phenotype has been termed $PhLOPS_A$ (47). More recently, variants of the *cfr* gene, termed *cfr*(B) (48, 49), *cfr*(C) (50), *cfr*(D) (51), and *cfr*(E) (52) have been described (Table 1). For *cfr*(B), it was shown that this gene confers multiple antimicrobial resistance by the same mechanisms as the *cfr* gene (53). The Cfr(B), Cfr(C), Cfr(D), and Cfr(E) proteins shared 74%, 64%, 55%, and 51% amino acid identity with Cfr.

In 2015, the oxazolidinone resistance gene *optrA* was identified on a plasmid in an *E. faecalis* isolate of human origin in China (54). Cloning and expression of this gene in *E. faecalis* JH2-2 and *S. aureus* RN4220 showed that *optrA* conferred resistance not only to both oxazolidinones, linezolid and tedizolid, but also to fluorinated and nonfluorinated phenicols (54). The *optrA* gene codes for an ABC-F protein which confers resistance by ribosome protection (55) (Table 1).

The gene *poxtA* was detected in a MRSA isolate of clinical origin in Italy in 2018 (56). In the recipient strains *E. faecalis* JH2-2 and *S. aureus* RN4220, the cloned *poxtA* gene conferred only 2-fold increases of the MICs to linezolid, tedizolid, chloramphenicol, tetracycline, and doxycycline, all of which are below the Clinical and Laboratory Standards Institute (CLSI)-approved breakpoints for the "resistant" category. Solely for florfenicol, an 8-fold increase in the MICs of both host strains was observed. Thus, it appears a bit questionable to consider *poxtA* to be a phenicol-oxazolidinone-tetracycline resistance gene. The PoxtA protein is also a member of the ABC-F family, but it is only 32% identical to OptrA (56) (Table 1).

Recently, Hua and colleagues performed comparative transcriptome analyses of a low-level linezolid resistant *E. faecalis* isolate and two susceptible *E. faecalis* isolates, including the reference strain ATCC 29212. Among the differentially expressed genes, three genes were significantly upregulated and were predicted to be associated with drug resistance through active efflux pumps and biofilm formation. Whether these genes play a role in the development of low-level linezolid resistance remains to be clarified (57).

MONITORING OF LINEZOLID RESISTANCE

Clinical Breakpoints and Quality Control Ranges

The monitoring of linezolid and tedizolid susceptibility requires approved clinical breakpoints and quality control ranges. The two main organizations worldwide, the Clinical and Laboratory Standards Institute (CLSI) (http://em100.edaptivedocs.net/dashboard.aspx) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (https://www.eucast.org/clinical_breakpoints/), have both set clinical breakpoints for linezolid and tedizolid that are applicable to staphylococci, enterococci, and streptococci, which are freely accessible from the respective websites. The breakpoints set by these two organizations are similar, but not identical, and they occasionally also differ in the target bacteria for which they have been approved. For agar disk diffusion assays, CLSI recommends the use of 30-μg linezolid disks, whereas EUCAST recommends the use of 10-μg linezolid disks. CLSI provides no approved tedizolid breakpoints for agar disk diffusion and also recommends that bacteria identified as linezolid resistant by agar disk diffusion should be confirmed by a MIC method. This suggests that broth microdilution is the recommended method to determine oxazolidinone susceptibility in routine diagnostics.

The quality control (QC) ranges set for the reference strains also differ in part between CLSI and EUCAST. The main differences are that (i) CLSI uses specific staphylococcal reference strains for disk diffusion (*S. aureus* ATCC 25923) and MIC determination (*S. aureus* ATCC 29213), whereas EUCAST uses the same strain (*S. aureus* ATCC 29213) for both antimicrobial susceptibility testing (AST) methods, and (ii) that the aforementioned disks with different linezolid contents are recommended by CLSI and EUCAST, respectively. Thus, the use of different clinical breakpoints and different QC parameters can result in slight differences between the monitoring results when following CLSI or EUCAST methodologies.

FIG 2 Percentages of linezolid-susceptible *S. aureus*, coagulase-negative staphyloccoci (CoNS), and *Enterococcus* isolates as determined in the worldwide Zyvox Annual Appraisal of Potency and Spectrum (ZAAPS) program during the years 2002 to 2016 (58–66, 69–71).

Monitoring Programs for Linezolid Susceptibility

In 2002, the worldwide Zyvox Annual Appraisal of Potency and Spectrum (ZAAPS) program was initiated to monitor trends in the linezolid susceptibility patterns of six groups of bacterial pathogens (58). These include *S. aureus*, CoNS, *Enterococcus* spp., *S. pneumoniae*, streptococci of the beta-hemolytic group, and streptococci of the viridans group. In the initial study, a total of 7,971 bacterial isolates were investigated for their susceptibility to linezolid and 21 other antimicrobial agents by broth microdilution according to the CLSI recommendations. The isolates originated from North America (two nations, 30 sites), South America (two nations, five sites), Europe (six nations, 16 sites), and the Asia-Pacific region (two nations, three sites) and comprised 3,687 *S. aureus* isolates, 870 CoNS, 1,070 enterococci, 1,770 *S. pneumoniae* isolates, 387 beta-hemolytic streptococci, and 187 viridans group streptococci (58). In total, four linezolid-resistant isolates with linezolid MICs of $\geq$8 μg/ml, one each of *S. aureus*, *S. epidermidis*, *E. faecium*, and the viridans group *Streptococcus*, were identified. All of these isolates had the mutation G2576U in their 23S rRNA (58).

Over the next 10 consecutive years, the ZAAPS program was continued and expanded (59–68). An excellent addition to the pure phenotypic monitoring is that all isolates identified as linezolid resistant were investigated for the underlying resistance mechanisms. After a break in 2013, the program continued for the following years (69–71). The last report dates from 2018 and reports the susceptibility data from 2016 (71). Here, 8,325 bacterial isolates were obtained from 76 medical centers in 42 countries (excluding the United States). Among them, 17 isolates were confirmed as linezolid resistant. A single *S. aureus* isolate from Panama carried the *cfr* gene, and eight *E. faecalis* isolates from Europe, Latin America, and Southeast Asia, as well as one *Streptococcus gallolyticus* isolate from Thailand, harbored the *optrA* gene. The remaining resistant isolates, including four *S. epidermidis* isolates from Germany and Italy, two *E. faecium* isolates from Italy, and one *Streptococcus mitis* group isolate from Slovenia, had the 23S rRNA mutation G2576U and occasionally insertions or exchanges in the genes coding for the ribosomal proteins L3 or L4 (71).

Figure 2 shows the percentages of linezolid susceptibility of the three groups of bacteria, *S. aureus*, CoNS, and *Enterococcus* spp. Streptococci were—with a few exceptions—completely susceptible over the entire monitoring period from 2002 to 2016. In general, the susceptibility rates for *S. aureus* ranged between 99.93 and 100%, for

CoNS between 98.81 and 100%, and for *Enterococcus* spp. between 98.83 and 100%. Even though resistant target bacteria were identified occasionally, these data confirmed that after more than 15 years of clinical use, there is no trend toward increasing resistance visible worldwide.

In addition to the ZAAPS program, the Linezolid Experience and Accurate Determination of Resistance (LEADER) surveillance program was started in 2004 (72). This program is focused exclusively on the situation in the United States, monitors the susceptibility of virtually the same set of target bacteria as ZAAPS, and also performs AST by broth microdilution according to CLSI recommendations. From 2004 on, isolates from the United States were excluded from the ZAAPS program. The initial LEADER study investigated 4,414 isolates provided by 50 medical centers from 34 states in the United States, including Washington, DC (72). Over the following years, results from the LEADER program have been published (43, 44, 73–79). The latest data are from 2015. A summary of the results from 2011 to 2015 showed very low rates of nonsusceptible *S. aureus* ($<$0.1 to 0.1%), CoNS (0.5 to 1.2%), *Enterococcus* spp. (0.3 to 0.7%), and viridans group streptococci (0.0 to 0.7%), whereas all *S. pneumoniae* and beta-hemolytic streptococci were susceptible to linezolid (79). A comparison of the susceptibility percentages between the ZAAPS and LEADER programs has been published for the years 2004 to 2012 and for *S. aureus*, CoNS, and *Enterococcus* spp. In general, the susceptibility rates for all three groups of bacteria from the United States were equal or lower than those of the international strain collections (80).

In addition to these linezolid-specific monitoring programs, detection of resistance to linezolid among bacteria of the genera *Enterococcus*, *Staphylococcus*, and *Streptococcus* of human origin is included in various country-specific monitoring programs, such as NethMap from the Netherlands (https://www.rivm.nl/bibliotheek/rapporten/2020-0065.pdf), the Danish Integrated Antimicrobial Resistance Monitoring and Research Program (DANMAP) (https://www.danmap.org/reports/2019), the Swedish Antibiotic Sales and Resistance in Human Medicine (SWEDRES) and the Swedish Veterinary Antibiotic Resistance Monitoring (SVARM) (https://www.sva.se/media/jzdlctnk/rapport_swedres-svarm_2018.pdf), the Norwegian Surveillance System for Antimicrobial Drug Resistance NORM/NORM-Vet (https://www.vetinst.no/overvaking/antibiotikaresistens-norm-vet), the British Society for Antimicrobial Chemotherapy (BSAC) Resistance Surveillance Programme for the UK and Ireland (https://bsacsurv.org/), and the PanEuropean EARS-Net annual surveillance reports on antimicrobial resistance published by the European Centre for Disease Prevention and Control (ECDC) (https://www.ecdc.europa.eu/en/antimicrobial-resistance/surveillance-and-disease-data/report), as well as the China Antimicrobial Surveillance Network (CHINET) (www.chinets.com) and the China Antimicrobial Resistance Surveillance System (CARSS) (http://carss.cn/). Similarly to ZAAPS and LEADER, all of these monitoring and surveillance programs reported very low percentages of linezolid-resistant target bacteria. However, they used in part different methodologies and interpretive criteria, which renders their results not directly comparable.

In the veterinary sector, only DANMAP, NORM-Vet, and the German national resistance monitoring program for veterinary pathogens, GE*RM*-Vet (https://www.bvl.bund.de/DE/Arbeitsbereiche/05_Tierarzneimittel/01_Aufgaben/05_AufgAntibiotikaResistenz/05_GERMvet/GERMvet_node.html), monitor linezolid resistance of selected Gram-positive pathogens from animals. Although all three programs use different standards for performing AST and interpreting the results, no linezolid-resistant target bacteria have been identified in DANMAP 2019 and GE*RM*-Vet 2018, while a single *Streptococcus canis* isolate from a clinical infection of a dog and three *E. faecalis* isolates from raw dog food samples were identified in NORM-Vet 2019.

THE MOBILE OXAZOLIDINONE RESISTANCE GENE *cfr*

Geographical Distribution and Host Bacteria of the *cfr* Gene

A database search that included PubMed (https://pubmed.ncbi.nlm.nih.gov/) and NCBI Nucleotide (https://www.ncbi.nlm.nih.gov/nucleotide/) databases was done for

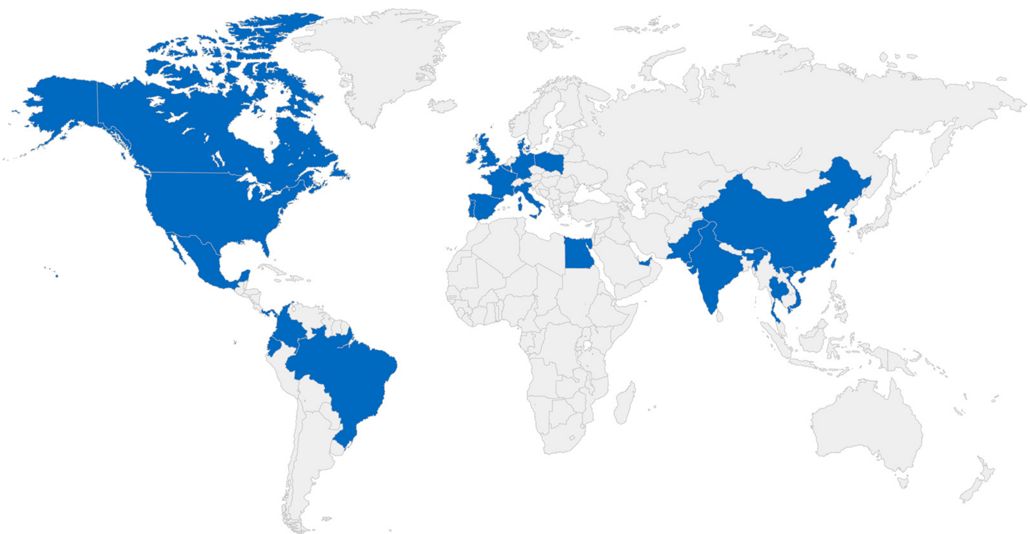

FIG 3 Geographical distribution of *cfr*-carrying bacteria. The countries in blue are those from which the occurrence of *cfr*-carrying bacteria has been reported.

all currently known mobile oxazolidinone resistance genes. It identified the gene *cfr* to be present in 25 countries in five continents (Fig. 3). It is important to understand that this geographical distribution reflects the participation in monitoring programs, such as ZAAPS and LEADER, and the areas in which research groups were particularly active in the search for oxazolidinone resistance genes and the analysis of linezolid-resistant bacteria, such as China, Germany, Ireland, Italy, and Spain. The host bacteria in which the *cfr* gene has been identified include the Gram-positive genera *Staphylococcus*, *Streptococcus*, *Enterococcus*, *Bacillus*, *Jeotgalicoccus*, *Macrococcus*, and *Mammaliicoccus*, as well as the Gram-negative genera *Escherichia*, *Proteus*, *Providencia*, *Morganella*, and *Pasteurella* (81–84). These *cfr*-carrying bacteria originated from humans, cattle, pigs, horses, dogs, cats, chickens, turkeys, ducks, or geese, but also from chicken meat, beef, and pork, as well as from environmental samples.

Occurrence of the *cfr* gene in *Staphylococcus* spp. The staphylococcal species *Staphylococcus sciuri*, *Staphylococcus lentus*, *Staphylococcus vitulinus*, *Staphylococcus fleurettii*, and *Staphylococcus stepanovicii* have recently been reclassified as new species within the genus *Mammaliicoccus* (85). Members of two of these species, *S. sciuri* and *S. lentus*, carry mobile oxazolidinone resistance genes. Since all references referring to the corresponding publications and database entries list these two species as members of the genus *Staphylococcus*, we decided to keep the former genus assignment here for the sake of convenience.

Although initially identified in an *S. sciuri* isolate of bovine origin in Germany about 20 years ago (45), this gene has since been found in a total of 19 staphylococcal species, including coagulase-positive, coagulase-variable, and coagulase-negative species. Some of these *cfr*-carrying staphylococci occur exclusively in humans, such as *Staphylococcus capitis* and *Staphylococcus hominis*. *cfr*-carrying *S. capitis* isolates have been identified in China (86–88) and in the United States (74), whereas *S. hominis* isolates carrying the *cfr* gene have been detected in Italy (89) and the United States (79).

Another group of *cfr*-carrying staphylococci has so far only been identified in a specific staphylococcal species from one defined animal host in a specific country; these include *Staphylococcus pseudintermedius* from a dog in Portugal (90), *Staphylococcus rostri* from ducks in China (91, 92), *Staphylococcus warneri* and *Staphylococcus hyicus* from pigs in Denmark (93), and *Staphylococcus auricularis*, *Staphylococcus chromogenes*, and *Staphylococcus kloosii* from calves in Germany (94).

A third group of *cfr*-positive staphylococci has been described in both human and animal hosts. These include *S. aureus*, *Staphylococcus haemolyticus*, *Staphylococcus saprophyticus*,

Staphylococcus cohnii, and *Staphylococcus arlettae*. *S. aureus* isolates (including MRSA isolates) have been seen in humans from Brazil (95), China (96–98), Colombia (99, 100), Ecuador (GenBank accession no. KY448337), Ireland (101, 102), Pakistan (103), Panama (71), Spain (104–111), the United Arab Emirates (112), and the United States (74, 79, 113–116). However, such isolates have also been obtained from a horse in Germany (117), beef in Egypt (118), and pigs in Belgium (119), China (98, 120–122), and South Korea (123, 124). *S. epidermidis* isolates of human origin containing the *cfr* gene have been found in Brazil (125), China (126, 127), France (65, 128, 129), Germany (130, 131), Ireland (132, 133), Italy (65, 67, 68, 70, 89, 134–137), Mexico (65, 67), Poland (138), Spain (65, 139–142), and the United States (74, 79, 113, 143–146). In addition, a single porcine *cfr*-positive *S. epidermidis* isolate was recently detected in China (147). *cfr*-carrying *S. haemolyticus* strains have been found in humans from Brazil (125), China (97, 147), Egypt (148), India (149–152), Mexico (65), Spain (111), and Vietnam (40). Porcine and bovine *cfr*-carrying *S. haemolyticus* isolates have only been detected in China (91, 92, 147). *S. saprophyticus* isolates have been found in pigs from China (122, 153), in turkeys from Egypt (154), and in humans and calves from Germany (94). *S. cohnii* isolates have been obtained from humans in China (88, 97, 147, 155), Germany (94), Mexico (67), India (151), and Vietnam (40). They have also been found in pork and chicken meat (156), as well as in pigs (122, 153) and chickens (91, 92) from China, calves from Germany (94), and environmental samples from Spain (157). *S. arlettae* isolates have been found in humans from India (151), turkeys from Egypt (154), pigs (122), ducks (91, 92), and chickens (158) from China, as well as in samples from a swine farm environment in Spain (157).

The fourth group of *cfr*-carrying staphylococci includes the CoNS species *Staphylococcus equorum*, *Staphylococcus lentus*, *S. sciuri*, and *Staphylococcus simulans*, which have been exclusively found in animals, food of animal origin, and/or environmental samples. *S. equorum* isolates have been detected in pork, chicken meat, and chickens from China (147, 156) and in environmental samples from Spain (157). *S. lentus* isolates have been found in cattle from Belgium (159, 160) and Germany (94, 117), turkeys from Egypt (154), chickens from China (91, 92, 147), and pigs from China (153) and Germany (117), as well as samples from a pig farm environment in Germany (161). *S. sciuri* isolates were present in cattle from Germany (45, 94, 117), Belgium (159, 160), and China (147), and in pigs (91, 92, 122, 153, 162, 163), ducks and chickens (91, 92, 147), chicken meat (156), dogs (153, 164), and cats (153) from China, as well as in turkeys from Egypt (154). Finally, *cfr*-carrying *S. simulans* isolates have been identified in pigs (91, 92), pork, and chicken meat (156) from China, cattle in Germany (94, 117), and pigs in Denmark (93).

Occurrence of the *cfr* gene in *Enterococcus* spp. In contrast to the widespread occurrence of the gene *cfr* in staphylococci, this gene has comparatively rarely been detected among members of the genus *Enterococcus*. So far, it has been found in the six species *E. faecalis*, *E. faecium*, *Enterococcus thailandicus*, *Enterococcus casseliflavus*, *E. gallinarum*, *Enterococcus hirae*, and *Enterococcus avium*. *cfr*-carrying *E. faecalis* strains have been identified not only in humans from China (165), Thailand (166), and the United States (113, 114, 167), but also among pigs from Brazil (168, 169), China (170–173), and Italy (174), as well as in sewage from a pig farm in China (175). In contrast, *cfr*-positive *E. faecium* strains have mainly been isolated from humans. The corresponding reports were from Canada (176), China (177), Germany (178), Ireland (132), Italy (179, 180), Poland (181), and the United Kingdom (182). Thus far, the only report about the gene *cfr* in *E. faecium* from an animal is from the United States, where such an isolate had been detected in cattle (183). Reports about *cfr*-carrying *E. thailandicus* from pigs (174, 175) and sewage from a pig farm (175), as well as *E. casseliflavus* (184), *E. avium* (174), *E. hirae* (GenBank accession no. MK798156), and *E. gallinarum* from pigs (174, 184) all originated from China and/or Italy.

Occurrence of the *cfr* gene in other Gram-positive bacteria. There are only a few reports of the presence of the *cfr* gene in other Gram-positive bacteria. The *cfr* gene was found in *Streptococcus suis* (82, 185) and in *Bacillus* spp. (186–188), as well as in *Macrococcus caseolyticus* and *Jeotgalicoccus pinnipedialis* (189), all from pigs in China.

Occurrence of the *cfr* gene in Gram-negative bacteria. Despite the fact that many Gram-negative bacteria, especially those of the order *Enterobacterales*, are intrinsically resistant to some of the antimicrobial classes to which the gene *cfr* confers resistance, such as lincosamides, oxazolidinones, and streptogramin A antibiotics, this multiresistance gene has been found occasionally in the species *Escherichia coli*, *Proteus vulgaris*, *Proteus mirabilis*, *Proteus cibarius*, *Providencia rettgeri*, *Morganella morganii*, and *Pasteurella multocida*, all from food-producing animals in China. The *cfr*-carrying *E. coli* isolates were from pigs (190–196) or chickens (197), whereas the corresponding *P. vulgaris* isolates were all from pigs (198–200). One report described the presence of *cfr* in a porcine *P. mirabilis* isolate (201) while another report mentioned it in a *Proteus* isolate of not further specified food animal origin (202). All *P. cibarius* isolates originated from geese (203). A *cfr*-carrying plasmid (GenBank accession no. CP060728) was identified in a *P. rettgeri* isolate of duck origin. The *cfr*-positive *M. morganii* isolate was of pig origin (83) and the *P. multocida* isolate of duck origin (84).

Mobile Genetic Elements That Are Involved in the Dissemination of the *cfr* Gene

The *cfr* gene has most frequently been located on mobile genetic elements (MGEs). Among these, plasmids are the preferred location. Plasmids have also been shown to act as vectors for *cfr*-carrying transposons and translocatable units (TUs). In addition, chromosomally located *cfr*-carrying transposons, TUs, and integrative and conjugative elements (ICEs) have also been described. Numerous plasmids harboring the *cfr* gene have been detected in various Gram-positive and Gram-negative genera. In the following descriptions of *cfr*-carrying plasmids, focus is put on those plasmids for which complete plasmid sequences are available. These plasmids differ in size, structure, and organization, as well as in the numbers and types of colocated antimicrobial, heavy metal, or biocide resistance genes (Tables 2 and 3).

Plasmids carrying the *cfr* gene in *Staphylococcus* spp. The completely sequenced *cfr*-carrying plasmids among staphylococci ranged in size between 6,962 and 76,991 bp. All of them—except plasmid pSA8589—harbored one to eight additional resistance genes.

Plasmid pSA8589 from human *S. aureus* isolate 1900 in the United States is the smallest currently known *cfr*-carrying plasmid in staphylococci. It is composed only of the *cfr* gene, a plasmid replication gene (*rep*), a recombinase gene, a plasmid recombination/mobilization gene (*pre*/*mob*), and a truncated *tnpB* gene for a Tn*554*-associated transposase (116, 204). The four slightly larger plasmids—pMSA16 from bovine *S. aureus* in China, pSS-03 from porcine *S. cohnii* and *S. arlettae* in China, and pSAM13-0451 from human *S. aureus* in Ireland—share a common structure comprising the *cfr* gene, a *repU* gene for plasmid replication, a *pre*/*mob* gene, and different *erm* genes [*erm*(A) in pMSA16, *erm*(C) in pSS-03, and *erm*(T) in pSAM13-0451] for combined resistance to macrolides, lincosamides, and streptogramin B (MLS_B) antibiotics (Table 2). Plasmid pSAM13-0451 is about 1.5 kbp larger than the other plasmids and harbors a truncated *lsa*(B) gene and a second complete *pre*/*mob* gene, of which only remnants are present in the other three plasmids (Fig. 4A).

Another five plasmids—pK8D55P-cfr, pERGB, pSS-01, pSCFS1, and pH8C110P-cfr—range in size between 12,724 and 24,103 bp and display largely different organizations, which is also reflected by the different colocated resistance genes (Table 2). The three plasmids, pK8D55P-cfr from an *S. sciuri* isolate of duck origin in China, pERGB from a human *S. aureus* isolate in Spain (106) (Fig. 4B), and pH8C110P-cfr from an *S. sciuri* isolate of animal feed origin in China, share the resistance genes *aadD* and *tet*(L), which have also been found on other staphylococcal plasmids, such as the multiresistance plasmid pAFS11 (205), but they differ in the additional resistance genes *erm*(C), *dfrK*, and *erm*(B), respectively. Plasmid pSS-01 from a porcine *S. cohnii* isolate in China harbored the aminoglycoside resistance gene *aacA-aphD* on a Tn*4401*-like nonconjugative transposon and the phenicol exporter gene *fexA* on a Tn*558* transposon, in addition to *cfr* (122). Plasmid pSCFS1 from a bovine *S. sciuri* in Germany was the first completely sequenced *cfr*-carrying plasmid (206). This plasmid

TABLE 2 Characteristics of completely sequenced *cfr*-carrying plasmids in Gram-positive bacteria

Plasmid(s)	Origin	Size (bp)	Colocated resistance gene(s)	GenBank accession no.
pSA8589	*S. aureus*, human, USA	6,962		KC561137
pMSA16	*S. aureus*, cattle, China	7,054	*erm*(A)	JQ246438
pSS-03	*S. cohnii*, pig, China	7,057	*erm*(C)	JQ219851
pSS-03	*S. arlettae*, pig, China	7,122	*erm*(C)	JF834911
pSAM13-0451	*S. epidermidis*, human, Ireland	8,558	*erm*(T), Δ*lsa*(B)	KY579373
pHNCR35	*S. simulans*, human, China	9,880	*fexA*	KF861983
pK8D55P-cfr	*S. sciuri*, duck, China	12,724	*erm*(C), *aadD*, *tet*(L)	CP065963
pSS-01	*S. cohnii*, pig, China	15,703	*fexA*, *aacA-aphD*	JF834909
pERGB	*S. aureus*, human, Spain	15,259	*aadD*, *tet*(L), *dfrK*	JN970906
pSCFS1	*S. sciuri*, cattle, Germany	17,108	*erm*(33), *lsa*(B), *spc*	NC_005076
pH8C110P-cfr	*S. sciuri*, animal feed, China	24,103	*erm*(B), *aadD*, *tet*(L)	CP065796
pSAM13-0401	*S. aureus*, human, Ireland	27,502	*lsa*(B)	KU510528
p12-00322	*S. epidermidis*, human, Germany	36,754	*lsa*(B)	KM521836
Unnamed 1	*S. aureus*, pig, China	37,510	*fexA*	CP065195
p12-02300	*S. epidermidis*, human, Germany	38,864	*fexA*	KM521837
pY96A	*S. aureus*, pig, China	39,212	*fexA*	CP065516
p14-01514	*S. epidermidis*, human, Germany	39,243	*fexA*	KX520649
pSA737, pSEPI8573	*S. aureus*, human, USA	39,287	*fexA*	KC206006, KC222021
pSR01	*S. aureus*, human, China	39,500	*aacA-aphD*	CP048644
pLRSA417	*S. aureus*, human, China	39,504	*aacA-aphD*	KJ922127
pSX01	*S. xylosus*, pig, China	39,969	*aacA-aphD*	KP890694
pY8P168P-cfr	*S. saprophyticus*, pig, China	41,503	*fexA*, *aacA-aphD*	CP065798
pSAM12-0145	*S. aureus*, human, Ireland	41,590	*fexA*	KU521355
pGMI17-006	*S. aureus*, human, Denmark	45,885	*fexA*, *lsa*(B)	CP028164
pH29-46	*S. lentus*, chicken, China	46,167	*fexA*, *aacA-aphD*	CP059680
pk8D6P-cfr	*S. sciuri*, duck, China	53,742	*fexA*, *aacA-aphD*, *aadD*, *ble*	CP065793
pWo27-9	*S. sciuri*, pig, China	55,724	*optrA*, *aadD*, *ble*	KX982169
pWo28-1	*S. sciuri*, pig, China	60,565	*optrA*, *fexA*, *aadD*, *aacA-aphD*, *ble*	KX982171
pWo28-3	*S. sciuri*, pig, China	60,563	*optrA*, *fexA*, *aadD*, *aacA-aphD*, *ble*	KT601170
pSA-01	*S. arlettae*, chicken, China	63,558	*fexB*, *erm*(B), *erm*(C), *erm*(T), *aadD*, *aacA-aphD*, *tet*(L), *fosD*	KX274135
pSP01	*S. epidermidis*, human, Italy	76,991	*lsa*(B), *blaZ*, *msr*(A), *cop*	KR230047
Unnamed	*E. faecalis*, cattle, China	11,940		CP028840
pCPPF5	*E. faecalis*, pig, China	12,270		KC954773
pE30	*E. faecalis*, unknown, China	12,270		KT717888
pFSIS1608820	*E. faecium*, cattle, USA	28,222	*optrA*, *fexA*, *erm*(A), *erm*(B), *aphA3*, *spc*	CP028728
pEF-01	*E. faecalis*, cattle, China	32,388	*fexA*	CP002208
pE35048-oc	*E. faecium*, human, Italy	41,816	*optrA*, *erm*(B), Δ*lnu*(E)	MF580438
pF120805	*E. faecium*, human, Ireland	72,924	*optrA*, *erm*(A), *erm*(B), *aphA3*, *aadE*, *lnu*(A), *lnu*(B)	KY579372
pFas4-2	*E. hirae*, pig, China	85,629	*fexA*, *lsa*(B), *ars* operon	MK798156
p4	*E. faecalis*, pig, China	95,693	*erm*(B), *aacA-aphD*, *aphA3*, *ble*	MH830362
pBS-03	*Bacillus* sp., pig, China	7,446	*aadY*	JQ394981
pBS-01	*Bacillus* sp., pig, China	16,492	*erm*(B)	NC_013963
pBS-02	*Bacillus* sp., pig, China	16,543		NC_014557

carries the additional resistance gene *lsa*(B) for elevated MICs to lincosamides, the spectinomycin resistance gene *spc*, and the MLS_B resistance gene *erm*(33) (206) (Fig. 4C). The gene *erm*(33) is a naturally occurring fusion product between *erm*(A) and *erm*(C) (207).

Seven plasmids, ranging in size between 37 and 41 kbp and including pSAM12-0145 from a human *S. aureus* isolate in Ireland (102), p12-02300 and p14-01514 from human *S. epidermidis* in Germany (130), and the two identical plasmids pSA737 and pSEPI8573 from human *S. aureus* isolates in the United States (204), as well as an unnamed plasmid and plasmid pY96A, both from *S. aureus* of porcine origin, shared large portions of similarity, including the *cfr* region. All seven plasmids harbored only the *fexA* gene as an additional resistance gene. Large parts of these plasmids exhibit reading frames for proteins with unknown functions. Plasmid pSAM13-0401 from a human *S. aureus* isolate in Ireland (102) shared the IS*21-558-cfr* part of this region with several of the aforementioned plasmids (Fig. 4D).

TABLE 3 Characteristics of completely sequenced *cfr*-carrying plasmids in Gram-negative bacteria

Plasmid	Origin	Size (bp)	Colocated resistance gene(s)	GenBank accession no.
Unnamed 4	*E. coli*, pig, China	28,519		CP037908
pFSEC-01	*E. coli*, pig, China	33,885		KR779901
pHNEP129	*E. coli*, pig, China	35,336	*mcr-1.1*	MT667261
pSD11	*E. coli*, pig, China	37,672		KM212169
pEC14cfr	*E. coli*, pig, China	37,663		KY865319
pGXEC6	*E. coli*, pig, China	38,405		KM580533
pGXEC3	*E. coli*, pig, China	41,646	$bla_{CTX-M-14b}$	KM580532
pFT130-1	*E. coli*, migratory bird, China	52,088	*floR*, *aphA3*, *tet*(A), $bla_{TEM-176}$	CP040091
pHNEP124	*E. coli*, pig, China	60,430	bla_{TEM-1}, *mcr-1.1*	NZ_MT667260
pEC295cfr	*E. coli*, pig, China	67,077	*erm*(B)	KY865320
pEC12	*E. coli*, pig, China	70,158		MG677985
pHNFP671	*E. coli*, pig, China	82,807		KP324830
pHNEP28_cfr	*E. coli*, livestock farm, China	108,837	*tet*(M), *qnrS1*, bla_{TEM-1}	KT845955
pSCEC2	*E. coli*, pig, China	135,615	*floR*, *strA*, *strB*, *tet*(A), *sul2*	KF152885
pYPR25-2	*P. rettgeri*, duck, China	35,276		CP060728
plas1.1.1	*P. mirabilis*, pig, China	12,795		CP047113
pJPM35-2	*P. mirabilis*, duck, China	35,276		CP053900
pG11-51 (p52)	*P. cibarius*, goose, China	51,644	*ble*	CP047287
pG32-51	*P. cibarius*, goose, China	51, 686	*ble*	CP053373
pZF1-cfr	*P. cibarius*, pig, China	59,168	*ble*	CP047341
pZF2-cfr	*P. cibarius*, pig, China	59,167	*ble*	CP045009
pZN3-cfr-121kb	*P. vulgaris*, pig, China	121,294	*floR*, *msr*(E), *mph*(E), *lnu*(F), *aadA2*, *aacC4*, *hph*, *aphA1*, *tet*(B), *sul1*, *sul2*, *dfrA12*, *ble*, *qacE*Δ*1*	CP047346
pPvSC3	*P. vulgaris*, chicken/pig, China	284,528	*floR*, *catB3*, *aadA1*, *strA*, *strB*, *tet*(B), *sul1*, *sul2*, bla_{OXA-10}, *qacE*Δ*1*, *ars* operon, *mer* operon	CP034667

Another five *cfr*-carrying plasmids, namely p12-00322 from a human *S. epidermidis* isolate in Germany (130), pSR01 (GenBank accession no. CP048644) and pLRSA417 (208) from human *S. aureus* isolates in China, pSX01 from a porcine *S. xylosus* isolate in China (GenBank accession no. KP890694), and pH29-46 from a chicken *S. lentus* isolate in China (GenBank accession no. CP059680) ranged between 36 and 46 kbp and were related in their structure and organization. The plasmids pY8P168P-cfr (GenBank accession no. CP065798) from a porcine *S. saprophyticus* isolate and pk8D6P-cfr (GenBank accession no. CP065793) from an *S. sciuri* isolate of duck origin, both from China, shared the region comprising the genes *cfr* and *aacA-aphD* with several of the other plasmids and the *fexA* region with plasmid pH29-46 (Fig. 4E). Four of these plasmids harbored only the gene *aacA-aphD* or *lsa*(B) as an additional resistance gene, while the remaining plasmids carried the genes *aacA-aphD* and *fexA* or *aacA-aphD*, *fexA*, *aadD*, and *ble* in addition to *cfr* (Table 2). The 45,885-bp plasmid pGMI17-006 (GenBank accession no. CP028164) from a human *S. aureus* isolate in Denmark carried only the resistance genes *fexA* and *lsa*(B) and differed in its structure from the aforementioned plasmids.

The three staphylococcal plasmids that harbored the *cfr* gene and the oxazolidinone/phenicol resistance gene *optrA* all originated from porcine *S. sciuri* isolates in China. They were similar in size and structure (Fig. 4F). While the two larger plasmids, pWo28-1 and pWo28-3, harbored the same set of additional resistance genes, i.e., *ble*, *aadD*, *aacA-aphD*, *fexA*, and *optrA* (162, 163), the approximately 5-kbp-smaller plasmid pWo27-9 (GenBank accession no. KX982169) lacked the resistance genes *aacA-aphD* and *fexA*.

The *cfr*-carrying plasmid pSA-01 from a chicken *S. arlettae* isolate in China had a size of 63,558 bp and a unique structure and carried eight additional resistance genes, including *aacA-aphD*, *aadD*, *erm*(B), *erm*(C), *erm*(T), *fexB*, *tet*(L), and *fosD* (158) (Fig. 4G). The largest completely sequenced *cfr*-carrying plasmid in staphylococci known thus far is plasmid pSP01 from a human *S. epidermidis* isolate in Italy (135). This conjugative plasmid harbored not only the additional antimicrobial resistance genes *lsa*(B), *blaZ*,

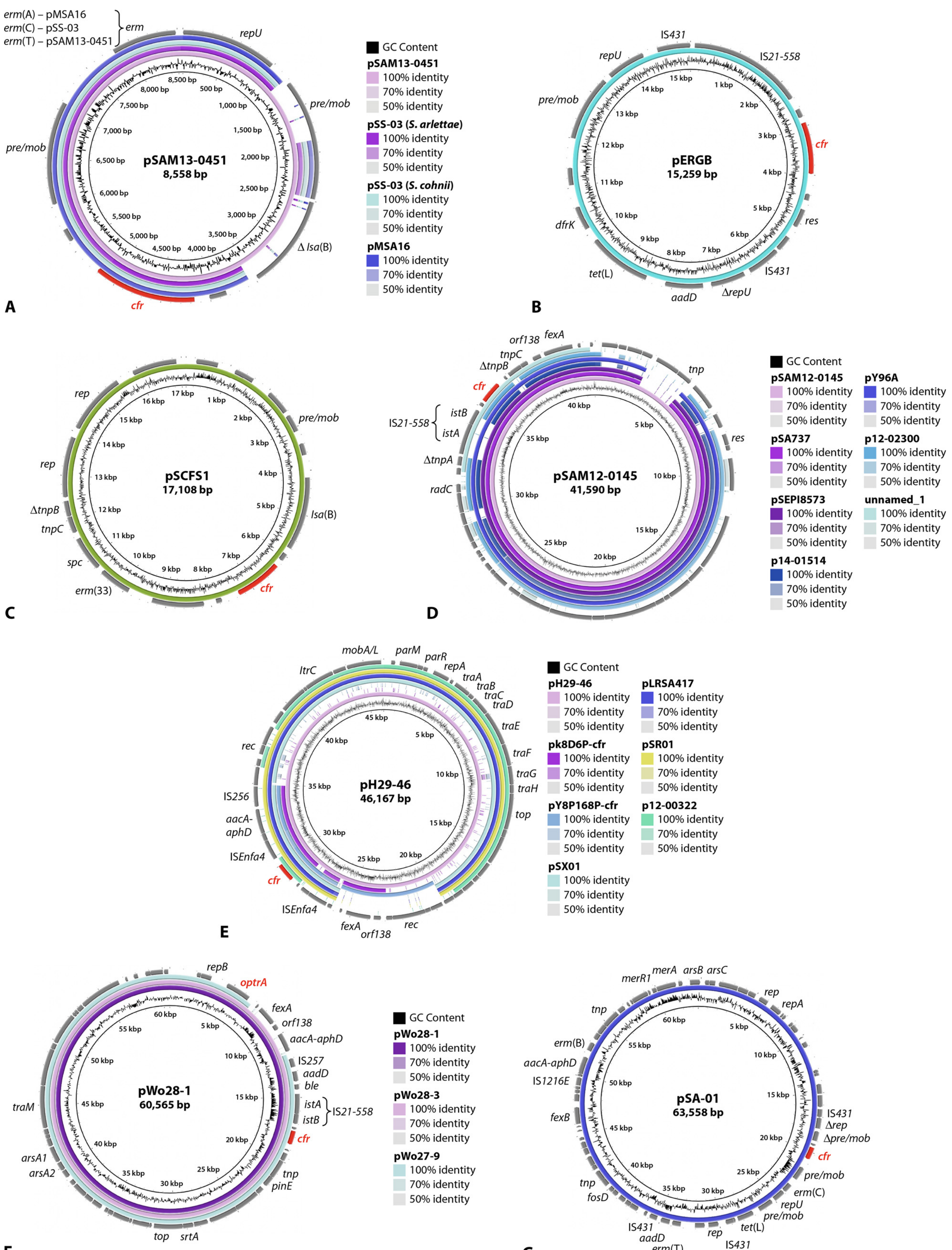

FIG 4 Structural comparison of *cfr*-carrying staphylococcal plasmids (constructed by BLAST Ring Image Generator [BRIG]). Relevant genes with known functions and insertion elements are indicated for the respective reference plasmid in the outer ring. The *cfr* and the *optrA* genes are

(Continued on next page)

and *msr*(A), but also a gene for copper resistance. The *lsa*(B)-IS*21-558-cfr* region of plasmid pSP01 shared 99% identity with that of plasmid p12-00322 from a human *S. epidermidis* isolate in Germany (130, 135).

Partial sequences of numerous *cfr*-carrying plasmids have been deposited in databases. These sequences range in size from 2,570 to 37,848 bp. The shorter sequences of <10 kbp usually comprise only the *cfr* gene and its immediate flanking regions, as present in plasmids pSCFS4 from a bovine *S. simulans* isolate in Germany (2,570 bp; GenBank accession no. AM086400), pSCFS7-like from a human *S. epidermidis* isolate in Spain (3,824 bp; GenBank accession no. KP229554), pSCFS7 from a human *S. aureus* isolate in Ireland (4,043 bp; GenBank accession no. FR675942), pHNZT2 from a porcine *S. simulans* isolate in China (5,086 bp; GenBank accession no. KF861985), pMHZ from a human *S. capitis* isolate in China (5,247 bp; GenBank accession no. JX232067), pRM01 from a human *S. cohnii* isolate in China (5,247 bp; GenBank accession no. KC820815); pHNLKJC2 from an *S. sciuri* isolate from raw pork in China (5,635 bp; GenBank accession no. KF751701), p7LC from a human *S. epidermidis* isolate in the United States (5,882 bp; GenBank accession no. JX910899), p1128105 from a human *S. aureus* isolate in the United States (7,020 bp; GenBank accession no. KJ866414), pHNKF3 from a porcine *S. simulans* isolate in China (7,320; GenBank accession no. KF861984), pHNTLD18 from an *S. equorum* isolate from retail meat in China (8,510 bp; GenBank accession no. KF751702), and pSS-02 from a porcine *S. saprophyticus* isolate in China (8,850 bp; GenBank accession no. JF834910), as well as pSCFS3 from a bovine *S. aureus* isolate in Germany (9,497 bp; GenBank accession no. AM086211). These short plasmid segments occasionally included—besides *cfr*—another antimicrobial resistance gene, such as *aacA-aphD* (p7LC, pHNKF3), *erm*(C) (pHNLKJC2), or *fexA* (pSCFS4, pSS-02, pHNTLD18, and pSCFS3). Larger plasmid sequences were available from plasmids pWo48-2 from a porcine *S. sciuri* isolate in China (13,244 bp; GenBank accession no. KX982175), pSS-02 from a human *S. haemolyticus* isolate in China (13,976 bp; GenBank accession no. JX827253), pJP1-like from a chicken *S. lentus* isolate in China (14,318 bp; GenBank accession no. KF129408), pSS-01 from a porcine *S. cohnii* isolate in China (15,703 bp; GenBank accession no. JF834909), p45547X from a human *S. aureus* isolate in Brazil (16,848 bp; GenBank accession no. KJ192337), pJP2 from an *S. rostri* isolate of duck origin in China (18,065 bp; GenBank accession no. KC989517), pSS-04 from a porcine *S. sciuri* isolate in China (18,496 bp; GenBank accession no. KF129410), pSCFS6 from a porcine *S. warneri* isolate in Denmark (22,010 bp; GenBank accession no. AM408573), and pHK01 from a human *S. cohnii* isolate in China (37,848 bp; GenBank accession no. KC820816). All of these larger sequences contained one or more additional resistance genes, such as *fexA* (pSS-02 and pHK01), *fexA* and *aacA-aphD* (pSS-01), *fexA* and *lnu*(B) (pSCFS6), *aacA-aphD* and *aadD* (pWo48-2), *fexA*, *aacA-aphD*, and *erm*(B) (pSS-04), *fexA*, *aacA-aphD*, *aadD*, and *ble* (pJP1-like), *aadY*-like, *aadD*, *erm*(B), and *tet*(L) (p45547X), as well as *fexA*, *aacA-aphD*, *aadD*, *erm*(B), *ble*, and *fosD* (pJP2).

Plasmids carrying the *cfr* gene in *Enterococcus* spp. The completely sequenced *cfr*-carrying plasmids from *E. faecalis* deposited in the databases are all from food-producing animals in China (Table 3). Three plasmids, ranging in size between 11,940 and 12,270 bp, were structurally closely related to one another and to the corresponding region of the larger plasmid pEF-01 (Fig. 5A) (170). They include an unnamed plasmid from bovine *E. faecalis* (GenBank accession no. CP028840), plasmid pCPPF5 from a porcine *E. faecalis* isolate (171), and plasmid pE30 from a not further specified food-producing animal (GenBank accession no. KT717888). None of these plasmids harbored additional resistance genes. It should be noted that plasmid pCPPF5 was unable to

FIG 4 Legend (Continued)

indicated in red. The innermost circle provides a size scale, while the next innermost circle shows the GC content. Other plasmids used for comparison (if available) are indicated by color-coded rings, with the reference plasmid representing the innermost colored ring. (A) pSAM13-0451, pSS-03 (*Staphylococcus arlettae*), pSS-03 (*Staphylococcus cohnii*), and pMSA16, (B) pERGB, (C) pSCFS1, (D) pSAM12-0145, pSA737, pSEPI8573, p14-01514, pY96A, p12-02300, and unnamed_1, (E) pH29-46, pk8D6P-*cfr*, pY8P168P-*cfr*, pSX01, pLRSA417, pSR01, and p12-00322, (F) and pWo28-1, pWo28-3, and pWo27-9, as well as (G) pSA-01.

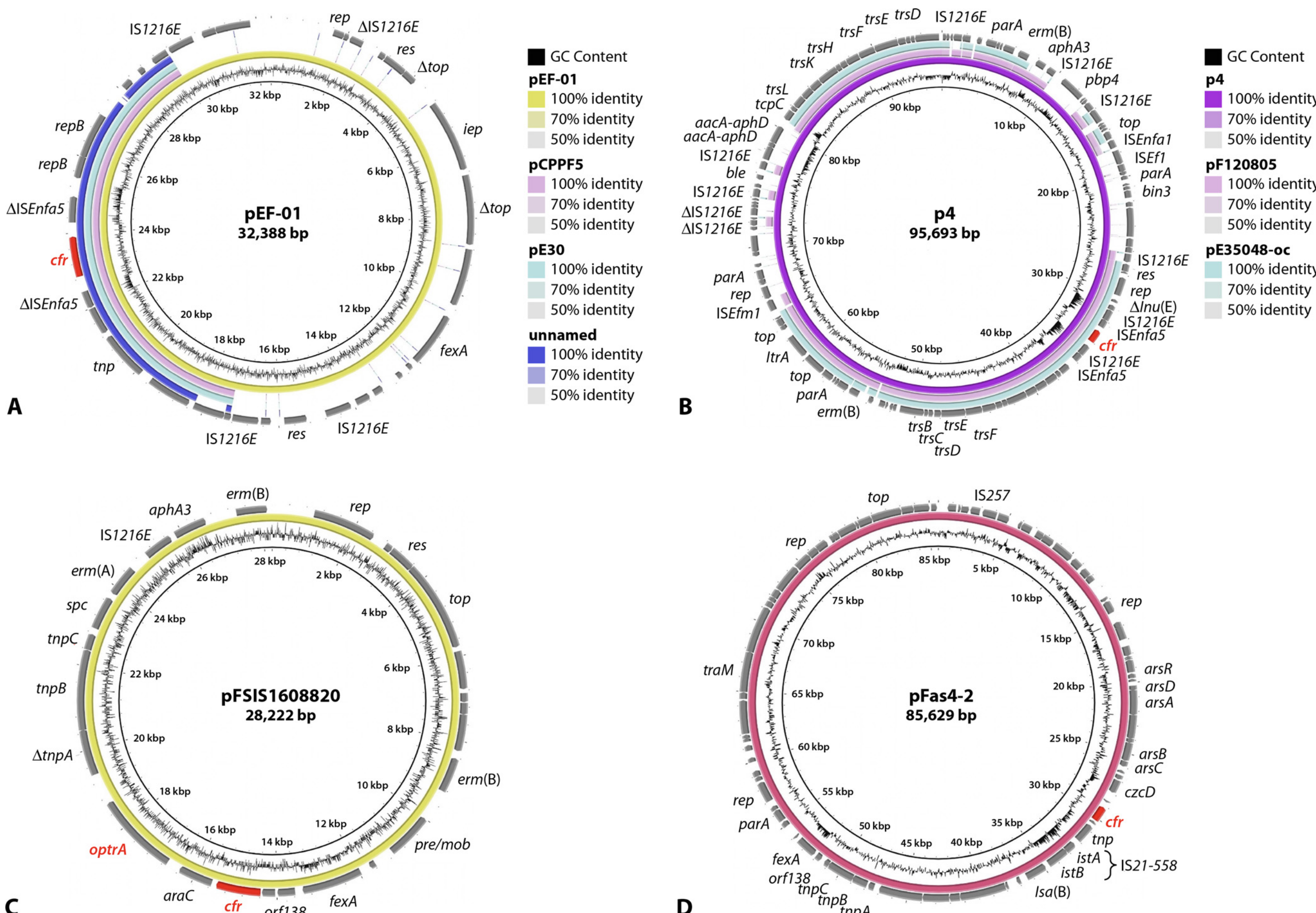

FIG 5 Structural comparison of *cfr*-carrying enterococcal plasmids (constructed by BRIG). Relevant genes with known functions and insertion elements are indicated for the respective reference plasmid in the outer ring. The *cfr* gene is indicated in red. The innermost circle provides a size scale, while the next innermost circle shows the GC content. Other plasmids used for comparison (if available) are indicated by color-coded rings, with the reference plasmid representing the innermost colored ring. (A) pEF-01, pCPPF5, pE30, and unnamed, (B) p4, pF120805 and pE35048-oc, (C) pFSIS1608820, and (D) pFas4-2.

confer the PhLOPS$_A$ phenotype in *E. faecalis*, but when the respective *cfr* gene was cloned in *E. coli*, it conferred elevated MICs to chloramphenicol and florfenicol (171). Plasmid pEF-01 was the first completely sequenced *cfr*-carrying plasmid in enterococci (170). This plasmid originated from bovine *E. faecalis*, had a size of 32,388 bp, and carried the additional resistance gene *fexA* (170) (Fig. 5A). The largest completely sequenced *cfr*-carrying plasmid in *E. faecalis* to date is plasmid p4 from a porcine *E. faecalis* isolate (GenBank accession no. MH830362). This plasmid had a size of 95,693 bp and harbored the additional resistance genes *erm*(B), *aacA-aphD*, *aphA3*, and *ble* (Fig. 5B). Partial sequences of *cfr*-carrying plasmids deposited in GenBank include pHOU-cfr from a human *E. faecalis* isolate in China (3,494 bp; GenBank accession no. JQ660368) and pW9-2 from a porcine *E. faecalis* isolate in China (25,761 bp; GenBank accession no. JQ911741). In the latter sequence, the MLS$_B$ gene *erm*(B) was also present.

Three completely sequenced *cfr*-carrying plasmids have been identified so far in *E. faecium* (Table 2). The smallest plasmid, pFSIS1608820 from bovine *E. faecium* in the United States, has a size of 28,222 bp. It harbored the additional resistance genes *aphA3*, *erm*(A), *erm*(B), *fexA*, *optrA*, and *spc* (183) (Fig. 5C). The 41,816-bp plasmid pE35048-oc originated from a human *E. faecium* isolate in Italy. It carried the additional resistance genes *erm*(B) and *optrA*, as well as a truncated *lnu*(E) gene (180). This plasmid shared large portions of similarity, including the *cfr* region, with the 72,924-bp plasmid pF120805 of a human *E. faecium* isolate from Ireland (132) (Fig. 5B). Plasmid pF120805 harbored seven additional resistance genes, including *aadE*, *aphA3*, *erm*(A),

erm(B), *lnu*(A), *lnu*(B), and *optrA*. Plasmids pE35048-oc and pF120805 from *E. faecium* were in part related to each other and to plasmid p4 from *E. faecalis* (Fig. 5B).

The only completely sequenced *cfr*-carrying plasmid from *E. hirae* known thus far is the 85,629-bp plasmid pFas4-2 (209). This plasmid carried the additional antimicrobial resistance genes *fexA* and *lsa*(B), as well as an *ars* operon for arsenic resistance (Fig. 5D).

Incomplete sequences of the plasmids p3-38 (21,116 bp; GenBank accession no. JQ911740) and pW3 (27,360 bp; GenBank accession no. JQ911739), both from porcine *E. thailandicus* isolates in China, and plasmid pEn24cfr from a porcine *E. casseliflavus* isolate in China (13,614 bp; GenBank accession no. KF792823) are available. No additional resistance genes were detected in any of these three sequences.

Plasmids carrying the *cfr* gene in *Bacillus* spp. So far, three complete *cfr*-carrying plasmids have been reported from not further specified *Bacillus* spp. of porcine origin in China (Table 2) (186–188). Two of the plasmids, pBS-01 and pBS-02, were similar in size (~16.5 kbp) and shared about 10 kbp of their sequences, including the *cfr* upstream region. Plasmid pBS-01 carried a complete transposon Tn*917* with the MLS_B resistance gene *erm*(B) (186). In contrast, plasmid pBS-02 did not harbor additional resistance genes and also showed a *cfr* downstream part that differed from the genetic context in plasmid pBS-01 (187). The distinctly smaller plasmid pBS-03 (~7.5 kbp) shared only the *cfr* gene and its upstream-located Δ*pre*/*mob* gene with the other two plasmids (188). However, plasmid pBS-03 harbored a novel streptomycin resistance gene, designated *aadY* (188).

Plasmids carrying the *cfr* gene in other Gram-positive bacteria. Only incomplete sequences of the *cfr*-carrying plasmid pStrcfr from porcine *S. suis* in China are available. One sequence describes the *cfr* region (8,762 bp; GenBank accession no. KC844836) and showed the presence of the *cfr* gene bracketed by IS*Enfa5* elements and inserted into the *lnu*(E) reading frame (82). The other sequence (13,837 bp; GenBank accession no. KF129409) describes the colocated *fexA*-carrying transposon Tn*558* on pStrcfr.

The incomplete sequence of the ca. 53-kbp plasmid pJP1 from a porcine *J. pinnipedialis* isolate in China (8,896 bp; GenBank accession no. JQ320084) shows the *cfr* gene upstream of the resistance genes *aadD* and *ble*, as well as the insertion sequence IS*21-558*. Further PCR screening identified the additional resistance genes *aacA-aphD* and *erm*(C) on plasmid pJP1. A plasmid indistinguishable from pJP1 was also identified in porcine *M. caseolyticus* from China (189).

Plasmids carrying the *cfr* gene in *Escherichia coli*. Completely sequenced *cfr*-carrying plasmids from *E. coli*, as deposited in the databases, differ distinctly in size and structure (Table 3). All of these plasmids originated from *E. coli* isolates of animal origin. An unnamed plasmid with a size of 28,519 bp (GenBank accession no. CP037908) is to date the smallest completely sequenced *cfr*-carrying plasmid from *E. coli*. This plasmid was of pig origin and did not exhibit additional resistance genes. The five plasmids pHNEP129 (GenBank accession no. MT667261), pSD11 (210), pEC14cfr (196), pGXEC6 (194), and pGXEC3 (194) were all from porcine *E. coli* isolates in China, ranged in size from 35,336 to 41,646 bp, and were closely related in their structure and organization (Fig. 6A). In contrast to the other plasmids, which did not harbor additional antimicrobial resistance genes, pHNEP129 carried a *mcr-1.1* gene for colistin resistance and pGXEC3 had a $bla_{CTX-M-14b}$ gene for an extended-spectrum β-lactamase (ESBL) (194). The 82,807-bp plasmid pHNFP671 from porcine *E. coli* in China closely resembled in part the 33,885-bp plasmid pFSEC-01 and the 60,430-bp plasmid pHNEP124, both also from porcine *E. coli* isolates in China. The comparison of the maps of all three plasmids showed that there are overlapping areas between pFSEC-01 and pHNEP124 and suggested that the large plasmid pHNFP671 may have developed from a recombination between the two smaller plasmids (Fig. 6B), during which the additional antimicrobial resistance genes on plasmid pHNEP124—*mcr-1.1* and bla_{TEM-1}—were lost. A detailed analysis of the sequence of plasmid pFSEC-01 showed that the *cfr* gene, bracketed by two IS*26* elements in the same orientation, was inserted into a plasmid closely related to pEA3 from the plant pathogen

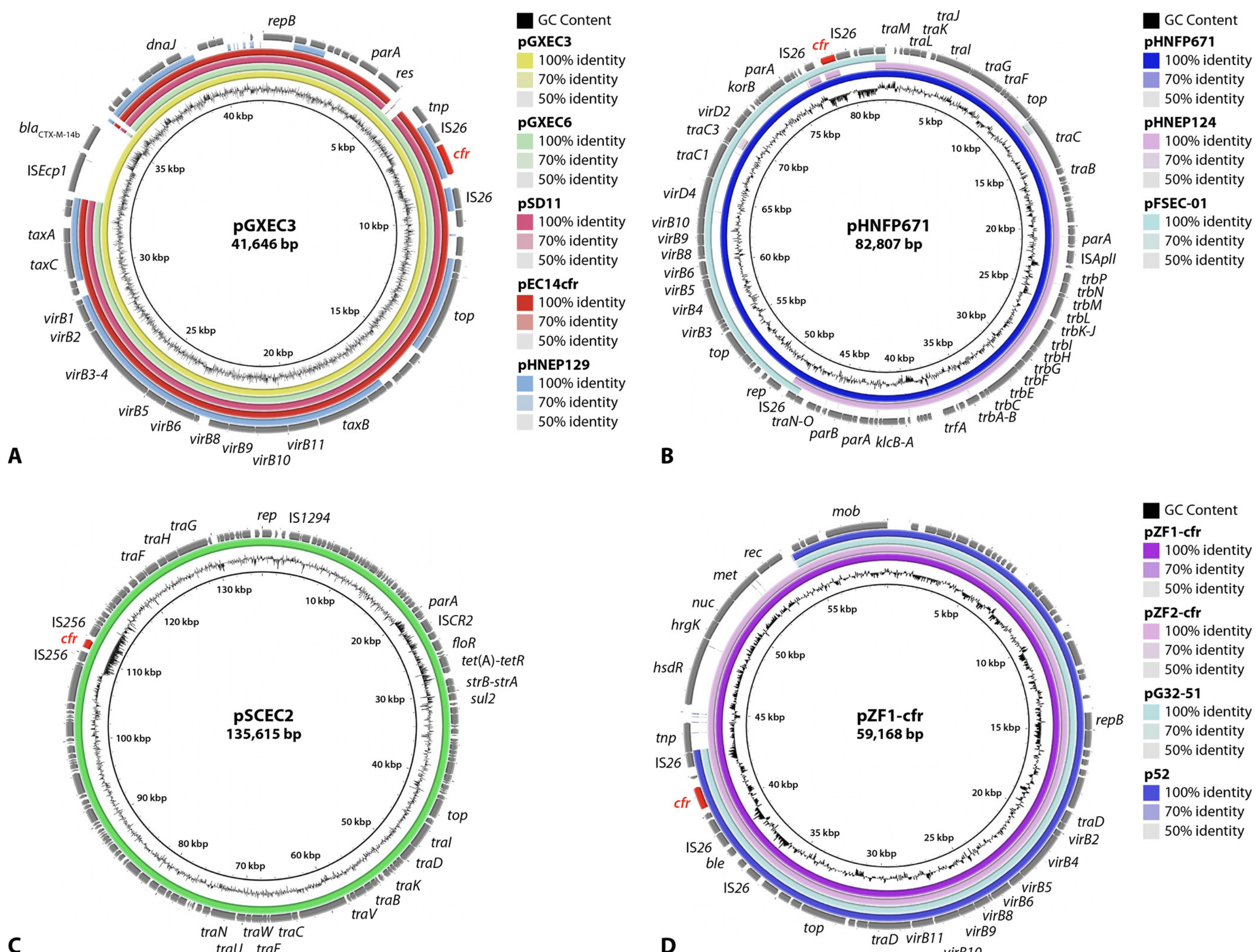

FIG 6 Structural comparison of *cfr*-carrying plasmids in *E. coli* and *Proteus* spp. (constructed by BRIG). Relevant genes with known functions and insertion elements are indicated for the respective reference plasmid in the outer ring. The *cfr* gene is indicated in red. The innermost circle provides a size scale, while the next innermost circle shows the GC content. Other plasmids used for comparison (if available) are indicated by color-coded rings, with the reference plasmid representing the innermost colored ring. (A) pGXEC3, pGXEC6, pSD11, pEC14cfr, and pHNEP129 (all *E. coli*), (B) pHNFP671, pHNEP124, and pFSEC-01 (all *E. coli*), (C) pSCEC2 (*E. coli*), and (D) pZF1-cfr, pZF2-cfr, pGE32-51, and p52 (all *P. cibarius*).

Erwinia amylovora. This observation suggests that plasmid pFSEC-01 may have been transferred between different bacterial genera of both animal and plant origins (193).

The remaining five plasmids included the plasmid pFT130-1 (52,088 bp; GenBank accession no. CP040091) from an *E. coli* isolate from a migratory bird in China, as well as the plasmids pHNEP28_cfr (108,837 bp) (211), pEC295cfr (67,077 bp) (196), pEC12 (70,158 bp; GenBank accession no. MG677985) (196), and pSCEC2 (135,615 bp) (191), all from porcine *E. coli* isolates in China. While plasmid pEC12 did not harbor additional antimicrobial resistance genes, plasmid pEC295cfr carried an *erm*(B) gene. The remaining three plasmids had three to five additional antimicrobial resistance genes, such as *qnrS1*, *tet*(M), and $bla_{\text{TEM-1}}$ (pHNEP28_cfr) (211), *aphA3*, $bla_{\text{TEM-176}}$, *floR*, and *tet*(A) (pFT130-1), or *floR*, *strA*, *strB*, *sul2*, and *tet*(A) (pSCEC2) (Fig. 6C) (191).

In addition, the 12,390-bp segment of the ca. 110-kbp plasmid pEC-01 from a porcine *E. coli* isolate in China showed that the *cfr* gene was bracketed by IS*26* elements in the same orientation (190). Plasmid pEC-01 was the first *cfr*-carrying plasmid described in *E. coli*.

Plasmids carrying the *cfr* gene in *Proteus* and *Providencia* spp. Members of the three species *P. mirabilis*, *P. cibarius*, and *P. vulgaris* have been identified as carriers of plasmid-borne *cfr* genes. The respective isolates were all of food animal origin in China

(Table 3). The 12,795-bp plasmid plas1.1.1 (GenBank accession no. CP047113) from a porcine *P. mirabilis* isolate is the smallest *cfr*-carrying plasmid within the genus *Proteus*. Another two plasmids of 35,276 bp, pJPM35-2 (GenBank accession no. CP053900) from *P. mirabilis* and pYPR25-2 (GenBank accession no. CP060728) from *P. rettgeri*, both of duck origin, were identical in their nucleotide sequences. None of these three plasmids harbored additional antimicrobial resistance genes.

The complete sequences of four *cfr*-carrying plasmids from *P. cibarius* have been deposited in databases. All four plasmids also carried the bleomycin resistance gene *ble*. The two smaller plasmids, pG11-51 (p52) (51,644 bp) (203) and pG32-51 (51,686 bp) (212), from *P. cibarius* of goose origin, were almost identical in their structure and organization, as were the two larger plasmids pZF1-cfr (59,168 bp) and pZF2-cfr (59,167 bp) (213). A comparison of the four plasmids showed that the two smaller plasmids shared large parts of their sequences with those of the two larger plasmids (Fig. 6D). This also included the *cfr* region, which revealed the *cfr* gene being bracketed by IS*26* elements in the same orientation (213). It should be noted that plasmid pG32-51 is described in the database entry as originating from *P. cibarius*, but in the associated publication to be from *P. vulgaris* (213). Moreover, the presence of an intact *erm*(C) gene in plasmid pG11-51 (p52) is indicated in the publication (203), whereas only a truncated *erm*(B) gene is present in the database entry (GenBank accession no. CP047287).

The two *cfr*-carrying plasmids from *P. vulgaris* were distinctly larger than the aforementioned other plasmids from members of the genus *Proteus*. In addition, they harbored a large number of additional antimicrobial resistance genes. The 121,294-bp plasmid pZN3-cfr-121kbp (GenBank accession no. CP047346) originated from a porcine *P. vulgaris* isolate and carried the additional antimicrobial resistance genes *aacC4* [*aac(3)-IV*], *aadA2*, *hph* [*aph(4)-Ia*], *aphA1* [*aph(3′)-Ia*], *ble*, *dfrA12*, *floR*, *lnu*(F), *mph*(E), *msr*(E), *sul1*, *sul2*, and *tet*(B), as well as the biocide resistance gene *qacE*Δ*1*. While the database entry states that plasmid pPvSC3 originated from a *P. vulgaris* isolate of chicken origin, it is referred to as originating from a pig in the corresponding publication. This conjugative plasmid was 284,528 bp in size and harbored the additional antimicrobial resistance genes *aadA1*, bla_{OXA-10}, *catB3*, *floR*, *strA*, *strB*, *sul1*, *sul2*, and *tet*(B), and the biocide resistance gene *qacE*Δ*1*, as well as an *ars* operon for arsenic resistance and a *mer* operon for mercury resistance (199) (Table 3).

Transposons and integrative and conjugative elements carrying the *cfr* gene. So far, only three *cfr*-carrying transposons have been described, one in the Gram-negative genus *Morganella* (83) and the other two in the Gram-positive genus *Staphylococcus* (214).

The *cfr*-carrying transposon Tn*6451* was recently identified in a porcine *M. morganii* isolate in China (83). Tn*6451* has a size of 111,238 bp. As a derivative of transposon Tn*7*, it contains a typical Tn*7* transposition module comprising the five genes *tnsABCDE* (83, 215). Tn*6451* is inserted into the chromosomal *att*Tn*7* site, which is located in the transcriptional terminator of the gene *glmS*, and produces 5-bp direct repeats at the integration site (5′-AGATA-3′) (83). Usually, Tn*7* transposons utilize a "cut-and-paste" transposition mechanism (215, 216), although Tn*6451* apparently was not able to excise from its chromosomal location, as no excision product was detected by PCR (83). The *cfr* gene in Tn*6451* was located in a novel genetic structure (IS*26*-*cfr*-ΔTn*554 tnpB*-ΔTn*3 tnpA*-IS*26*), which was bracketed by two IS*26* copies in the same orientation (83). Recombination of the two IS*26* copies resulted in the formation of a TU, which consisted of the gene *cfr*, ΔTn*554 tnpB*, ΔTn*3 tnpA*, and one copy of IS*26* (83), as shown by PCR and sequence analysis. In addition to *cfr*, Tn*6451* harbored another 14 antimicrobial resistance genes, including *aac(6′)-Ib-cr*, *aacC4*, *aadA1*, *arr-3*, bla_{OXA-1}, *catB3*, *dfrA1*, *dfrA27*, *floR*, *hph*, *sat2*, *sul1*, *sul2*, *tet*(B), and the biocide resistance gene *qacE*Δ*1* (83).

Tn*6349* is a composite transposon of 48,350 bp recently described in a clinical sequence type 5 (ST5)-MRSA-II strain in Italy (214). Tn*6349* was bounded by two copies of IS*1216* in the same orientation. It inserted into a ΦN315-like prophage present in the chromosome of the ST5-MRSA-II strain and created 8-bp direct repeats (5′-AAACAAAT-3′) at the integration site (214). The Tn*6349* transposon displayed a mosaic structure,

which was possibly generated from the recombination between a pE35048-oc-like plasmid (180) and the novel *poxtA*- and *fexB*-carrying transposon Tn*6657* (214). Concerning the transferability of Tn*6349*, the formation of a Tn*6349*-associated TU, most likely resulting from the recombination of the terminal IS*1216*, was shown. However, neither the transfer of this TU to either *E. faecalis* or *S. aureus* by electrotransformation or conjugation nor the activation of the ΦN315-like prophage was observed (214). Most recently, a review was published in which a novel view was proposed about structures that were bounded by members of the IS*26* family orientated in the same direction. Such structures were not considered true composite transposons and should be termed as "pseudo-compound transposons" (PCTs) (217). Based on this new nomenclature, Tn*6349* should be also classified as a PCT, as the insertion sequence IS*1216* bounding Tn*6349* belongs to the IS*26* family.

Within Tn*6349*, another *cfr*-carrying composite transposon, Tn*6644*, was identified (214). The 5,091-bp transposon Tn*6644* was bounded by identical IS*Enfa5* copies in the same orientation. Tn*6644* was inserted into the lincomycin resistance gene *lnu*(E) and bracketed by 3-bp direct repeats (5′-GAT-3′) (214). This structure has already been described in plasmids or chromosomal DNA of *S. suis* (218), *E. casseliflavus* (184), and *E. faecium* (180), but only recently received the designation Tn*6644* (214). A TU of 3.4 kbp which comprised the *cfr* gene and one copy of IS*Enfa5* was demonstrated by PCR (214).

To date, only two *cfr*-harboring ICEs, namely ICE*PmiChnBCP11* (201) and ICE*PvuChnBC22* (200), were identified in *P. mirabilis* and *P. vulgaris*, respectively. Both strains were isolated from fecal swabs of diarrheal pigs in China (200, 201). ICE*PmiChnBCP1* was 139,487 bp in size and carried—in addition to *cfr*—19 other antimicrobial resistance genes, including *aac(6′)-Ib-cr*, *aacC4*, *aadA2*, *aphA1*, *arr-3*, $bla_{CTX-M-65}$, bla_{OXA-1}, *catB3*, *dfrA32*, *ereA*, *fosA3*, *floR*, *hph*, *strA*, *strB*, *sul1*, *sul2* (two copies), and *tet*(C). ICE*PvuChnBC22* was 148,751 bp in size and harbored the additional resistance genes *aac(3)-IV* (two copies), *aac(6′)-Ib-cr* (two copies), *aadA2*, *aphA1*, *arr-3* (two copies), bla_{DHA-1}, bla_{NDM-1}, bla_{OXA-1}, ble_{MBL}, *catB4*, *dfrA32*, *ereA*, *hph* (two copies), *sul1* (three copies), *qacE*Δ*1* (three copies), and *tet*(A). Both ICE*PmiChnBCP11* and ICE*PvuChnBC22*, belong to the SXT/R391 family, which is one of the largest ICE families (219). Similarly to other members of the SXT/R391 family, the two *cfr*-carrying ICEs also integrate into the 5′ end of the *prfC* gene, which codes for the peptide chain release factor 3 (219). In both *cfr*-bearing ICEs, the *cfr* gene was surrounded by two copies of IS*26*, which is widespread among Gram-negative bacteria (217). This means that IS*26* may play a crucial role in the integration of the *cfr* gene into these ICEs. As expected for functionally active ICEs, both ICEs could successfully transfer to the recipient *E. coli* EC600 strain via conjugation (200, 201). According to the currently published literature, transposons and ICEs—in comparison to plasmids—appear to play a less prominent role in the dissemination of the *cfr* gene within and beyond species and genera.

Insertion sequences generating *cfr*-carrying translocatable units. Insertion sequences (IS) are the simplest mobile genetic elements in the bacterial genomes (220). They typically consist of at least one reading frame that codes for the transposase required for mobility and a pair of terminal inverted repeats at both ends (221). IS elements play a vital role in the transfer and spread of antimicrobial resistance genes, since two identical or closely related copies of the IS elements flanking one or more resistance genes can form a "composite transposon"-like structure (222), many of which are now referred to as PCTs (217). Such structures are capable of moving a variety of antimicrobial resistance genes, thereby fostering the dissemination of antimicrobial resistance. So far, seven different IS elements have been identified to bracket the *cfr* gene and generate such structures, including IS*256*, IS*21-558*, IS*431*, IS*1216E*, IS*Enfa4*, IS*Enfa5*, and IS*26* (Fig. 7).

(i) IS*256*-*cfr*-IS*256*. IS*256*, first detected in *S. aureus*, is composed of a single open reading frame (ORF) that codes for a transposase flanked by imperfect inverted repeats. The IS*256* element typically creates 8- or 9-bp target site duplications (TSDs)

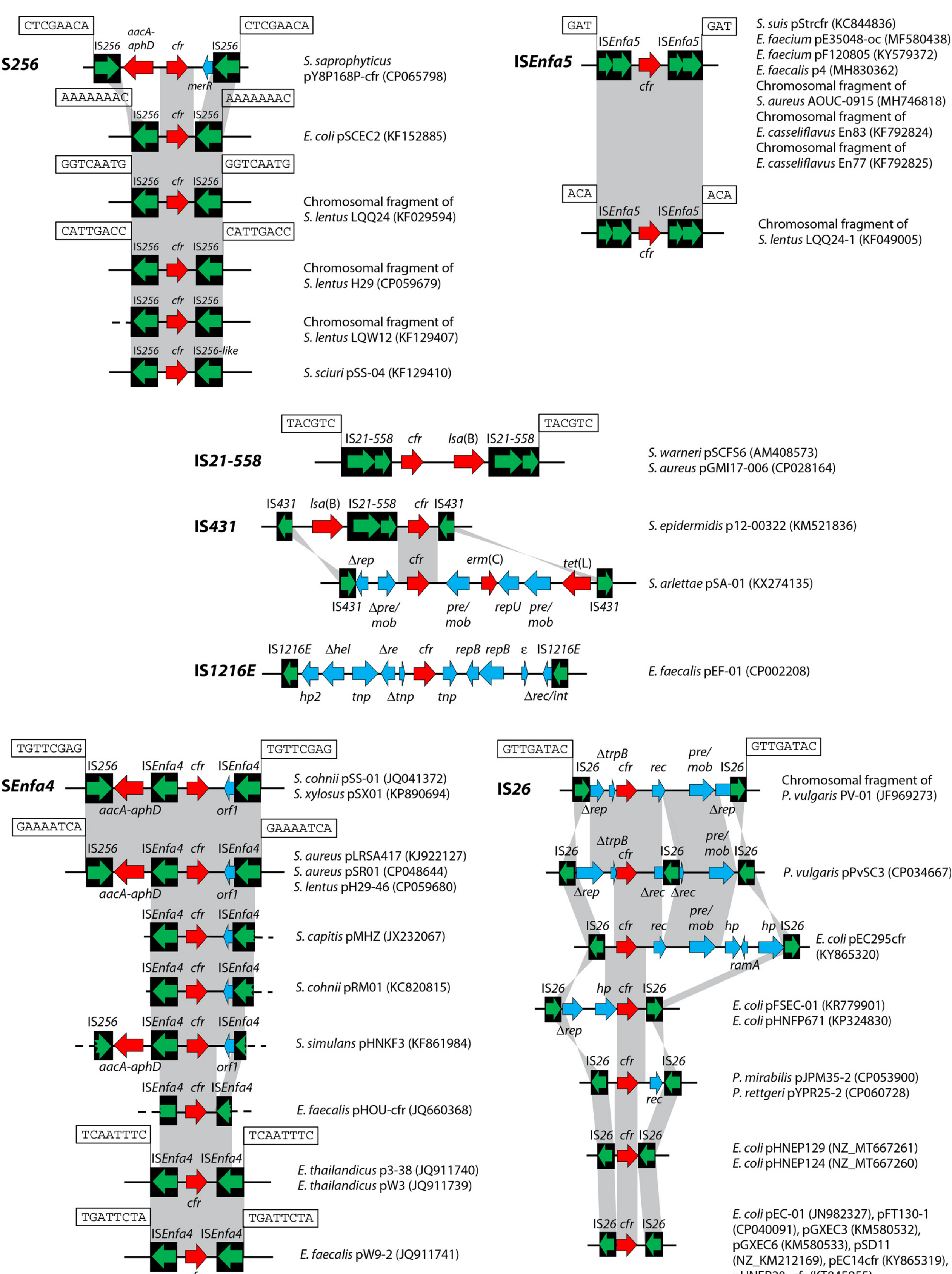

FIG 7 Insertion sequences flanking the *cfr* gene. The insertion sequences IS*256*, IS*Enfa5*, IS*21-558*, IS*431*, IS*1216E*, IS*Enfa4*, and IS*26* are displayed as black boxes, with the green arrow(s) inside symbolizing the respective transposase gene(s). The *cfr* gene is shown as a red arrow. Additional genes

(Continued on next page)

upon transposition (223). The element is widespread in both chromosomal DNA and plasmids among Gram-positive bacteria, such as *Staphylococcus* spp. and *Enterococcus* spp., and is rarely reported in *Enterobacteriaceae* (190). The *cfr* gene bracketed by IS*256* in the same orientation was initially detected on the IncA/C plasmid pSCEC2 from a porcine *E. coli* isolate in China (190). The IS*256*-flanked structure was not stable, as the *cfr*-carrying central region plus one IS*256* could form a TU via IS*256*-mediated recombination (190). Shortly after this report was published, an identical structure bounded by IS*256* was also described in the chromosomal DNA of chicken *S. lentus* LQQ24, LQW5, and LQW12, as well as on plasmid pSS-04 from porcine *S. sciuri* GN5-1 in China (92). It should be noted that on plasmid pSS-04, the ORF of the righthand IS*256* element (designated here "IS*256*-like") exhibited only 95% (1,115/1,173) nucleotide sequence identity to that of the IS*256* first identified in *S. aureus*, and no direct repeats were detected (92). Moreover, a BLASTN search revealed that the "IS*256*-*cfr*-IS*256*" structure was also found in chromosomal DNA of *S. lentus* H29 of chicken origin in China (Fig. 7). In *S. lentus* LQQ24, *S. lentus* H29, and plasmid pSCEC2, the characteristic 8-bp TSDs were observed immediately downstream of the left IS*256* and upstream of the right IS*256*; however, their nucleotide sequences are distinct from each other (Fig. 7). In *S. lentus* LQW12, it is not possible to determine whether there are direct repeats at both ends of the IS*256*-*cfr*-IS*256* structure, since the complete sequence of the right-hand IS*256* element was not obtained (92). Recently, an IS*256*-flanked structure that comprised the resistance genes *cfr* and *aacA-aphD*, as well as a *merR*-like transcriptional regulator gene, was detected on the *S. saprophyticus* plasmid pY8P168P-cfr.

(ii) IS*21-558*-*cfr*-IS*21-558*. IS*21-558*, also called IS*Sau9*, is a member of the IS*21* family. The IS*21-558* element was originally identified on plasmid pSCFS3 recovered from a *S. aureus* strain of pig origin in Germany (117). It consists of two overlapping ORFs, *istAS* and *istBS*, encoding 445-amino-acid (aa) and 250-aa proteins, respectively (93). The *cfr*-harboring segment bounded by two directly oriented copies of IS*21-558* was initially described in a variant of the transposon Tn*558* that was located on the ca. 43-kbp plasmid pSCFS6 from both porcine *S. warneri* and *S. simulans* isolates in Denmark (93). The clindamycin resistance gene *lsa*(B) was also present in the ~4.7-kbp *cfr*-carrying central region and was located immediately downstream of the *cfr* gene. A 6-bp TSD (5′-TACGTC-3′) was found at both ends of the IS*21-558*-*cfr*-IS*21-558* structure (93). In addition, a BLASTN search showed that the same *cfr*-carrying structure was also present on the ca. 46-kbp plasmid pGMI17-006 from *S. aureus* strain CFSAN064038 in Denmark (Fig. 7). This observation suggests that the IS*21-558*-*cfr*-IS*21-558* structure has the ability to spread between different staphylococcal species.

(iii) IS*431*-*cfr*-IS*431*. IS*431*, also termed IS*257*, is found in *Staphylococcus* species. For many years, the two names IS*431* and IS*257* have been used to refer to the same or closely related IS elements (224). To date, two *cfr*-positive plasmids in which the *cfr*-carrying structure bracketed by IS*431* orientated in the same direction is present, p12-00322 from human *S. epidermidis* in Germany and pSA-01 from chicken *S. arlettae* in China, have been identified (130, 158). On p12-00322, the *cfr*-carrying central region, surrounded by IS*431* in the same orientation, contained one copy of the IS*21-558* element and the gene *lsa*(B) conferring low-level clindamycin resistance (130). Whether the IS*431*-*cfr*-IS*431* structure in p12-00322 can form a TU remains to be tested. On pSA-01, an approximately 11.5-kbp segment surrounded by IS*431* comprises, besides the *cfr* gene, the MLS_B resistance gene *erm*(C), the tetracycline resistance gene *tet*(L), two intact copies of the recombination/mobilization gene *pre/mob*, a truncated Δ*pre/mob* gene, and two replication-associated genes, namely the plasmid replication gene *repU* and a truncated Δ*rep* gene (158). Moreover, the structure flanked by IS*431* on pSA-01

FIG 7 Legend (Continued)
are shown as blue arrows. In all cases, the arrowhead indicates the direction of transcription. Whenever direct repeats were identified at the termini of the IS elements that flank the *cfr* region, they are indicated in boxes. The gray-shaded area indicates >99% nucleotide sequence identity. For each specific IS-*cfr*-IS arrangement, the bacterial species, the location (plasmid/chromosomal DNA) and the database accession number (in brackets) are given on the righthand side.

has been confirmed to be unstable, as the presence of a 12,481-bp circular intermediate was confirmed by PCR (158). On both plasmids, no direct repeats are found at either end of the IS*431*-*cfr*-IS*431* structure (Fig. 7). Additional resistance genes are colocated in the IS*431*-*cfr*-IS*431* structure in both *cfr*-positive plasmids. Coselection and cotransfer of the *cfr* gene possibly may occur under the selective pressure imposed by the use of the respective antimicrobial agents.

(iv) IS*1216E*-*cfr*-IS*1216E*. The IS*1216E* element with a size of 808 bp was first found in *E. faecium* and belongs to the IS*6* family. The involvement of IS*1216E* in the mobility of the gene *cfr* was first described in the nonconjugative plasmid pEF-01 from *E. faecalis* strain EF-01, which was isolated from bovine feces in China (170). On plasmid pEF-01, two directly oriented copies of the IS*1216E* element flanked a 12.4-kbp segment that carried the *cfr* gene. However, no direct repeats were observed at both ends of the IS*1216E*-*cfr*-IS*1216E* structure (170). This structure was regarded as an active TU due to the presence of a circular form containing one intact IS*1216E* element and the sequence between the two copies of IS*1216E* (170). However, there are to date no other reports about the IS*1216E*-*cfr*-IS*1216E* structure apart from plasmid pEF-01. The definitive role of IS*1216E* elements in spreading the oxazolidinone resistance gene *cfr* remains to be confirmed.

(v) IS*Enfa4*-*cfr*-IS*Enfa4*. The IS*256* family element IS*Enfa4* encodes a single transposase of 390 aa and harbors imperfect 26-bp terminal inverted repeats (175). Originally, the IS*Enfa4* element (initially also designated IS*256*-like) was found in close proximity to *cfr* on plasmid pSS-01 from porcine *S. cohnii* (122). On plasmid pSS-01, an approximately 8.5-kbp segment harboring a Tn*4001*-like transposon, *cfr*, *orf1*, and IS*Enfa4* was flanked by 8-bp direct repeats (5′-TGTTCGAG-3′) at its ends (122). This identical structure was also present on plasmid pSX01 from *S. xylosus* (Fig. 7). The same *cfr*-harboring segment, flanked by other direct repeats (5′-GAAAATCA-3′), was observed on several other plasmids from *Staphylococcus*, including pLRSA417 and pSR01 from *S. aureus*, and pH29-46 from *S. lentus* (208). A similar genetic context flanking the *cfr* gene was found on another three plasmids with partial sequence, including pMHZ from *S. capitis*, pRM01 from *S. cohnii*, and pHNKF3 from *S. simulans* (208). In addition, the "IS*Enfa4*-*cfr*-IS*Enfa4*" structure was also described on three conjugative plasmids of different sizes from porcine *Enterococcus* isolates, namely, pW9-2 from *E. faecalis*, as well as p3-38 and pW3, both from *E. thailandicus* (175). On the three plasmids, a 4,447-bp *cfr*-bearing region bounded by two IS*Enfa4* elements in the same orientation revealed the presence of 8-bp TSDs. Two different 8-bp TSDs were observed in the three plasmids (175). A free circular intermediate form containing the *cfr* gene region and one intact IS*Enfa4* element could be obtained via PCR assays (175). The partial sequence of the *cfr*-harboring plasmid pHOU-cfr from human clinical *E. faecalis* isolate 603-50427X in Thailand (166) shared high homology with the IS*Enfa4*-*cfr*-IS*Enfa4* structure on the aforementioned three plasmids (Fig. 7).

(vi) IS*Enfa5*-*cfr*-IS*Enfa5*. IS*Enfa5*, a member of the IS*3* family, was originally identified on plasmid pStrcfr from a porcine *S. suis* isolate in China (82). It is composed of two overlapping reading frames encoding proteins of 224 aa and 299 aa, respectively (82). The IS*Enfa5* element has imperfect terminal inverted repeats of 39 bp (right inverted repeat [IRR]) and 40 bp (left inverted repeat [IRL]) at its termini (82). On the plasmid pStrcfr, the gene *cfr* was initially found to be flanked by two copies of IS*Enfa5* in the same orientation, and 3-bp direct repeats (5′-GAT-3′) were present immediately upstream and downstream of the IS*Enfa5*-*cfr*-IS*Enfa5* structure (82). Subsequently, the same genetic structure was also reported on plasmids or in the chromosomal DNA of enterococci and staphylococci of different origin, such as human *S. aureus* AOUC-0915 in Italy (225), porcine *E. casseliflavus* En83 and En77 in China (184), plasmid pE35048-oc from an *E. faecium* isolate of human origin in Italy (180), plasmid p4 of a porcine *E. faecalis* (GenBank accession no. MH830362), and plasmid pF120805 from an *E. faecium* isolate of human origin in Ireland (132) (Fig. 7). In addition, another 3-bp direct repeat (5′-ACA-3′) flanking the IS*Enfa5*-*cfr*-IS*Enfa5* structure was found in the chromosomal

DNA of *S. lentus* LQQ9 of chicken origin in China (92). The chicken *S. lentus* isolates LQW11, LQW6, LQQ37, and LQQ27-2, although not sequenced, also showed this structure in their chromosomal DNA (92). The IS*Enfa5*-*cfr*-IS*Enfa5* structure is functionally active, as shown by the presence of a TU containing the gene *cfr*, one copy of IS*Enfa5*, and the sequences between *cfr* and the two IS*Enfa5* copies (82). This may in part explain the wide distribution of this structure.

(vii) IS*26*-*cfr*-IS*26*. The insertion sequence IS*26* is 820 bp in size and consists of a 705-bp reading frame that encodes a single transposase. IS*26* was originally assigned to the IS*6* family. However, recently the IS*6* family has been redefined as the IS*26* family (226). The IS*26* element is most commonly found associated with antimicrobial resistance genes in Gram-negative bacteria (227, 228). The involvement of IS*26* elements in the movement of the gene *cfr* was first identified in porcine *P. vulgaris* strain PV-01 in China in 2011 (198). An approximately 7-kbp *cfr*-containing fragment bracketed by two IS*26* copies in the same orientation was integrated into the chromosomal *fimD* gene of PV-01 (198). Some other genes were also identified in the *cfr*-containing fragment, including a recombination/mobilization gene (*pre/mob*), a truncated Tn*554* transposase B gene (Δ*tnpB*), a recombinase gene (*rec*), and two truncated plasmid replication-associated genes (Δ*rep*) (198). Direct repeats (8 bp; 5′-GTTGATAC-3′) were detected immediately upstream and downstream of the inserted region (198). During the following years, another six *cfr*-carrying fragments with distinct structures bounded by IS*26* have been identified in the chromosomal DNA or on plasmids of *Proteus* spp. and *E. coli* (Fig. 7). The second type of IS*26*-*cfr*-IS*26* structure was described on a ca. 284-kbp plasmid pPvSC3 from a *P. vulgaris* isolate of pig origin in China (199). The *cfr*-bearing central region on pPvSC3 displayed partial homology to that in *P. vulgaris* PV-01. In pPvSC3, the *rec* gene was disrupted into two parts by another IS*26* element, and the IS*26* elements were oriented in the opposite direction compared to the gene *cfr* (199). Similarly to the situation in *P. vulgaris* PV-01, a *cfr*-carrying segment plus an IS*26* element could also be looped out via IS*26*-mediated recombination (199). The third type of IS*26*-*cfr*-IS*26* was reported on plasmid pEC295cfr from the porcine *E. coli* isolate LN310P in China (196). In addition to the genes *rec* and *pre/mob*, the IS*26*-flanked region on pEC295cfr also contained a transcriptional activator gene, *ramA*, and an ORF for a putative inner membrane protein. It should be mentioned that on plasmid pEC295cfr, the two IS*26* copies were in the opposite orientation (196). The fourth type of IS*26*-*cfr*-IS*26* structure was first found on the conjugative plasmid pFSEC-01 from porcine *E. coli* in China (193). Unlike the first three types, the ORFs between the gene *cfr* and the righthand IS*26* element were missing, and a reading frame encoding a 342-aa truncated Rep protein was present immediately upstream of the *cfr* gene on pFSEC-01. No *cfr*-carrying intermediate circular forms were detected on pFSEC-01 (190). The same *cfr*-harboring structure identified on pFSEC-01 was also found on the 82,807-bp plasmid pHNFP671 (GenBank accession no. KP324830) from *E. coli* of pig origin in China. The fifth type of IS*26*-*cfr*-IS*26* structure was detected on plasmid pJPM35-2 (GenBank accession no. CP053900) from *P. mirabilis* and pYPR25-2 (GenBank accession no. CP060728) from *P. rettgeri*, both of duck origin in China. In addition to the *cfr* gene, only the *rec* gene exists in the IS*26*-flanked segment (Fig. 7). The sixth type of IS*26*-*cfr*-IS*26* structure was found on the two plasmids pHNEP129 (GenBank accession no. MT667261) and pHNEP124 (GenBank accession no. MT667260), both of which were obtained from porcine *E. coli* isolates in China. To our knowledge, the IS*26*-*cfr*-IS*26* structure on pHNEP129 and pHNEP124 is the simplest type, and no other ORFs were in the IS*26*-bracketed central region except the *cfr* gene (Fig. 7). The seventh type of IS*26*-*cfr*-IS*26* structure, most commonly found in *E. coli*, consists of a 1,545-bp *cfr*-carrying region surrounded by IS*26.* Since it was first reported on plasmid pEC-01 (190), this structure has been successively identified on other plasmids, all of which were derived from *E. coli*, including pSD11 (210), pEC14cfr (196), pHNEP28_cfr (211), pFT130-1 (GenBank accession no. CP040091), pGXEC3, and pGXEC6 (194). In addition, this structure was confirmed to be unstable, as the *cfr*-carrying central region plus one IS*26*

copy can easily be excised (190, 210). It is worth pointing out that, except for in PV-01, no TSDs were found immediately upstream and downstream of the IS*26*-*cfr*-IS*26* structure. Recently, a novel mode of IS*26* movement was described to explain the formation of this structure (228). A TU, composed of an IS*26* element and an adjacent DNA fragment, preferentially targets another copy of IS*26* via a conservative process without duplication of the IS and of the target site (228).

Chromosomal *cfr* Genes

Plasmids, TUs, transposons, and ICEs carrying *cfr* genes can integrate in whole or in part into the chromosomal DNA of bacteria. In the previous subchapters, multiple *cfr* genes associated with the aforementioned MGEs have been described. In this subchapter, only a few examples of chromosomally located *cfr* genes are depicted in more detail.

The MRSA isolate CM05, which originated from a linezolid-treated patient in Colombia, was the first clinical *cfr*-carrying staphylococcal isolate (229). The *cfr* gene in this isolate was located within a 15,511-bp region of most likely plasmidic origin that was inserted between bases 1256 and 1257 of the 23S rRNA allele 4 (100). Two regions of 1,546 bp, which included the *erm*(B) gene and were located in the same orientation, flanked the *cfr* gene and its downstream IS*21-588* element. Recombination between these two identical regions resulted in the formation of a 5,987-bp TU, which comprised the *cfr* gene, the IS*21-558* element, and one copy of the *erm*(B) region. The loss of this TU yielded a CM05 deletion variant which was linezolid susceptible but had a fitness advantage over CM05 in the absence of a selective pressure (100).

The gene *cfr* was also found in the chromosomal DNA of a porcine MRSA ST9 isolate from China (120). Detailed analysis of the integration site revealed that a 5,334-bp segment had inserted downstream of the *ccr* genes into the staphylococcal cassette chromosome *mec* element (SCC*mec*) type IVb. This segment comprised the *cfr* gene bracketed by an IS*256* element in the downstream part and an IS*Enfa4* element in the upstream part, both in opposite orientations (120). Bearing in mind that oxazolidinones are important for the treatment of infections caused by MRSA, the finding of the methicillin resistance gene *mecA* together with the gene *cfr* located in the same SCC*mec* element is alarming. This strain has been reported in 2014; since then, no other MRSA isolates with this particular SCC*mec* element have been reported, suggesting that this strain with its SCC*mec* element has not further disseminated.

The analysis of *cfr* being bracketed by IS elements showed that such structures—PCTs or true transposons—are not only present on plasmids, but also in the chromosomal DNA. Examples for such chromosomal IS-bounded *cfr* genes are found in (i) an *S. lentus* isolate from a chicken in China (92), where *cfr* was bracketed by IS*256*, (ii) various *S. lentus* isolates from chickens in China (92), an *S. aureus* isolate from a human in Italy (214) and *E. casseliflavus* isolates from pigs in China (184), where *cfr* was bracketed by IS*Enfa5*, and (iii) *P. vulgaris* from a pig in China (198), where a larger *cfr*-carrying segment was bracketed by IS*26*.

In porcine *S. sciuri* from China, the *cfr* gene has been detected in close vicinity to a chromosomal *optrA* gene in a different genetic environment (163). Here, both oxazolidinone resistance genes were located next to each other but in different orientations. The insertion sequence IS*Enfa5* was located upstream of *cfr*, whereas upstream of *optrA*, the transcriptional regulator gene *araC* and either complete or partially truncated Tn*558* transposons were found (163). In porcine *S. cohnii* and *S. sciuri* from China, the *cfr* gene and its associated IS*21-558* element were located in the chromosomal DNA, and two different genetic environments were detected (153). In the *S. cohnii* isolate, the IS*21-558-cfr* segment was inserted into a Tn*558* transposon, thereby deleting both the *tnpA* and *tnpB* genes of this transposon. In the *S. sciuri* isolate, the resistance genes *aadD* and *ble* were found upstream of the IS*21-558-cfr* segment, while in the downstream region, genes for a resolvase and a transposase, as well as a complete Tn*558*, were found. Interestingly, exactly the same *cfr* region was found on a plasmid

TABLE 4 Characteristics of completely sequenced plasmids carrying *cfr*(B), *cfr*(C), or *cfr*(D) genes

cfr gene	Plasmid	Origin	Size (bp)	Colocated resistance gene(s)	GenBank accession no.
cfr(B)	Plasmid 2	*E. faecium*, human, Netherlands	293,851		LR135358
cfr(C)	pCd13_cfrC	*C. difficile*, human, Greece	6,961	*aphA3*	MH229772
	pTx-40	*C. coli*, cattle, USA	48,048	*aphA3*, Δ*aadE*, *hph*, *tet*(O)	KX686749
	pN61925F	*C. coli*, cattle, USA	48,049	*aphA3*, Δ*aadE*, *hph*, *tet*(O)	MK541989
	pN61740F	*C. coli*, cattle, USA	48,049	*aphA3*, Δ*aadE*, *hph*, *tet*(O)	MK541988
	pN46788F	*C. coli*, cattle, USA	50,413	*aphA3*, Δ*aadE*, *hph*, *tet*(O)	MK541987
	pSH89	*C. coli*, pig, China	57,366	*aphA3*, *tet*(O)	CP047217
	pJZ_1_79	*C. coli*, pig, China	62,417	*aphA3*, *tet*(O)	CP047213
cfr(D)	p15-307-1_02	*E. faecium*, human, France	103,074	*erm*(A)-like, *erm*(B), *optrA*	CP044318
	pM17/0314	*E. faecium*, human, Ireland	103,600	*erm*(A)-like, *erm*(B), *optrA*	MN831413
	pBP5067_P1	*E. faecium*, human, India	122,126	*optrA*, *erm*(B), *vanA* gene cluster (*vanRSHAXYZ*)	CP059807
	pBA17124_P1	*E. faecium*, human, India	130,516	*optrA*, *erm*(B), *vanA* gene cluster (*vanRSHAXYZ*)	CP059785

from porcine *S. sciuri*, suggesting that the chromosomal location resulted from the partial or complete integration of a former *cfr*-carrying plasmid (153).

In *S. suis* from pigs in China, the *cfr* gene was found in a 57,542-bp chromosomal antibiotic-resistance-associated genomic island, designated ARGI4 (185). The *cfr* gene was bracketed by a complete IS*1216E* in the upstream part and a truncated IS*Enfa5* and a complete IS*Teha2* in the downstream part. The ARGI4 proved to be not transferable by conjugation (185).

MOBILE OXAZOLIDINONE RESISTANCE GENE *cfr*(B)

Geographical Distribution and Host Bacteria of the *cfr*(B) Gene

The gene *cfr*(B) has so far only been detected in two genera, *Clostridioides* and *Enterococcus*. All *cfr*(B)-carrying isolates known thus far are from humans. *cfr*(B)-positive *Clostridioides difficile* (formerly known as *Clostridium difficile* or *Peptoclostridium difficile*) isolates have been identified in China (GenBank accession no. CP003939), Spain (49), Greece (230), Sweden (GenBank accession no. MPDX01000657), and the United Kingdom (GenBank accession no. HG002396 and HG002389), as well as in Honduras and Chile (52). *E. faecalis* isolates harboring the *cfr*(B) gene were found in Japan (231), as well as in Panama and the United States (167), whereas the corresponding *E. faecium* isolates were detected in Germany (232), the Netherlands (GenBank accession no. NXPC01000098 and NXPD01000081), and the United States (48, 79). In available information, all *cfr*(B)-carrying bacteria exhibited linezolid MICs above the clinical breakpoint for resistance.

Mobile Genetic Elements That Are Involved in the Dissemination of the *cfr*(B) Gene

Plasmids carrying the *cfr*(B) gene. To the best of our knowledge, the sequence of a single complete plasmid that harbors the *cfr*(B) gene has been deposited in GenBank and associated databases (GenBank accession no. LR135358). This plasmid, designated plasmid 2, is 293,851 bp in size, originates from an *E. faecium* isolate of human origin in the Netherlands, and—besides *cfr*(B)—does not carry additional resistance genes (Table 4). Large plasmids of ≥200 kbp that carry the gene *cfr*(B) in variants of Tn*6218* were described in *E. faecium* isolates of human origin in Germany (232).

Transposons carrying the *cfr*(B) gene. So far, the gene *cfr*(B) has been associated with the nonconjugative transposon Tn*6218* (48, 233). This transposon or variants thereof have been detected at various positions in the chromosomal DNA of *C. difficile*, as well as in *E. faecium* and *E. faecalis* (48, 232, 233). The prototype Tn*6218* from *C. difficile* strain Ox2167 (GenBank accession no. HG002396) is 8,495 bp in size and originated from the United Kingdom (233). A slightly smaller Tn*6218* variant of 8,407 bp (GenBank accession no. KR610408) has been identified in clinical *E. faecium* isolates from the

United States (48). This variant differed distinctly from the other Tn*6218* variants in its structure and composition (232). In general, the Tn*6218* elements found in *E. faecium* and *C. difficile* were essentially the same, except for the integrase genes, whose gene products showed only 86% identity (48). Bender and coworkers found among five German clinical *E. faecium* isolates Tn*6218* elements that were highly similar or even identical to the original Tn*6218* (232). The *cfr*(B) genes in Greek *C. difficile* isolates were located on chromosomal Tn*6218* elements (230). The *cfr*(B) genes of *C. difficile* isolates from Honduras were also located on Tn*6218*-like elements in the chromosomal DNA (52). A Japanese *E. faecalis* isolate carried a Tn*6218* element that was closely related (98.62 to 99.97%) to the corresponding elements from *C. difficile* isolates in the United Kingdom and China, as well as from *E. faecium* isolates from Germany and the Netherlands (231). The *cfr*(B) genes in *C. difficile* from Chile were located in a not further described chromosomal genetic structure that contained transposase and integrase genes (52).

A comparison of the Cfr(B) proteins found in *E. faecalis*, *E. faecium*, and *C. difficile* revealed 99.7 to 100% identity (48). Similar results by comparing the *cfr*(B) nucleotide sequences were seen by Kuroda and coworkers, who identified only single-nucleotide polymorphisms (SNPs) at four defined positions between all *cfr*(B) genes (231).

MOBILE OXAZOLIDINONE RESISTANCE GENE *cfr*(C)

Geographical Distribution and Host Bacteria of the *cfr*(C) Gene

The gene *cfr*(C) has been identified in only three species to date—*C. difficile*, *Clostridium bolteae*, and *Campylobacter coli*. While the *cfr*(C)-carrying *C. difficile* isolates were from humans in Belgium (234), France (234), Germany (234), Italy (234), Greece (230), Honduras (52), and Costa Rica (52), the *C. bolteae* isolate originated from a human in France (234). In contrast, all *cfr*(C)-harboring *C. coli* isolates were of animal origin, i.e., from cattle in the United States (50, 235) and from pigs in China (236, 237).

Mobile Genetic Elements That Are Involved in the Dissemination of the *cfr*(C) Gene

Plasmids carrying the *cfr*(C) gene. The smallest plasmid carrying the gene *cfr*(C) was identified in a *C. difficile* strain of human origin in Greece. This plasmid of 6,961 bp, designated pCd13_cfrC, comprised a plasmid replication gene, a plasmid recombination gene, and the *aphA3* gene for resistance to kanamycin, neomycin, and amikacin besides *cfr*(C). Distinctly larger plasmids carrying the *cfr*(C) gene have been detected in *C. coli* (Table 4). In bovine *C. coli* from the United States, the *cfr*(C) gene was found on the conjugative 48,048-bp plasmid pTx-40 (50). The tetracycline resistance gene *tet*(O), the kanamycin/neomycin/amikacin resistance gene *aphA3*, the hygromycin resistance gene *hph* and a truncated streptomycin resistance gene *aadE* were detected in the vicinity of the *cfr*(C) gene (50). Two plasmids, pN61925F and pN61740F, which closely resembled pTx-40 in size (48,049 bp) and structure and also carried the same set of antimicrobial resistance genes, were identified in bovine *C. coli* in 2014 as part of the U.S. National Antimicrobial Resistance Monitoring System (NARMS) program (235). A slightly larger plasmid from *C. coli* in the United States, pN46788F with a size of 50,413 bp, closely resembled the aforementioned plasmids (Fig. 8). In *C. coli* from pigs in China, two conjugative plasmids of 62,417 bp (pJZ_1_79) and 57,366 bp (pSH89) were found. Both of them harbored the *tet*(O) gene as well as the genes *cfr*(C) and *aphA3*, albeit with the insertion sequence IS*607** upstream of *cfr*(C) (237).

Integrative and conjugative elements carrying the *cfr*(C) gene. Candela and coworkers described three different types of ICE-like elements in *C. difficile*, designated ICE_{DA275}, ICE_{F548}, and ICE_{DA203}. Partial structures have been reported for all three ICEs, and a size of 24,150 bp has been indicated for ICE_{DA203} (234). Unfortunately, the sequences of these three ICEs have not been deposited in any databases. According to the authors, GenBank searches identified the ICE_{90B3}, which differed from ICE_{DA203} by one base pair exchange, in *C. bolteae* (234). PCR assay with primers located at the termini of ICE_{90B3} and directed toward the flanking regions showed that a TU, which points

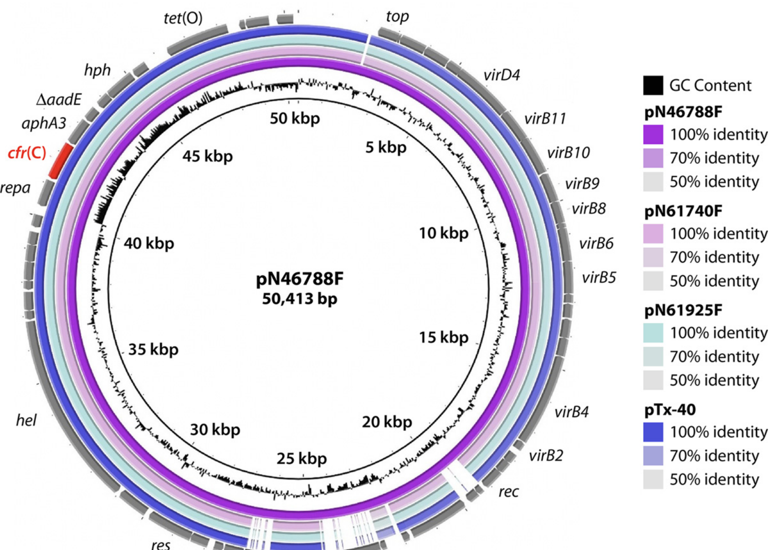

FIG 8 Structural comparison of *cfr*(C)-carrying plasmids in *C. coli* (constructed by BRIG). Relevant genes with known functions and insertion elements are indicated for the respective reference plasmid in the outer ring. The *cfr*(C) gene is indicated in red. The innermost circle provides a size scale, while the next innermost circle shows the GC content. Other plasmids used for comparison are indicated by color-coded rings, with the reference plasmid representing the innermost colored ring. The plasmids used for this comparison are pN46788F, pN61740F, pN61925F, and pTx-40.

toward the mobility of the element, was formed in *C. bolteae* (234). The *cfr*(C) genes in *C. difficile* from Honduras and Chile have also been reported to be located on ICE$_{F548}$-like elements (52).

Chromosomal *cfr*(C) Genes

Liu and coworkers identified the gene *cfr*(C) in the chromosomal DNA of four *C. coli* isolates from pigs in China (236). Detailed sequence analysis revealed that the *cfr*(C)-carrying genomic regions represented three novel multidrug resistance genomic islands (MDRGIs) of different sizes. MDRGI1 was 17,277 bp in size and was inserted into a gene for a hypothetical protein (236). It harbored the resistance genes Δ*tet*(O), *lnu*(C), *aac*, *aacA-aphD*, *aadE*, Δ*sat4*, *aphA3*, *cfr*(C), and *tet*(O). MDRGI2 was 20,074 bp in size and was inserted between two genes for hypothetical proteins (236). It carried the resistance genes *tet*(O), *lnu*(C), *spc*, *aphA7*, Δ*aadE*, *sat4*, *aphA3*, *cfr*(C), *aadE*, and Δ*tet*(O). Part of the resistance gene region in MDRGI2 was flanked by 629-bp direct repeats. PCR assays confirmed the presence of a TU of 5,815 bp, which was generated by recombination between the direct repeats. This TU comprises the resistance genes Δ*aadE*, *sat4*, *aphA3*, and *cfr*(C) in addition to another four genes (236). The formation of such TUs might explain why the same (or a very similar) set of resistance genes is found in *cfr*(C)-carrying *C. coli*. MDRGI3 was inserted into the CRISPR-associated gene. The available partial sequence revealed that MDRGI3 included at least the resistance genes Δ*aadE*, *sat4*, *aphA3*, and *cfr*(C) (236).

In another study, five different chromosomal regions in which the *cfr*(C) gene had been inserted were identified among porcine *C. coli* isolates from China. In all genetic environments, the *cfr*(C) gene was located in close proximity to the *aphA3* gene. The insertion sequence IS*607** was located upstream of *cfr*(C)-*aphA3* in one of the five regions, while the insertion sequence IS*Cco7* was found downstream of *cfr*(C) in two regions. Whether or not these insertion sequences play a role in the mobility of *cfr*(C) remains to be answered. Further antimicrobial resistance genes present in the five chromosomal regions included *tet*(O) in all but one region, as well as *sat4*, *aadE*, and *aph(2″)*, which were found in two of the five environments in one or two copies (237).

MOBILE OXAZOLIDINONE RESISTANCE GENE *cfr*(D)

Geographical Distribution and Host Bacteria of the *cfr*(D) Gene

Comparatively little information is currently available about the gene *cfr*(D). It has been identified only in *E. faecium* and *E. faecalis* isolates from humans. The corresponding *E. faecium* isolates originated from Australia (51), France (238), Ireland (239), and the Netherlands (GenBank accession no. LR135354). The *cfr*(D) gene was identified in *E. faecalis* isolates from Italy and Spain (174, 240). In all *cfr*(D)-carrying isolates, the phenicol/oxazolidinone resistance gene *optrA* was also present.

Mobile Genetic Elements That Are Involved in the Dissemination of the *cfr*(D) Gene

Plasmids carrying the *cfr*(D) gene. Only a few completely sequenced plasmids carrying *cfr*(D) have been described. The 103,074-bp plasmid p15-307-1_02 originated from a French *E. faecium* isolate (238) and the 103,600-bp plasmid pM17/0314 (239) from an Irish *E. faecium* (Table 4). Both plasmids also carried the resistance genes *erm*(A)-like, *optrA*, and *erm*(B). The 122,126-bp plasmid pBP5067_P1 (GenBank accession no. CP059807) and the 130,516-bp plasmid pBA17124_P1 (GenBank accession no. CP059785) were from human *E. faecium* isolates from India. These plasmids were related in their structures and carried the additional resistance genes *optrA* and *erm*(B), as well as the *vanA* gene cluster (*vanRSHAXYZ*) (Table 4). A partial sequence, which comprises the *cfr*(D) region, is available for plasmid 4 of the Dutch *E. faecium* isolate E8014. In the three plasmids pM17/0314, p15-307-1_02, and plasmid 4, the *cfr*(D) gene and a complete or a truncated *guaA* gene, which encodes a glutamine-hydrolyzing GMP synthase, are flanked by IS*1216* elements in the same orientation (238). In the two Indian plasmids, the *guaA* gene is missing, but the IS*1216* elements are present. In the Australian *E. faecium* isolate E637001, the *cfr*(D) contig exhibited 100% nucleotide sequence identity with the corresponding region of plasmid 2 from France (51). The Spanish *E. faecalis* isolate X528 carried the *cfr*(D) gene on a 4,545-bp contig, which was identical to the respective region of the plasmid 4 from the Dutch *E. faecium* E8014. Whether or not the *cfr*(D) gene in the Spanish *E. faecalis* isolate is plasmid-borne remains to be answered, as conjugation assays failed to show the transferability of *cfr*(D) (240).

MOBILE OXAZOLIDINONE RESISTANCE GENE *cfr*(E)

Geographical Distribution and Host Bacteria of the *cfr*(E) Gene

The gene *cfr*(E) is the youngest member in the *cfr* family. It has so far only been described in a single *C. difficile* isolate of human origin from Mexico (52).

Mobile Genetic Elements Associated with the *cfr*(E) Gene

The *cfr*(E) gene is potentially part of a mobile genetic element, since genes for a DNA invertase, a recombinase, an ATP binding protein, a transcriptional regulator, and two hypothetical proteins have been detected in the close vicinity to *cfr*(E). The entire segment has been reported to be inserted into the chromosomal gene *adeC*, which codes for an adenine deaminase (52).

MOBILE OXAZOLIDINONE RESISTANCE GENE *optrA*

Geographical Distribution and Host Bacteria of the *optrA* Gene

According to the PubMed and NCBI Nucleotide databases, the gene *optrA* is present in 29 countries/regions of six continents (Fig. 9), 18 of which were also positive for *cfr*-carrying bacteria. The host bacteria carrying the *optrA* gene are mainly *Enterococcus* spp., although several studies have also identified *optrA* in the genera *Staphylococcus*, *Streptococcus*, *Clostridium*, and *Campylobacter*. In addition, *optrA* genes have been identified in the genomes of members of the genera *Fusobacterium*, *Listeria*, and *Salmonella*. Similarly to the situation with the *cfr* gene, *optrA*-positive bacteria also originated from humans, various animals (cattle, pigs, chickens, turkeys, ducks, dogs, and cats), and food of animal origin (eggs, pork, beef, and chicken and turkey meat), as

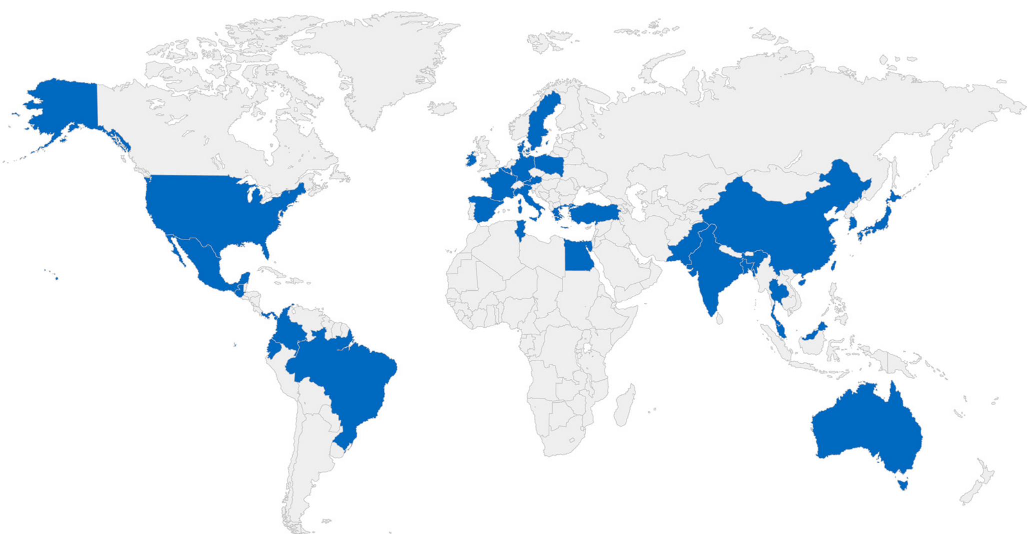

FIG 9 Geographical distribution of *optrA*-carrying bacteria. The countries in blue are those from which the occurrence of *optrA*-carrying bacteria has been reported.

well as from environmental sources. In addition, *optrA*-positive enterococci also originated from vegetables, such as caraway seeds, cucumber, and onion.

Occurrence of the *optrA* gene in *Enterococcus* spp. Initially identified in *E. faecalis* and *E. faecium* from humans, pigs, and chickens in China in 2015 (54), the *optrA* gene is widespread in *Enterococcus* spp., especially in *E. faecalis* and *E. faecium*. *optrA*-carrying *E. faecalis* strains have been detected in humans from Australia (51), Austria (241), Bangladesh (242), Belgium (243), China, including Taiwan (54, 71, 165, 167, 244–251), Colombia (252), Denmark (253), Ecuador (167), Egypt (254), France (167, 255), Germany (256), Greece (257), Guatemala (71, 167), Ireland (69, 70, 167, 239), Japan (231), South Korea (258, 259), Malaysia (69, 167), Mexico (71, 167), Panama (167), Poland (260), Spain (240, 261, 262), Sweden (167), Thailand (167), Tunisia (263), Turkey (264), and the United States (79, 264). *E. faecalis* isolates carrying the *optrA* gene were also found in various animals, including pigs from Brazil (169, 265), China (173, 246, 266–270), Italy (174), South Korea (271), and the United States (183), chickens from China (246), Colombia (272), South Korea (271), and Tunisia (273, 274), cattle from the United States (183), and dogs from China (164). In addition, *optrA*-positive *E. faecalis* isolates have also been obtained from animal food (beef, chicken meat, pork, and egg) and vegetables (caraway seeds and cucumber) in China (164), beef in Denmark (275), and chicken meat in South Korea (276, 277), as well as from wastewater in Tunisia (278). In China, *optrA*-positive *E. faecalis* isolates have also been identified on shared bicycles (279).

Similarly, *optrA*-positive *E. faecium* isolates were also widely identified in humans from Australia (51), Belgium (243), China (245, 248–250), France (255), Germany (256), Greece (280), Ireland (132, 239), Italy (180), South Korea (259), Pakistan (281), Spain (262), Turkey (264), and the United States (264). The *optrA*-positive *E. faecium* isolates were also isolated from pigs in China (266, 267) and Italy (174), cattle in South Korea (271) and the United States (183), chickens (271), ducks (282) and chicken meat (276) in South Korea, and turkey meat in Denmark (275), as well as from environmental samples in Spain (283). Apart from *E. faecalis* and *E. faecium*, the *optrA* gene has also been detected in *E. casseliflavus* isolates from onions, beef, and chicken meat (164), in *E. gallinarum* from a pig (284), and in *E. avium*, *E. hirae*, *E. thailandicus*, *E. gallinarum*, and not further specified *Enterococcus* isolates of human origin, all from China (248, 249). A recent study described the presence of *optrA*-positive *E. avium*, *E. thailandicus*, and *E. gallinarum* isolates from pigs in Italy (174).

Occurrence of the *optrA* gene in *Staphylococcus* spp. Unlike the widespread occurrence of the gene *optrA* in the genus *Enterococcus*, this gene has been rarely reported in the genus *Staphylococcus*. *optrA*-carrying MRSA isolates were found both in humans and animals (pigs, chickens, and ducks) from China (98). However, the *optrA*-positive *S. sciuri* isolates occurred exclusively in animals, such as dogs (153, 164), pigs (153, 162, 163), and cats (153) from China and turkeys from Egypt (154). Another group of *optrA*-carrying CoNS species, including *S. xylosus*, *S. lentus*, *S. saprophyticus*, and *S. epidermidis*, has so far only been found in turkeys from Egypt (154).

Occurrence of the *optrA* gene in other Gram-positive bacteria. There are several reports on the presence of the *optrA* gene in the genus *Streptococcus*, including *S. agalactiae*, *S. gallolyticus*, and *S. suis*. The *optrA*-positive *S. agalactiae* and *S. gallolyticus* were found exclusively in humans from China (285) and Thailand (71), respectively, whereas the corresponding *S. suis* isolates were present only in pigs from China (185, 286–288). Another species of *optrA*-carrying bacteria is *Clostridium perfringens*, so far only identified in chickens from China (289). Moreover, whole-genome sequences of several *Listeria monocytogenes* isolates (GenBank accession no. AARQTE010000003, AARQTE010000015, AARQTG010000014, AARQTK010000001, AARQTK010000013, and AARQTG010000006) from environmental swabs in the United States revealed the presence of an *optrA* gene.

Occurrence of the *optrA* gene in Gram-negative bacteria. So far, there are only two published reports about the identification of the *optrA* gene in Gram-negative bacteria, both in the genus *Campylobacter*. The respective *optrA*-positive *C. coli* originated either from chickens and ducks (290) or from pigs (291) in China. However, complete *optrA* genes have also been identified in the whole-genome sequences of *Campylobacter jejuni* from duck meat in China (GenBank accession no. CP048771), *Fusobacterium* sp. from a human fecal sample in China (GenBank accession no. CP060637), and *Salmonella* sp. from a cloacal swab of a chicken in China (GenBank accession no. QFLJ01000014).

Mobile Genetic Elements That Are Involved in the Dissemination of the *optrA* Gene

Numerous plasmids carrying the gene *optrA* have been described in various Gram-positive bacteria. As done for the *cfr*-carrying plasmids, we focused the description of the *optrA*-harboring plasmids on their size, structure, and organization, as well as the colocated additional genes that conferred resistance to antimicrobial agents, heavy metals, or biocides (Table 5).

Plasmids carrying the *optrA* gene in *Enterococcus* spp. The completely sequenced *optrA*-carrying plasmids in *Enterococcus* spp. varied in size from 28,222 bp to 142,820 bp (Table 5). All of these plasmids carried 1 to 13 additional resistance genes. The five identical plasmids with a size of 36,311 bp—pKUB3007-4 and pKUB3006-4 (231) from human *E. faecalis* isolates in Japan, pM17/0149 (239) and p6742_1 (260) from human *E. faecalis* isolates in Ireland and Poland, respectively, and pEFs17-1 (GenBank accession no. MT223178) from an *E. faecalis* isolate of not further specified animal origin in South Korea—showed large portions of similarity with pN48037F-3 and pN60443F-2 from porcine and bovine *E. faecalis* isolates in the United States (183). These latter plasmids are about 3.9 and 5.3 kbp larger than the aforementioned five plasmids. All of these plasmids harbored only the *fexA* gene as additional resistance gene, except for pN60443F-2, which also harbored an *erm*(B) gene (Fig. 10A).

The 45,581-bp plasmid pC25-1 shared large portions of similarity with the 64,500-bp plasmid pC54 (266). Both plasmids were from porcine *E. faecalis* in China and carried the additional resistance genes *fexA* and *erm*(A) (266) (Fig. 10B). The 45,603-bp plasmid pAF379 from *E. faecalis* isolated from urban wastewater in Tunisia is so far the only completely sequenced *optrA*-carrying plasmid from environmental samples. This plasmid showed a distinct structure compared to the other completely sequenced plasmids and carried the Tn*554*-associated resistance genes *erm*(A) and *spc* genes as well (278). Similarly, the 58,593-bp plasmid pL9 (GenBank accession no. CP041776)

TABLE 5 Characteristics of completely sequenced *optrA*-carrying plasmids in bacteria

Plasmid	Origin	Size (bp)	Colocated resistance gene(s)	GenBank accession no.
pKUB3007-4	*E. faecalis*, human, Japan	36,331	*fexA*	AP018547
pKUB3006-4	*E. faecalis*, human, Japan	36,331	*fexA*	AP018542
pM17/0149	*E. faecalis*, human, Ireland	36,331	*fexA*	MN831410
p6742_1	*E. faecalis*, human, Poland	36,331	*fexA*	KY513280
pEFs17-1	*E. faecalis*, animal, South Korea	36,331	*fexA*	MT223178
pN48037F-3	*E. faecalis*, pig, USA	40,269	*fexA*	CP028723
pN60443F-2	*E. faecalis*, cattle, USA	41,597	*fexA*, *erm*(B)	CP028725
pC25-1	*E. faecalis*, pig, China	45,581	*fexA*, *erm*(A)	CP030043
pAF379	*E. faecalis*, urban wastewater, Tunisia	45,603	*erm*(A), *spc*	NHNF01000009
pEF10748	*E. faecalis*, human, China	53,178	*fexA*	MK993385
pL9	*E. faecalis*, pig, Brazil	58,593	*fexA*, *tet*(S)	CP041776
pC54	*E. faecalis*, pig, China	64,500	*fexA*, *erm*(A)	CP030046
pS7316optrA	*E. faecalis*, human, Japan	68,368	*fexA*, *erm*(B), *tet*(L), *tet*(M)	LC499744
p1	*E. faecalis*, pig, China	74,536	*fexA*, *erm*(A), *spc*, copper resistance operon	MH830363
pE211	*E. faecalis*, pig, China	77,562	*fexA*	MK425644
pEF123	*E. faecalis*, chicken, China	79,682	*fexA*, *catA*, *erm*(A), *erm*(B), *aphA3*, *str*, *tet*(M), *tet*(L), *sat4*, *bcrABR*, *dfrG*	KX579977
pL15	*E. faecalis*, pig, Brazil	82,898	*catA*, *erm*(A), *erm*(B), *tet*(M), *tet*(L), *spc*, *tcr* operon, copper resistance operon	CP042214
pE508	*E. faecalis*, pig, China	84,468	*fexA*, *erm*(A), *aacA-aphD*, *tet*(L), *tet*(O/W/32/O)	MK425645
pE211-2	*E. faecalis*, pig, China	87,785	*fexA*, *erm*(A), *erm*(B), *aacA-aphD*, *aadE*, *aphA3*, *tet*(L), *tet*(M), *sat4*, *lnu*(B), *lsa*(E), *spw*, *dfrG*	MK784777
pL8	*E. faecalis*, pig, Brazil	91,525	*erm*(A), *erm*(B), *lnu*(C), *spc*	CP042217
pE035	*E. faecalis*, pig, China	121,524	*poxtA*, *fexB*, *erm*(A), *erm*(B), *aacA-aphD*, *lnu*(G), *bcrABDR*, *dfrG*	MK140641
pFSIS1608820	*E. faecium*, cattle, USA	28,222	*cfr*, *fexA*, *erm*(A), *erm*(B), *aphA3*, *spc*	CP028728
pE35048-oc	*E. faecium*, human, Italy	41,816	*cfr*, *erm*(B), Δ*lnu*(E)	MF580438
pEfmO_03	*E. faecium*, human, Ireland	58,684	*fexA*	MT261365
pF120805	*E. faecium*, human, Ireland	72,924	*cfr*, *erm*(A), *erm*(B), *aphA3*, *aadE*, *lnu*(A), *lnu*(B)	KY579372
p15-307-1_02	*E. faecium*, human, France	103,074	*cfr*(D), *erm*(A), *erm*(B)	CP044318
pM17/0314	*E. faecium*, human, Ireland	103,600	*cfr*(D), *erm*(A), *erm*(B)	MN831413
pBP5067_P1	*E. faecium*, human, India	122,126	*cfr*(D), *erm*(B), *vanA* gene cluster (*vanRSHAXYZ*)	CP059807
pBA17124_P1	*E. faecium*, human, India	130,516	*cfr*(D), *erm*(B), *vanA* gene cluster (*vanRSHAXYZ*)	CP059785
Unnamed	*E. faecium*, human, India	142,820	*erm*(B), *aadE*, *aphA3*, *tet*(S), *sat4*, *vanA* gene cluster (*vanRSHAXYZ*)	CP040238
pWo27-9	*S. sciuri*, pig, China	55,724	*cfr*, *aadD*, *ble*	KX982169
pWo28-3	*S. sciuri*, pig, China	60,563	*cfr*, *fexA*, *aadD*, *aacA-aphD*, *ble*	KT601170
pWo28-1	*S. sciuri*, pig, China	60,565	*cfr*, *fexA*, *aadD*, *aacA-aphD*, *ble*	KX982171
p2C45	*C. perfringens*, chicken, China	148,618	*fexA*, *erm*(A), *lnu*(P)	NZ_JAAQTM010000004

from a porcine *E. faecalis* in Brazil had a unique structure and harbored the additional resistance genes *fexA* and *tet*(S).

A group of five *E. faecalis* plasmids, ranging in size between 68,368 and 87,785 bp, included the plasmids pS7316optrA from a human in Japan, pEF123 from a chicken in China, p1 and pE211-2 from pigs in China, and pL15 from a pig in Brazil. These five plasmids showed limited sequence similarity to one another and carried 3 to 13 additional genes, which conferred resistance to phenicols, MLS_B antibiotics, tetracyclines, aminoglycosides, trimethoprim, pleuromutilins, and/or bacitracin (Table 5). Two of these plasmids, p1 and pL15, also had copper resistance genes. Another group of plasmids from *E. faecalis*, which shared limited nucleotide sequence similarity, ranged in size from 53,178 bp to 121,524 bp and included pEF10748 from a human in China, pE211, pE508, and pE035 from pigs in China, and pL8 from a pig in Brazil. Plasmids pEF10748 and pE211 carried only *fexA* as an additional resistance gene, while the other three larger plasmids harbored four to eight additional resistance genes, including *lnu*(C), *erm*(B), *erm*(A), and *spc* in pL8 (GenBank accession no. CP042217), *aacA-aphD*, *fexA*, *tet*(L), *tet*(O/W/32/O), and *erm*(A) in pE508 (269), and *erm*(B), *aacA-aphD*, *bcrABDR*, *erm*(A), *lnu*(G), *dfrG*, *fexB*, and *poxtA* in pE035 (174).

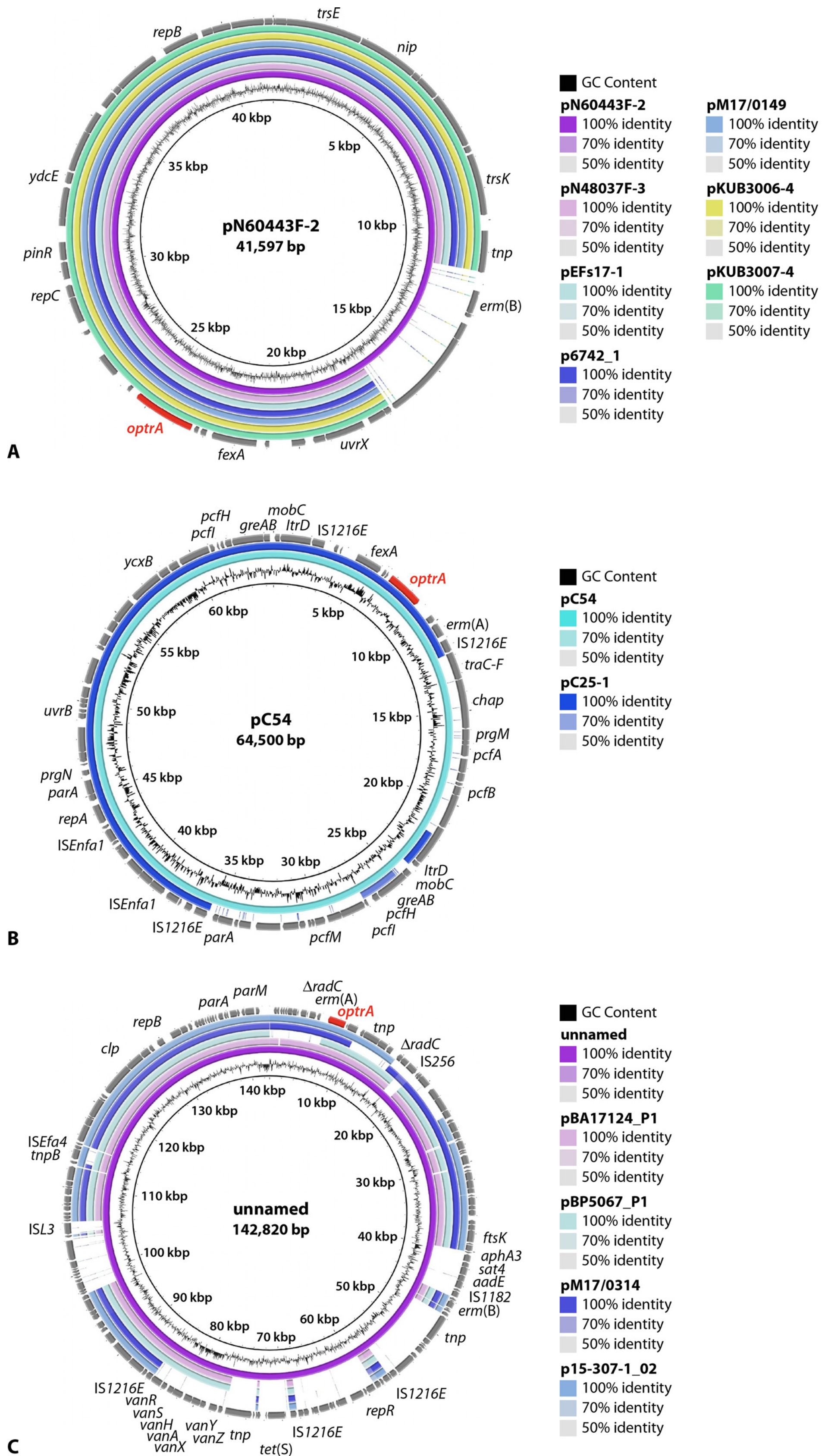

FIG 10 Structural comparison of *optrA*-carrying plasmids in enterococci (constructed by BRIG). Relevant genes with known functions and insertion elements are indicated for the respective reference plasmid in the

(Continued on next page)

The smallest completely sequenced *optrA*-harboring plasmid from *E. faecium* to date is the 28,222-bp plasmid pFSIS1608820 (183). This plasmid originated from cattle in the United States and carried the additional antimicrobial resistance genes *erm*(B), *fexA*, *erm*(A), *spc*, *aphA3*, and *cfr*. Three plasmids from human *E. faecium* isolates, ranging in size from 41,816 bp to 72,924 bp, differed in their structure and organization. These three plasmids carried distinct additional resistance genes. Plasmid pE35048-oc from Italy also harbored Δ*lnu*(E), *cfr*, and *erm*(B) (180); pEfmO_03 from Ireland only harbored *fexA* (292), while another Irish plasmid, pF120805, carried the seven additional resistance genes *cfr*, *lnu*(A), *erm*(A), *erm*(B), *aphA3*, *aadE*, and *lnu*(B) (132).

Four *optrA*-carrying plasmids from human *E. faecium*, that also harbored the oxazolidinone resistance gene *cfr*(D) and ranged in size from 103,074 bp to 130,516 bp, included plasmids pM17/0314 from Ireland (239), pBP5067_P1 (GenBank accession no. CP059807) and pBA17124_P1 (GenBank accession no. CP059785) from India, and p15-307-1_02 from France (255). They displayed a similar structure to that of a larger *cfr*(D)-lacking unnamed plasmid (142,820 bp; GenBank accession no. CP040238) from human *E. faecium* in India (Table 5). Apart from *optrA*, all of these five plasmids had three or more additional resistance genes, namely, *erm*(A), *erm*(B), and *cfr*(D) in pM17/0314 and p15-307-1_02, the *vanA* gene cluster (*vanZYXAHSR*), *erm*(B), and *cfr*(D) in pBA17124_P1 and pBP5067_P1, and the *vanA* gene cluster (*vanZYXAHSR*), *erm*(B), *aphA3*, *sat4*, *aadE*, and *tet*(S) in the unnamed plasmid (Fig. 10C).

Numerous partial sequences of *optrA*-carrying plasmids from *Enterococcus* spp. were found by database search. They ranged in size from 2,452 to 91,477 bp, and almost all were from *E. faecalis* isolates. Four of the 19 shorter sequences of <10 kbp comprised only the *optrA* gene, including plasmids p751258 (2,452 bp; GenBank accession no. MF443378) from a human *E. faecalis* isolate in Ecuador, p539673 (3,880 bp; GenBank accession no. MF443371) and p532444 (4,026 bp; GenBank accession no. MF443370) from human *E. faecalis* isolates in China, and pL14 (7,644 bp; GenBank accession no. CP043725) from a porcine *E. faecalis* isolate in Brazil, as well as the two larger incompletely sequenced plasmids, p_optrA 15–307-1_NODE_07 (10,411 bp; GenBank accession no. PHLC01000010) and p599799 (14,437 bp; GenBank accession no. MF443373), both from human *E. faecalis* isolates in France and Thailand, respectively. In the remaining 15 shorter sequences of <10 kbp, one or two additional resistance genes were detected, such as *fexA* in p719171 (4,550 bp; GenBank accession no. MF443375) and p898246 (6,171 bp; GenBank accession no. MF443382) from human *E. faecalis* isolates in Ireland, as well as in pXM2013_42321 (6,372 bp; GenBank accession no. MH225423), p570347 (6,499 bp; GenBank accession no. MF443372) and pWHXH (6,772 bp; GenBank accession no. MH225422), all from human *E. faecalis* isolates in China. The truncated or intact *erm*(A)-like gene was present in pFX13 (6,656 bp; GenBank accession no. KT862778) and p529360 (6,399 bp; GenBank accession no. MF443369) from porcine and human *E. faecalis* isolates in China, respectively; *fexA* and *erm*(A) occurred in six plasmids, all from human *E. faecalis* isolates in China, including pXM2013_71028 (8,128 bp; GenBank accession no. MH225424), p1207_26W003 (8,128 bp; GenBank accession no. MH225416), p19677 (8,138 bp; GenBank accession no. MH225418), pZJ11066 (8,817 bp; GenBank accession no. MH225425), pSZ21494 (8,875 bp; GenBank accession no. MH225420), and p1203_10W003 (9,146 bp; GenBank accession no. MH225415). *fexA* and truncated *erm*(A)-like genes were also present in pE419 (9,676 bp; GenBank accession no. KT862777) and pM18/0173 (9,742 bp; GenBank accession no. MN831415) from human *E. faecalis* isolates in China and Ireland. Some other larger sequences of >10 kbp also comprise

FIG 10 Legend (Continued)

outer ring. The *optrA* gene is indicated in red. The innermost circle provides a size scale, while the next innermost circle shows the GC content. Other plasmids used for comparison are indicated by color-coded rings, with the reference plasmid representing the innermost colored ring. (A) pN60443F-2, pN48037F-3, pEFs17-1, p6742_1, pM17/0149, pKUB3006-4, and pKUB3007-4, and (B) pC54 and pC25-1, as well as (C) unnamed, pBA17124_P1, pBP5067_P1, pM17/0314, and p15-307-1_02.

these two genes, *fexA* and *erm*(A), separately or simultaneously. The gene *erm*(A) was present in p_optrA 13–014_NODE_03 (10,835 bp; GenBank accession no. PHKZ01000005) from a human *E. faecalis* isolate in France, while the *fexA* gene was present in pSF35 (10,130 bp; GenBank accession no. KT862779) from a chicken *E. faecalis* isolate in China, pXY17 (11,036 bp; GenBank accession no. KT862780) from porcine *E. faecalis* isolates in China, and 10 plasmids from human *E. faecalis* isolates, namely, pM17/0240 (10,551 bp; GenBank accession no. MN831414), pM18/0497 (12,562 bp; GenBank accession no. MN831419) and p839260 (15,795 bp; GenBank accession no. MF443381) from Ireland, p29462 (21,568 bp; GenBank accession no. MH225419), p1202_21W014 (21,568 bp; GenBank accession no. MH225414), pE394 (36,331 bp; GenBank accession no. KP399637) and p452115 (36,458 bp; GenBank accession no. MF443368) from China, p_optrA 16–196_NODE_02 (35,057 bp; GenBank accession no. PHLE01000003) and p973450 (72,835 bp; GenBank accession no. MF443385) from France, and p441341 (35,059 bp; GenBank accession no. MF443367) from Sweden. Both genes, *fexA* and *erm*(A), were simultaneously detected in six plasmids from human *E. faecalis* isolates, namely, p838523 (10,006 bp; GenBank accession no. MF443380) from Malaysia, p986223 (12,051 bp; GenBank accession no. MF443387) and p986247 (13,157 bp; GenBank accession no. MF443388) from Mexico, p824270 (13,262 bp; GenBank accession no. MF443379) and p912300 (13,265 bp; GenBank accession no. MF443383) from the United States, and p739884 (13,262 bp; GenBank accession no. MF443376) from China. The gene *fexA*, accompanied by a truncated *erm*(A)-like gene, was present in plasmid pM18/0906 (11,697 bp; GenBank accession no. MN831417) from human *E. faecalis* in Ireland. The plasmids pE121 (22,854 bp; GenBank accession no. KT862776) and p10-2-2 (14,349 bp; GenBank accession no. KT862775), as well as p981649 (42,438 bp; GenBank accession no. MF443386) and p743142 (68,959 bp; GenBank accession no. MF443377), from porcine and human *E. faecalis* isolates in China (including Taiwan) harbored the *fexA* gene and a truncated *erm*(A)-like gene, as well as the spectinomycin resistance gene *spc* together with *fexA* and *erm*(A), respectively. In addition, the aminoglycoside resistance gene *aph(2″)-IIIa* was identified in p687671 (41,890 bp; GenBank accession no. MF443374) from a human *E. faecalis* isolate in Panama. The genes *fexA*, *erm*(A), and *erm*(B) were detected in p956343 (91,477 bp; GenBank accession no. MF443384) from a human *E. faecalis* isolate in Guatemala. Four additional resistance genes, *erm*(B), *aacA-aphD*, *spw*, and *erm* (A), were present in the incompletely sequenced plasmid p_optrA 16–164-1_NODE_01 (16,208 bp; GenBank accession no. PHLD01000003).

Bender and coworkers described diverse *optrA* genetic environments among *E. faecalis* and *E. faecium* isolates from humans in Germany. They most frequently found the *fexA* gene upstream of *optrA*, whereas in one isolate, an *erm*(B) gene was detected at this position. In several isolates, an *erm*(A) gene was detected downstream of *optrA*. One isolate harbored an *aadE-sat4-aphA3* resistance gene cluster in the vicinity of *optrA* (256). A study by Deshpande and coworkers described the genetic environment of mostly plasmid-borne *optrA* genes in human *E. faecalis* isolates from different countries/regions (167). They found 15 in part strikingly different *optrA* regions among 23 incompletely sequenced plasmids. Most frequently the genes *fexA* and/or *araC* were detected in the vicinity of *optrA*. Single copies of the insertion sequences IS*1216E* or IS*Enfa1* were detected in only four isolates each (167). Similar results were obtained for *E. faecalis* isolates from humans and various food-producing animals in China (246).

Plasmids carrying the *optrA* gene in *Staphylococcus* spp. To date, the sequences of three completely sequenced *optrA*-carrying plasmids from staphylococci have been deposited in the databases (Table 5). All three plasmids, pWo27-9 (163), pWo28-1 (163), and pWo28-3 (162), originated from porcine *S. sciuri* isolates in China and carried the gene *cfr* as well (Fig. 4F). In addition to the genes *optrA* and *cfr*, the additional resistance genes *ble* and *aadD* (pWo27-9), as well as *ble*, *aadD*, *aacA-aphD*, and *fexA* (pWo28-1 and pWo28-3) were present (Fig. 4F). The genetic environment of the plasmid-borne *optrA* and *cfr* genes was very similar to that found in the incompletely sequenced plasmid pWo35-20 (GenBank accession no. KX982166), also from a porcine *S. sciuri* isolate in China (163). Several other plasmids from porcine methicillin-resistant *S. sciuri* isolates in China were identified as carrying the *optrA* gene (163).

Plasmids carrying the *optrA* gene in other Gram-positive bacteria. Only one *optrA*-carrying plasmid, p2C45, from *C. perfringens* was found in the databases (Table 5). This plasmid had a size of 148,618 bp, originated from chicken in China, and carried the additional resistance genes *fexA*, *erm*(A), and *lnu*(P) (289). The segment carrying the *optrA* gene, with its downstream *erm*(A), the ferredoxin-encoding gene *fer*, a gene for a hypothetical protein, and IS*1216E* elements at both termini, displayed 99.9% nucleotide sequence identity to the corresponding region in the aforementioned plasmid pE508 from porcine *E. faecalis* isolates in China (289).

Transposons, integrative and conjugative elements, and prophages carrying the *optrA* gene. The 16,350-bp transposon Tn*6823* from *S. aureus* isolates of chicken origin in China is a variant of transposon Tn*558* and consists of the three transposase genes *tnpA*, *tnpB*, and *tnpC*, *orf138*, and the phenicol resistance gene *fexA*. An additional eight genes were inserted into the Tn*558* backbone, namely, four genes for hypothetical proteins *hp1* to *hp4*, the topoisomerase gene *top*, the mobilization gene *mob*, the transcriptional regulator gene *araC* and the *optrA* gene (293). Almost identical Tn*6823* sequences were present in the chromosomal DNA of a porcine *S. sciuri* isolate (GenBank accession no. KX447572) and a human *E. avium* isolate (GenBank accession no. MH018573), both from China. All of these Tn*6823* elements were integrated into the chromosomal *radC* gene coding for a DNA repair protein (293).

Another novel chromosome-borne *optrA*-carrying transposon, designated Tn*6674*, was found in a porcine *E. faecalis* isolate in China (270). Tn*6674* has a size of 12,932 bp (GenBank accession no. MK737778). As a Tn*554* derivative, it carries the transposase genes *tnpA*, *tnpB*, and *tnpC* and the Tn*554*-associated resistance genes *spc* and *erm*(A). In addition, Tn*6674* also harbored the resistance genes *fexA* and *optrA*. Like Tn*6823*, Tn*6674* was also inserted into the chromosomal *radC* gene (270). Circular forms of Tn*6674* were detected by PCR, suggesting that this transposon is functionally active (270). Transposon Tn*6674* was also found in the chromosomal DNA of the *E. faecalis* isolates A101 (GenBank accession no. MH018572), TZ2 (GenBank accession no. MH225421), EF294 (GenBank accession no. QDDM01000007), 33710 (GenBank accession no. QNHF01000012), and 743142 (GenBank accession no. MF443377) from humans in China (including Taiwan), and in *E. faecalis* Efl-952 from a human isolate in Greece (257).

The sequence of a third *optrA*-carrying transposon, designated Tn*6261*, from porcine *E. faecalis* in China is only available as a database entry (GenBank accession no. KU354267). This transposon is 8,886 bp in size and harbors Tn*558*-like *tnpA* and *tnpB* genes, the *erm*(A) gene, and a gene coding for an SAM-dependent methyltransferase, in addition to *optrA*. Tn*6261* was also integrated in the chromosomal *radC* gene of *E. faecalis*.

Two ICEs of the ICE*Sa*2603 family that carry the *optrA* gene have so far been identified in porcine *S. suis* isolates from China (287). ICE*Ssu*SC216 had a size of 53,020 bp and carried the additional antimicrobial resistance genes *aadD*, *erm*(B) (two copies), and *tet*(O). In contrast, the tandem ICE*Ssu*SC317 was 103,324 bp in size and harbored the tetracycline resistance genes *tet*(L) and *tet*(O), in addition to *optrA* (287).

The *optrA*-bearing prophage ΦSC181 had a size of 54,771 bp and carried the additional antimicrobial resistance genes *mef*(A), *aacA-aphD*, and *cat*. It also originated from a porcine *S. suis* isolate in China (287).

Insertion sequences generating *optrA*-carrying translocatable units. In contrast to the situation with the *cfr* gene, only three insertion sequences, namely IS*1216E*, IS*Efa15*, and IS*Chh1-like*, have been identified to bracket the gene *optrA* (Fig. 11).

(i) IS*1216E-optrA*-IS*1216E*. Most frequently, the gene *optrA*, with or without additional genes, was found to be bracketed by two identical IS*1216E* copies in the same orientation. When these IS*1216E* copies recombine, a TU is generated, which then can integrate into a plasmid, an ICE, or at different chromosomal sites. If integrated into a conjugative plasmid or an ICE, this may result in the dissemination of the *optrA* gene across strain, species, or even genus boundaries.

The simplest version of insertion sequences bracketing the *optrA* gene, in which only the *optrA* gene was located between the two IS*1216E* elements, was found in the

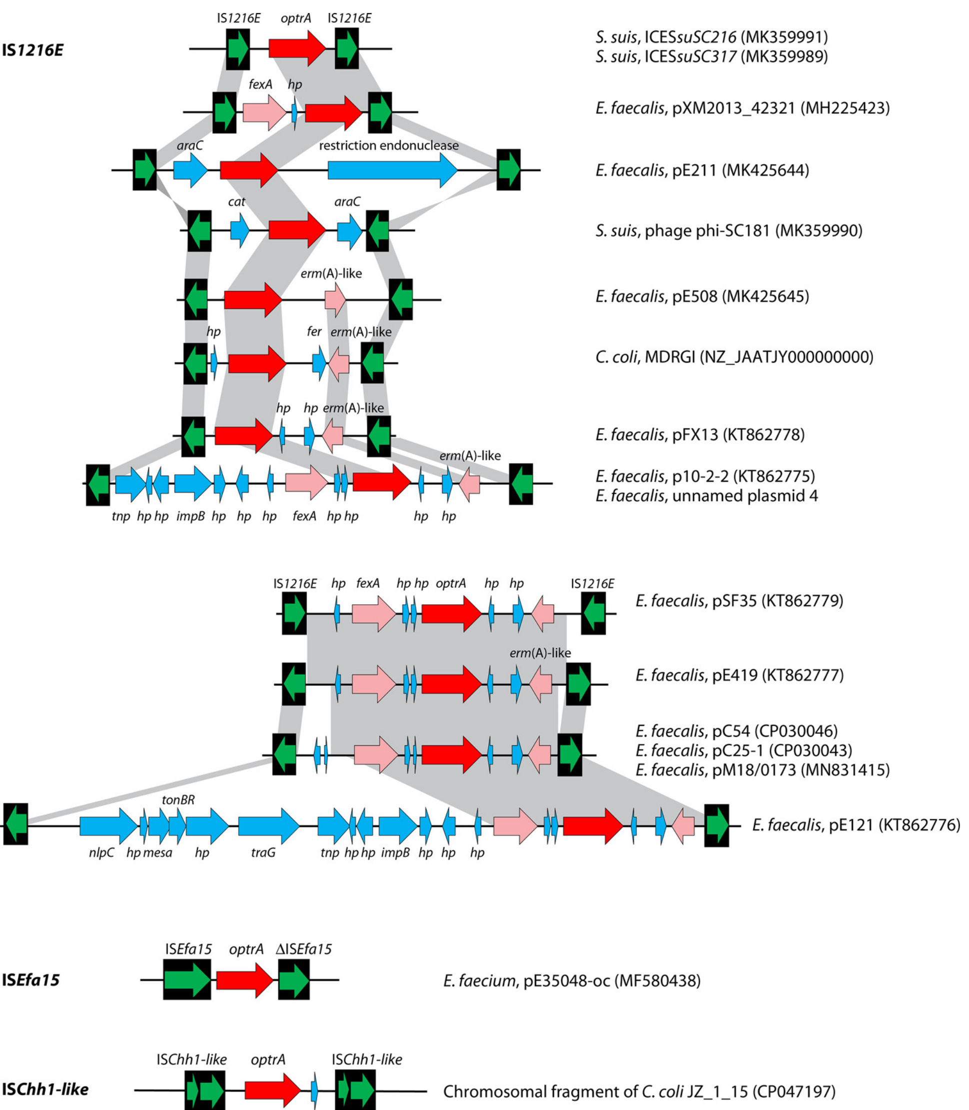

FIG 11 Insertion sequences flanking the *optrA* gene. The insertion sequences IS*1216E*, IS*Efa15*, and IS*Chh1-like* are displayed as black boxes with the green arrow(s) inside symbolizing the respective transposase gene(s). The *optrA* gene is shown as a red arrow. Additional resistance genes, such as *fexA* and *erm*(A)-like, are displayed as rose arrows, while other genes are shown as blue arrows. In all cases, the arrowhead indicates the direction of transcription. Whenever direct repeats were identified at the termini of the IS elements that flank the *cfr* region, they are indicated in boxes. The gray-shaded area indicates >99% nucleotide sequence identity. For each specific IS-*cfr*-IS arrangement, the bacterial species, the location (plasmid/integrative and conjugative element [ICE]/chromosomal multidrug resistance genomic island [MDRGI]) and the database accession number (in brackets) are given on the righthand side.

ICEs ICE*SsuSC216* (GenBank accession no. MK359991) and ICE*SsuSC317* (GenBank accession no. MK359989), both from porcine *S. suis* isolates in China (287). However, more complex arrangements usually carried two or more genes in addition to *optrA* and the two IS*1216E* elements. These genes included the resistance genes *erm*(A)-like, *cat*, and *fexA*; the transposase gene *tnp*; the DNA-directed DNA polymerase gene *impB*; the transcriptional regulator gene *araC*; the tyrosine kinase gene *fer*; and *hp* genes for hypothetical proteins. In plasmid pE508 (GenBank accession no. MK425645) from porcine *E. faecalis* in China, the array IS*1216E-optrA-erm*(A)-like-IS*1216E* was detected, whereas the array IS*1216E-fexA-hp-optrA*-IS*1216E* was present in plasmid pXM2013_42321 (GenBank accession no. MH225423) from a human *E. faecalis* isolate in China. The same arrangement was seen in three incompletely sequenced plasmids from canine

E. faecalis isolates in China (164). In other incompletely sequenced plasmids from *E. faecalis* originating from dogs, pork, or vegetables in China, a central region comprising the genes *hp-fexA-hp-optrA-hp-hp-hp-erm*(A) was bracketed by IS*1216E* elements in the same and in opposite orientations (164). The array IS*1216E-cat-optrA-araC*-IS*1216E* was identified in the prophage ΦSC181 from *S. suis* (287). Other arrays included IS*1216E-araC-optrA-Eco57I*-IS*1216E*, which also contained the gene for a restriction endonuclease, in plasmid pE211 (GenBank accession no. MK425644) and IS*1216E-optrA-hp-hp-erm*(A)-like-IS*1216E* in pFX13 (GenBank accession no. KT862778), both from porcine *E. faecalis* isolates in China. In a chromosomal MDRGI from *C. coli* (GenBank accession no. NZ_JAATJY000000000), the *optrA* gene was found to be embedded into the array IS*1216E-hp-optrA-fer-erm*(A)-like-IS*1216E* (290). The most complex plasmid-borne array, IS*1216E-tnp-hp-hp-impB-hp-hp-hp-fexA-hp-hp-optrA-hp-hp-erm*(A)-like-IS*1216E*, was present in plasmid p10-2-2 (GenBank accession no. KT862775) from a porcine *E. faecalis* isolate in China and in an unnamed plasmid of *E. faecalis* isolate 4 from a human in Spain (261), while a similar array, IS*1216E-tnp-hp-hp-impB-hp-hp-hp-fexA-hp-hp-optrA-hp-hp-erm*(A)-like-IS*1216E*, was found on a not further specified plasmid from a human *E. faecalis* isolate in Mexico (167). In *C. coli*, the array IS*1216E-tnp-hp-fexA-hp-optrA*-IS*1216E* was detected in the chromosomal DNA, and the formation of a TU, that comprised one IS*1216E* and the genes located between the two IS*1216E* copies, was confirmed (291).

There are also some arrangements in which the *optrA* gene with additional genes was bracketed by two identical IS*1216E* copies in opposite orientations. All of these arrays contained the segment *fexA-hp-hp-optrA-hp-hp-erm*(A)-like. Moreover, the arrays in plasmids pE419 (GenBank accession no. KT862777) from human *E. faecalis* and pSF35 (GenBank accession no. KT862779) from chicken *E. faecalis* isolates in China were closely related, except for the opposite orientation of IS*1216E* (246). The array IS*1216E-hp-hp-fexA-hp-hp-optrA-hp-hp-erm*(A)-like-IS*1216E* showed a very high similarity in plasmids pC54 (GenBank accession no. CP030046) and pC25-1 (GenBank accession no. CP030043) from porcine *E. faecalis* in China (266) and pM18/0173 (GenBank accession no. MN831415) from human *E. faecalis* in Ireland (239). The most complex array, IS*1216E-nlpC-hp-mesa-tonBR-hp-traG-tnp-hp-hp-impB-hp-hp-hp-fexA-hp-hp-optrA-hp-hp-erm*(A)-like-IS*1216E*, was present in plasmid pE121 (GenBank accession no. KT862776) from a human *E. faecalis* isolate in China (246).

(ii) IS*Efa15-optrA*-ΔIS*Efa15*. Apart from IS*1216E*, the insertion sequence IS*Efa15* was also shown to bracket the *optrA* gene in the array IS*Efa15-optrA*-ΔIS*Efa15* in plasmid pE35048-oc (GenBank accession no. MF580438) from a human *E. faecium* isolate in Italy (180). In the corresponding study, the formation of a TU comprising *optrA* and the ΔIS*Efa15* was confirmed (180). It should be noted that this IS*Efa15-optrA*-ΔIS*Efa15* segment has also been referred to as Tn*6628* (214).

(iii) IS*Chh1-like-optrA*-IS*Chh1-like*. Insertion sequences, designated IS*Chh1-like*, have been found to bracket the *optrA* gene in porcine *C. coli* isolates from China. The sizes of the IS*Chh1-like* flanked structures in different *C. coli* isolates varied between 6,802 and 6,807 bp (291). The formation of a TU, which might arise from the recombination of the IS*Chh1-like* elements upstream and downstream of *optrA*, could not be confirmed (291).

Chromosomal *optrA* Genes

The aforementioned *optrA*-carrying transposons, ICEs, and prophages are all integrated into the chromosomal DNA of the corresponding *Enterococcus* or *Staphylococcus* isolates. All three transposons had integrated into the *radC* gene. The ICE*SsuSC216* was inserted at the *rplL* locus. Upon integration, it generated perfect 15-bp direct TSDs at its termini (5′-TTATTTAAGAGTAAC-3′). The integration site for ICE*SsuSC317* was at the *rumA* locus. This ICE produced imperfect 14-bp direct TSDs at both termini (5′-CACATAGAAGTTGT-3′ [right terminus] and 5′-CACGTGGAGACGGT-3′ [left terminus]) (287). In *S. suis*, both loci—*rplL* and *rumA*—are well-known insertion hot spots of MGEs, including prophages and ICEs (294, 295). The *optrA*-carrying prophage ΦSC181 was also identified in the chromosomal DNA of a porcine *S. suis* isolate in China (287). It was

located at the *rumA* locus and produced imperfect 14-bp direct TSDs upon integration (5′-CACATAGAAGTTGT-3′ [right terminus] and 5′-CACGTGGAGTGTGT-3′ [left terminus]).

Several database entries (GenBank accession no. RXOX01000014, RHVS01000013, RHWF01000013, and RHVZ01000013, among others) identified the *optrA* gene in the chromosomal DNA of *E. faecalis* isolates from different sites in hospitals in Pakistan, including washroom sinks, bedside lights, nurse call button, and bedside rails. In all of these sequences, which ranged from 55,162 bp to 57,753 bp, only the *fexA* gene was present as an additional resistance gene. Moreover, the *optrA* gene was also found in the chromosomal DNA of eight *E. faecalis* isolates from animals and humans in China (246). The contigs harboring the *optrA* gene ranged in size from 6,088 bp to 29,141 bp. All but one (contig of isolate E079) of these contigs also harbored one complete additional antimicrobial resistance gene in the vicinity of the *optrA* gene. The four contigs from human isolates E147 and E381, as well as porcine isolates 5-7 and 10-120, carried the additional resistance gene *fexA*, while the contig from the chicken isolate LY4 carried the *erm*(A)-like gene. The remaining contigs from the porcine isolate G20 and the human isolate E016 harbored a truncated *erm*(A)-like gene and a complete Tn*558* transposon that included the *fexA* gene (246). In *E. faecalis* isolates from dogs and raw food (egg, beef, pork, and chicken meat) in China, the chromosomal *optrA* region occasionally identified the *erm*(A) gene downstream of *optrA*, whereas the complete *fex*(A)-carrying transposon Tn*558* was commonly found upstream of *optrA* (164). In a few cases, only the *fexA* gene without the remaining parts of Tn*558* was present, and a complete Tn*554* that included the *erm*(A) and *spc* genes was present upstream of the *optrA* and *fexA* genes (164). In *E. casseliflavus* isolates from beef and chicken meat in China, a complete Tn*558* was found in the vicinity of the *optrA* gene (164). In the latter two studies (164, 246), plasmid-borne *optrA* genes were also investigated for their genetic environment. The examples presented clearly showed that the genetic environments of the chromosomal *optrA* genes in *E. faecalis* differed distinctly from those on plasmids (164, 246).

The chromosomal *optrA* genes in *S. sciuri* and *S. simulans* isolates from dogs, cats, and pigs in China often showed the *optrA* gene with its upstream *araC* gene in close proximity to complete or truncated Tn*558* elements (164).

In *C. coli* isolates from ducks and chickens in China, the *optrA* genes were located within chromosomal MDRGIs (290). One MDRGI, with a size of 14,592 bp (GenBank accession no. NZ_JAATKE000000000), was inserted into the *C. coli* housekeeping gene *YSU_03710*, which codes for an acetyltransferase, and carried the additional resistance genes *fexA*, *tet*(O), *tet*(L), *spc*, and *aadE*. The other MDRGI (GenBank accession no. NZ_JAATJY000000000), which is about 3 kbp larger, had been inserted between the gene *YSU_02690* for a SAM-dependent methyltransferase and the gene *YSU_02685* for a hypothetical protein. This MDRGI harbored the additional resistance genes *tet*(O), *tet*(L), *catA*, and *erm*(A)-like (290). In a porcine *C. coli* isolate from China, the *optrA* gene was located in a chromosomal MDRGI composed of the IS*1216E*-*optrA*-*fexA*-*tnp*-IS*1216E* segment and the additional resistance genes *aadE*, *sat4*, and *aphA3* (291).

Variants of OptrA

The wild-type *optrA* gene, as identified in *E. faecalis* and *E. faecium*, is widely spread among *E. faecalis*, *E. faecium*, and *S. suis* isolates. However, since its first description, at least 69 variants of the *optrA* gene, which differed by 1 to 20 aa in their deduced OptrA sequences, have been detected (Table 6). This corresponds to an amino acid identity of 97.1 to 99.8% compared with the wild-type OptrA. Most frequently, amino acid substitutions at positions 176 (Y176D), 393 (G393D), 3 (K3E), and 40 (G40D) were observed (Table 6). In some studies, the MIC values for linezolid (and tedizolid) of the isolates carrying the different OptrA variants were determined (153, 164, 248, 249, 256). The comparison of the MIC values with the associated OptrA variants suggested that the different OptrA variants might have an impact on the relative oxazolidinone susceptibility/resistance of the respective isolates. Thus, the variants D, EDP, KD, KLDP, RD, RDK, and RDKP, as well as the wild-type OptrA, were commonly found in isolates that exhibited

TABLE 6 Comprehensive presentation of the OptrA variants identified to date

OptrA variant	Amino acid substitution(s)[a]	Species	GenBank accession no. or reference[b]
Wild type		*E. faecalis*	WP_063854496.1
		S. suis	WP_063854496.1
		E. faecium	WP_063854496.1
D_1	Y176**D**	*S. suis*	WP_099810410.1
		C. jejuni	WP_099810410.1
		C. coli	WP_099810410.1
		E. faecalis	WP_099810410.1
D_2	G40**D**, R239-	*S. suis*	WP_105150713.1
DC	Y176**D**, Y601**C**	*S. suis*	WP_105141008.1
DD_1	G40**D**, Y176**D**	*S. suis*	WP_105114403.1
DD_2	G40**D**, G393**D**	*S. suis*	WP_136628908.1
DD_3	Y176**D**, G393**D**	*S. suis*	WP_094467217.1
		E. faecium	WP_094467217.1
		E. faecalis	WP_094467217.1
		E. casseliflavus	WP_094467217.1
		S. sciuri	164
DD_4	Y176**D**, G394**D**	*S. suis*	WP_105209901.1
DK	Y176**D**, E256**K**	*E. faecalis*	AON96411.1
DM	Y176**D**, I622**M**	*E. faecalis*	164
		E. casseliflavus	164
DP	G40**D**, T481**P**	*S. suis*	WP_105138726.1
DP_2	Y176**D**, T481**P**	*S. suis*	WP_099809080.1
		E. faecalis	WP_099809080.1
		Enterococcaceae	QBA99765.1
DS	Y176**D**, G394**S**	*E. faecalis*	248
DDD	G40**D**, Y176**D**, G393**D**	*S. suis*	WP_050572105.1
		E. faecium	WP_050572105.1
DDM	Y176**D**, G393**D**, I622**M**	*E. faecium*	WP_002360182.1
DDP	G40**D**, Y176**D**, T481**P**	*S. suis*	WP_105116489.1
DDP_2	G40**D**, G393**D**, T481**P**	*E. faecalis*	WP_002415370.1
		S. suis	WP_002415370.1
DGP	Y176**D**, S411**G**, T481**P**	*S. suis*	WP_105129307.1
DVD	Y176**D**, I235**V**, G393**D**	*S. suis*	QEM40870.1
DVD_2	Y176**D**, A350**V**, G393**D**	*E. faecalis*	WP_141422915.1
		E. faecium	WP_141422915.1
DDKD	G40**D**, Y176**D**, I287**K**, G393**D**	*S. suis*	WP_170243993.1
DDTD	G40**D**, Y176**D**, P179**T**, G393**D**	*E. faecalis*	248
DNDM	Y176**D**, D247**N**, G393**D**, I622**M**	*S. sciuri*	164
DRDK	G40**D**, I104**R**, Y176**D**, E256**K**	*S. suis*	WP_105157283.1
DDKDP	G40**D**, Y176**D**, E290**K**, G393**D**, T481**P**	*S. suis*	WP_105095882.1
E	K3**E**	*E. faecalis*	QCC21367.1
E_2	D401**E**	*E. faecalis*	WP_172694219.1
ED	K3**E**, Y176**D**	*E. faecalis*	WP_078122664.1
		E. faecium	NTR32945.1
ED_2	K3**E**, G393**D**	*Enterococcaceae*	QBA99711.1
EDD	K3**E**, Y176**D**, G393**D**	*E. faecalis*	WP_078122475.1
		E. gallinarum	WP_078122475.1
		S. sciuri	AOQ25869.1
		E. faecium	QCX35246.1
EYD	K3**E**, N12**Y**, Y176**D**	*S. suis*	WP_050571857.1
EDM	K3**E**, Y176**D**, I622**M**	*E. faecalis*	WP_089202004.1
		E. faecium	WP_089202004.1
EDP	K3**E**, Y176**D**, T481**P**	*E. faecium*	WP_128704351.1
		E. faecalis	RXF20311.1
EDDD	K3**E**, G40**D**, Y176**D**, G393**D**	*S. suis*	WP_050571447.1
EDDD_2	K3**E**, G87**D**, Y176**D**, G393**D**	*E. faecalis*	NSO88909.1
EDDM	K3**E**, Y176**D**, G393**D**, I622**M**	*E. faecalis*	WP_153246992.1
EDVD	K3**E**, Y176**D**, I235**V**, G393**D**	*E. faecium*	248
EYDD	K3**E**, N12**Y**, Y176**D**, G393**D**	*S. suis*	WP_129406995.1
		S. sciuri	WP_129406995.1
		Salmonella sp.	RXY94784.1

(Continued on next page)

TABLE 6 (Continued)

OptrA variant	Amino acid substitution(s)[a]	Species	GenBank accession no. or reference[b]
EYDE	K3**E**, N12**Y**, G40**D**, Y176**E**	*S. suis*	WP_105142857.1
EYDM	K3**E**, N12**Y**, Y176**D**, I622**M**	*E. faecium*	248
EYDP	K3**E**, N12**Y**, G40**D**, T481**P**	*S. suis*	WP_105096713.1
EYDDD	K3**E**, N12**Y**, G40**D**, Y176**D**, G393**D**	*E. faecium*	WP_131648058.1
		S. suis phage ΦSC181	QEM40833.1
EYDDK	K3**E**, N12**Y**, Y176**D**, G393**D**, E583**K**	*S. sciuri*	153
EYDND	K3**E**, N12**Y**, Y176**D**, D247**N**, G393**D**	*S. simulans*	AVE17190.1
EYDRC	K3**E**, N12**Y**, G40**D**, K130**R**, Y135**C**	*S. suis*	WP_105119002.1
EYDNDM	K3**E**, N12**Y**, Y176**D**, D247**N**, G393**D**, I622**M**	*S. aureus*	WP_159314661.1
		S. sciuri	WP_159314661.1
		E. faecalis	AON96416.1
		E. avium	AXM43510.1
EYDVDM	K3**E**, N12**Y**, Y176**D**, I235**V**, G393**D**, I622**M**	*S. suis*	WP_105182874.1
EYDNKDM	K3**E**, N12**Y**, Y176**D**, D247**N**, Q310**K**, G393**D**, I622**M**	*L. monocytogenes*	EEX0182872.1
EYDDNDGPM	K3**E**, N12**Y**, G40**D**, Y176**D**, D247**N**, G393**D**, S411**G**, T481**P**, I622**M**	*E. faecalis*	WP_131639407.1
EDELYNKQLEIG	K3**E**, Y176**D**, Q541**E**, M552**L**, N560**Y**, K562**N**, Q565**K**, E614**Q**, I627**L**, D633**E**, N640**I**, R650**G**	*E. faecium*	WP_125276231.1
EYKKCDVASKELYNKQLEIG	K3**E**, N12**Y**, E37**K**, N122**K**, Y135**C**, Y176**D**, A350**V**, V395**A**, A396**S**, Q509**K**, Q541**E**, M552**L**, N560**Y**, K562**N**, Q565**K**, E614**Q**, I627**L**, D633**E**, N640**I**, R650**G**	*E. faecalis*	256
EYKWDVKELYNKQLEIG	K3**E**, N12**Y**, N122**K**, Y135**W**, Y176**D**, A350**V**, Q509**K**, Q541**E**, M552**L**, N560**Y**, K562**N**, Q565**K**, E614**Q**, I627**L**, D633**E**, N640**I**, R650**G**	*E. faecium*	WP_181727040.1
EYKWDVDASKELYNKQLEIG	K3**E**, N12**Y**, N122**K**, Y135**W**, Y176**D**, A350**V**, G393**D**, V395**A**, A396**S**, Q509**K**, Q541**E**, M552**L**, N560**Y**, K562**N**, Q565**K**, E614**Q**, I627**L**, D633**E**, N640**I**, R650**G**	*E. faecium*	WP_173495098.1
H	Q219**H**	*E. faecalis*	WP_138807048.1
I	T572**I**	*E. faecalis*	AWH59008.1
K	I287**K**	*S. suis*	WP_105126734.1
KD	T112**K**, Y176**D**	*E. faecalis*	WP_080477306.1
		S. suis	WP_080477306.1
KDP	T112**K**, Y176**D**, T481**P**	*E. faecalis*	WP_126267515.1
KDTP	T112**K**, Y176**D**, P179**T**, T481**P**	*E. faecalis*	248
KLDP	T112**K**, S147**L**, Y176**D**, T481**P**	*E. faecium*	248
KDDGP	T112**K**, Y176**D**, G393**D**, S411**G**, T481**P**	*S. suis*	WP_105120738.1
KDKGP	T112**K**, Y176**D**, E290**K**, S411**G**, T481**P**	*Fusobacterium* sp.	WP_187422904.1
P	T481**P**	*C. perfringens*	WP_170876513.1
		C. coli	WP_170876513.1
RD	I104**R**, Y176**D**	*S. suis*	WP_105145462.1
		E. faecalis	164
RDK	I104**R**, Y176**D**, E256**K**	*E. faecalis*	WP_105108188.1
		E. faecium	WP_105108188.1
		S. suis	WP_105108188.1
RDKP	I104**R**, Y176**D**, E256**K**, T481**P**	*S. suis*	WP_105134398.1
RDKGP	I104**R**, Y176**D**, E256**K**, S411**G**, T481**P**	*S. suis*	WP_105110522.1
SDDP	A27**S**, G40**D**, G393**D**, T481**P**	*S. gallolyticus* subsp. *pasteurianus*	ATM29806.1
T	A13**T**	*S. suis*	WP_099876735.1
YDD	N12**Y**, Y176**D**, G393**D**	*S. sciuri*	164

[a]Substituted amino acids are shown in bold and underlined; the hyphen in variant D_2 indicates that the respective amino acid is deleted.
[b]For every species in which the respective OptrA variant was detected, only one representative protein sequence is indicated. In cases where no OptrA sequences have been deposited in the databases, the publication that describes the respective OptrA variant is given.

high linezolid MIC values of ≥8 mg/liter, whereas the variants DDTD, EYDM, EYDDK, EYDNDM, and KDTP have usually been found in isolates that exhibited low linezolid MICs of ≤2 mg/liter (153, 164, 248, 249, 256). However, several OptrA variants were also found to be associated with variable linezolid MICs (153, 164, 248, 249), which

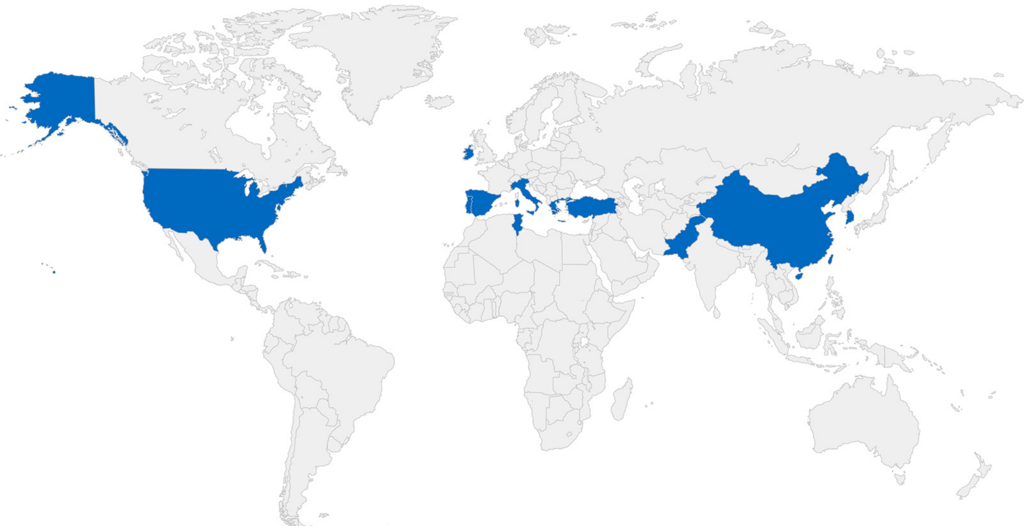

FIG 12 Geographical distribution of *poxtA*-carrying bacteria. The countries in blue are those from which the occurrence of *poxtA*-carrying bacteria has been reported.

suggests that not only the OptrA protein but also additional factors may account for the linezolid MIC.

MOBILE OXAZOLIDINONE RESISTANCE GENE *poxtA*

Geographical Distribution and Host Bacteria of the *poxtA* Gene

According to the PubMed and NCBI Nucleotide databases, the gene *poxtA* is present in 11 countries on four continents (Fig. 12). In most of these countries *cfr*- and/or *optrA*-carrying bacteria have also been detected. The host bacteria carrying the *poxtA* gene are so far exclusively *Enterococcus* spp. and *Staphylococcus* spp.

The *poxtA* gene was first described in a MRSA isolate of clinical origin in Italy in 2018 (56, 214). However, this gene was more frequently reported in *E. faecium* isolates obtained from humans in Greece (280), Ireland (239), Pakistan (281), Portugal (296), Spain (262, 296), Turkey (264), and the United States (264, 281), from pigs in China (297) and Italy (174, 298), from cattle in Spain (296), from air samples of a pig farm in Spain (283), from cattle, chicken, and ducks in South Korea (299), and from milk, retail meat, and food-producing animals in Tunisia (273, 296). Moreover, the *poxtA* gene was detected in *E. faecalis* isolates from humans in Ireland (239) and Spain (262), and also from chickens and ducks in South Korea (299). In addition, studies in China and Italy reported that the *poxtA* gene was detected in *E. hirae* isolates from pigs (174, 209, 300). Database searches also identified the *poxtA* gene in the whole-genome sequence of the *Pediococcus acidilactici* isolate BCC1, which was obtained from a chicken cecum sample in China (GenBank accession no. CP018763).

Mobile Genetic Elements That Are Involved in the Dissemination of the *poxtA* Gene

Plasmids carrying the *poxtA* gene in *Enterococcus* spp. Plasmids seem to play an important role in the dissemination of *poxtA* among enterococci. So far, *poxtA*-carrying plasmids have only been described in the three enterococcal species *E. faecalis*, *E. faecium*, and *E. hirae* (Table 7). Based on the transfer characteristics of the plasmids in enterococci, they are usually classified as (i) pheromone-responding plasmids, (ii) the pMG1 family, (iii) the Inc18 family, or (iv) the mobilizable plasmids (301). Except for the pMG1 family, *poxtA*-carrying plasmids have been detected in the following three types of plasmids: pheromone-responding plasmids (e.g., pE035), Inc18 family plasmids (e.g., pC27-2), and mobilizable plasmids (e.g., pE1077-23).

In *E. faecalis*, the complete sequences of four *poxtA*-carrying plasmids have been deposited in the databases (Table 7). Their sizes ranged from 18,280 bp to 121,524 bp.

TABLE 7 Characteristics of completely sequenced *poxtA*-carrying plasmids in enterococci

Plasmid	Origin	Size (bp)	Colocated resistance gene(s)	GenBank accession no.
pM18/0011	*E. faecalis*, human, Ireland	18,280		MN831412
pE076	*E. faecalis*, pig, China	19,832	*fexB*	MK140642
pC10	*E. faecalis*, pig, China	37,990	*fexB*, *tet*(M), *tet*(L), *cat*	MK861852
pE035	*E. faecalis*, pig, China	121,524	*erm*(B), *aacA-aphD*, *bcrABDR*, *erm*(A), *lnu*(G), *dfrG*, *fexB*, *optrA*	MK140641
pM16/0594	*E. faecium*, human, Ireland	21,849	*tet*(M), *tet*(L)	MN831411
pE1077-23	*E. faecium*, pig, China	23,710		MT074684
pSDGJQ5	*E. faecium*, chicken, China	30,457		CP038175
pT-E1077-31	*E. faecium*, pig, China	31,742		MT074685
pHB2-2	*E. faecium*, chicken, China	32,169	*tet*(M), *tet*(L)	CP038165
pGZ8	*E. faecium*, pig, China	36,911	*tet*(M), *tet*(L)	CP038162
pSC3-1	*E. faecium*, chicken, China	36,802	*tet*(M), *tet*(L)	CP038167
pSCBC1	*E. faecium*, pig, China	41,082	*tet*(M), *tet*(L)	CP038169
pYN2-1	*E. faecium*, pig, China	41,394	*tet*(M), *tet*(L)	CP038173
pSDGJP3	*E. faecium*, pig, China	51,661	*tet*(M), *tet*(L), *dfrG*	CP038171
pC27-2	*E. faecium*, pig, China	62,386	*fexB, erm*(B), *aphA3*, Δ*sat4*, *aadE*, *tet*(M), *tet*(L), *czcD*, *dfrG*	MH784602
pC25-1	*E. faecium*, pig, China	67,678	*fexB, erm*(B), *aphA3*, Δ*sat4*, *aadE*, *tet*(M), *tet*(L), *czcD*, *dfrG*	MH784601
pHN11	*E. faecium*, chicken, China	69,757	*fexB*, *erm*(B), *aphA3*, Δ*sat4*, *aadE*, *tet*(M), *tet*(L), *czcD*, *dfrG*	CP038176
pHDC14-2.27K	*E. hirae*, pig, China	27,303		CP042294
pCQP3-9_2	*E. hirae*, pig, China	33,132	*erm*(B), *tet*(M), *tet*(L)	CP037957
pFas4-1	*E. hirae*, pig, China	57,267	*fexB*, *erm*(B), *tet*(M), *tet*(L), *dfrG*, *vat*(E)	MK798157
pHDC14-2.133K	*E. hirae*, pig, China	133,362	*erm*(B), *tet*(M), *tet*(L), *catA8*, *dfr*, *aacA-aphD*, *spw*, *lsa*(E), *lnu*(B), *aphA3*, *sat4*, *aadE*	CP042290
pY80	*S. haemolyticus*, pig, China	55,758	*tet*(L), *aadD*, *fexB, czcD*	CP063444

The two smaller plasmids pM18/0011 (18,280 bp) and pE076 (19,832 bp) were from *E. faecalis* isolates of human origin in Ireland (239) and from porcine *E. faecalis* isolates (268) in China. Plasmid pM18/0011 did not harbor additional resistance genes, whereas plasmid pE076 carried a *fexB* gene. The 37,990-bp plasmid pC10 was found in a porcine *E. faecalis* isolate from China and carried the additional resistance genes *fexB*, *tet*(M), *tet*(L), and *cat*. The pheromone-responding conjugative plasmid pE035 is 121,524 bp in size and harbored the three florfenicol resistance genes *poxtA*, *optrA*, and *fexB*. In addition, it also carried the MLS_B resistance genes *erm*(A) and *erm*(B), the bifunctional aminoglycoside resistance gene *aacA-aphD*, the lincosamide resistance gene *lnu*(G), the trimethoprim resistance gene *dfrG*, and the bacitracin resistance operon *bcrABDR*. It proved to be transferable, with high transfer frequencies of $4.5 \times 10^{-3} \pm 0.3 \times 10^{-3}$. Three mobile loci, including a circularizable structure containing *aacA-aphD*, a mobile *bcrABDR* locus, and a mobile *dfrG* locus, were found on this plasmid, and all proved to be active. The presence of the three mobile loci on a *poxtA*-carrying multiresistance plasmid renders this plasmid flexible. In addition, these three loci will aid in the persistence and dissemination of this plasmid among enterococci and putatively also among other Gram-positive bacteria (268).

In *E. faecium*, the sizes of the *poxtA*-carrying plasmids ranged from 21,849 to 69,757 bp. Except for the smallest plasmid, pM16/0594, which was found in an isolate of human origin in Ireland (239), all other *poxtA*-carrying plasmids were found in isolates from pigs and chickens in China (266, 267, 297). Among them, plasmids carrying the rep_{2_pRE25} replication gene, such as plasmids pC27-2 and pC25-1 (Table 7), were commonly identified in food-producing animals in China (266, 267, 297). The rep_{2_pRE25} gene is associated with the Inc18 broad-host-range plasmid family, which seems to be involved in the dissemination of *poxtA* across different Gram-positive bacterial genera and species (301). Plasmid pM16/0594 had a size of 21,849 bp and carried the additional tetracycline resistance gene *tet*(M). Another three *poxtA*-carrying plasmids, pE1077-23, pSDGJQ5, and pT-E1077-31, which ranged in size from 23,710 bp to 31,742 bp and did not harbor additional resistance genes, were detected in *E. faecium* isolates from pigs and chickens in China. Among them, the 23,710-bp mobilizable *poxtA*-carrying plasmid pE1077-23 (302) was most likely generated by the integration

of a staphylococcal Tn*6657*-like transposon into a 9,317-bp plasmid, most closely related (99.9%) to the 9,312-bp enterococcal plasmid pISMMS_VER4_p6 (GenBank accession no. CP012453). Coinciding with replicative transposition, a characteristic 8-bp duplication of the sequence 5′-TTTGATAC-3′ was formed at the target site in the plasmid. Conjugation experiments revealed that pE1077-23 can be mobilized by pE1077-217, a 217,661-bp conjugative plasmid present in the same *E. faecium* isolate (302). The six plasmids pHB2-2, pGZ8, pSC3-1, pSCBC1, pYN2-1, and pSDGJP3 ranged in size from 32,169 bp to 51,661 bp. All of them carried the additional tetracycline resistance genes *tet*(M) and *tet*(L), with pSDGJP3 also harboring the trimethoprim resistance gene *dfrG*. They had been detected in *E. faecium* isolates from pigs and chickens in China. The five smaller plasmids shared substantial similarity with the larger plasmid pSDGJP3, which also comprised the region carrying the resistance genes *poxtA*, *tet*(L), and *tet*(M) (Fig. 13A). The last group of *poxtA*-carrying plasmids from *E. faecium* included the three plasmids pC27-2, pC25-1, and pHN11. These plasmids again originated from *E. faecium* isolates of chicken or pig origin in China, and their sizes varied between 62,386 bp and 69,757 bp. They were structurally related and had the additional antimicrobial resistance genes *fexB*, *erm*(B), *aphA3*, Δ*sat4*, *aadE*, *tet*(M), *tet*(L), and *dfrG*, as well as the cobalt/zinc/cadmium resistance gene *czcD*, in common (Fig. 13B).

In *E. hirae*, *poxtA*-carrying plasmids have only been detected in porcine isolates from China (209, 300) (Table 7). The sizes of the plasmids ranged from 27,303 bp to 133,362 bp. Among them, plasmid pHDC14-2.27K was the smallest and did not carry additional antimicrobial resistance genes. In contrast, plasmid pFas4-1 was 57,267 bp in size and harbored—besides *poxtA*—not only the resistance genes *fexB*, *tet*(M), and *tet*(L), but also the streptogramin A resistance gene *vat*(E), the MLS_B resistance gene *erm*(B), and the trimethoprim resistance gene *dfrG* (209). Three mobile loci, including a mobile *poxtA* locus, a mobile *fexB* locus, and a mobile *tet*(M)-*tet*(L) locus, were identified in plasmid pFas4-1 (209). Plasmid pFas4-1 from a porcine *E. hirae* isolate shared large regions with the smaller plasmids pCQP3-9_2 (also from porcine *E. hirae*), pC10 from porcine *E. faecalis*, and pM16/0594 from human *E. faecium*. The common regions within all four plasmids included the IS*1216E-poxtA*-IS*1216E* segment, as well as the IS*1216E*-bounded *tet*(L)- and *tet*(M)-containing segment. Moreover, the IS*1216E*-bounded *fexB* segment was found in plasmids pFas4-2, pCQP3-9_2, and pC10 (Fig. 13C). With a size of 133,362 bp, plasmid pHDC14-2.133K was the largest *poxtA*-carrying plasmid from *E. hirae* detected thus far. This plasmid harbored the additional antimicrobial resistance genes *erm*(B), *tet*(M), *tet*(L), *catA8*, *aacA-aphD*, *spw*, *lsa*(E), *lnu*(B), *aphA3*, *sat4*, *aadE*, and a not further specified *dfr* gene.

Plasmids carrying the *poxtA* gene in *Staphylococcus* spp. The 55,758-bp plasmid pY80, obtained from a porcine *S. haemolyticus* isolate in China, carried the genes *czcD, fexB*, *tet*(L), and *aadD* along with *poxtA* (Table 7).

Transposons carrying the *poxtA* gene. Three transposons carrying the *poxtA* gene have been described so far. The mosaic transposon Tn*6349* from *S. aureus* carries the *poxtA* and *cfr* genes along with other resistance genes (214). The small *poxtA*- and *fexB*-carrying transposon Tn*6657* was located within Tn*6349*. Both transposons are described in detail in the section dealing with *cfr*-carrying transposons. In addition, the *poxtA* gene was located together with the gene *fexB* in an IS*1216*-flanked Tn*6246*-like element in *E. faecium* from cow milk in Tunisia (296). Whether or not this structure with the composition IS*1216-poxtA*-IS*1216-hp-hp-fexB*-IS*1216* (with all genes in the same orientation) is a real transposon or a PCT needs to be clarified. Hybridization with *poxtA* and *fexB* probes suggested a location of this structure on plasmids of approximately 30 and 100 kb, of which the 30-kb plasmid could be transferred by conjugation into *E. faecium* BM4105RF (296).

Insertion sequences generating *poxtA*-carrying translocatable units. As previously seen with the gene *optrA*, IS*1216E* elements are the insertion sequences that bracket the *poxtA* gene. Numerous different contexts have been identified and are shown in Fig. 14. The most frequently observed context shows the *poxtA* gene and four small

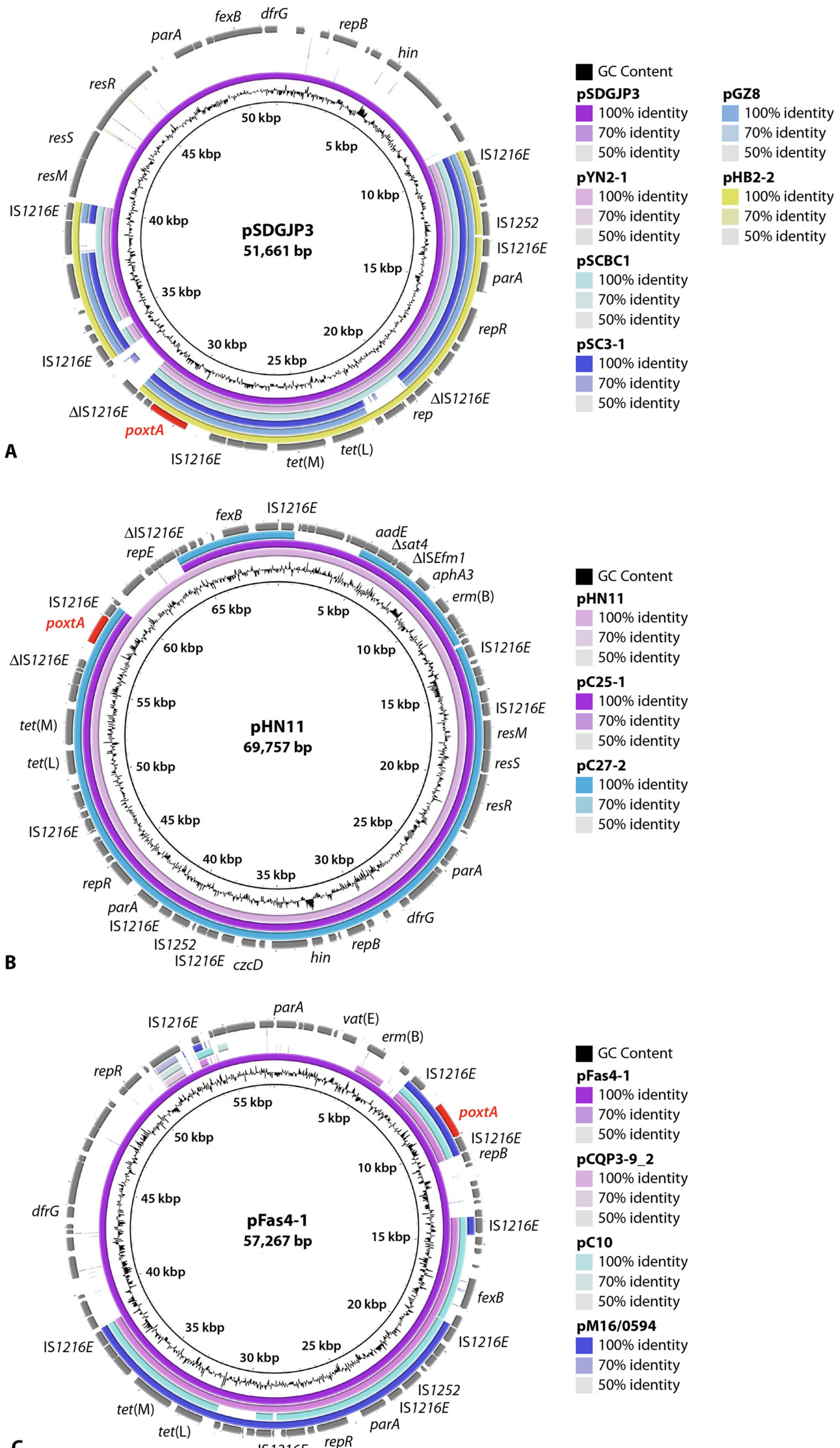

FIG 13 Structural comparison of *poxtA*-carrying plasmids in enterococci (constructed by BRIG). Relevant genes with known functions and insertion elements are indicated for the respective reference plasmid in the outer

(Continued on next page)

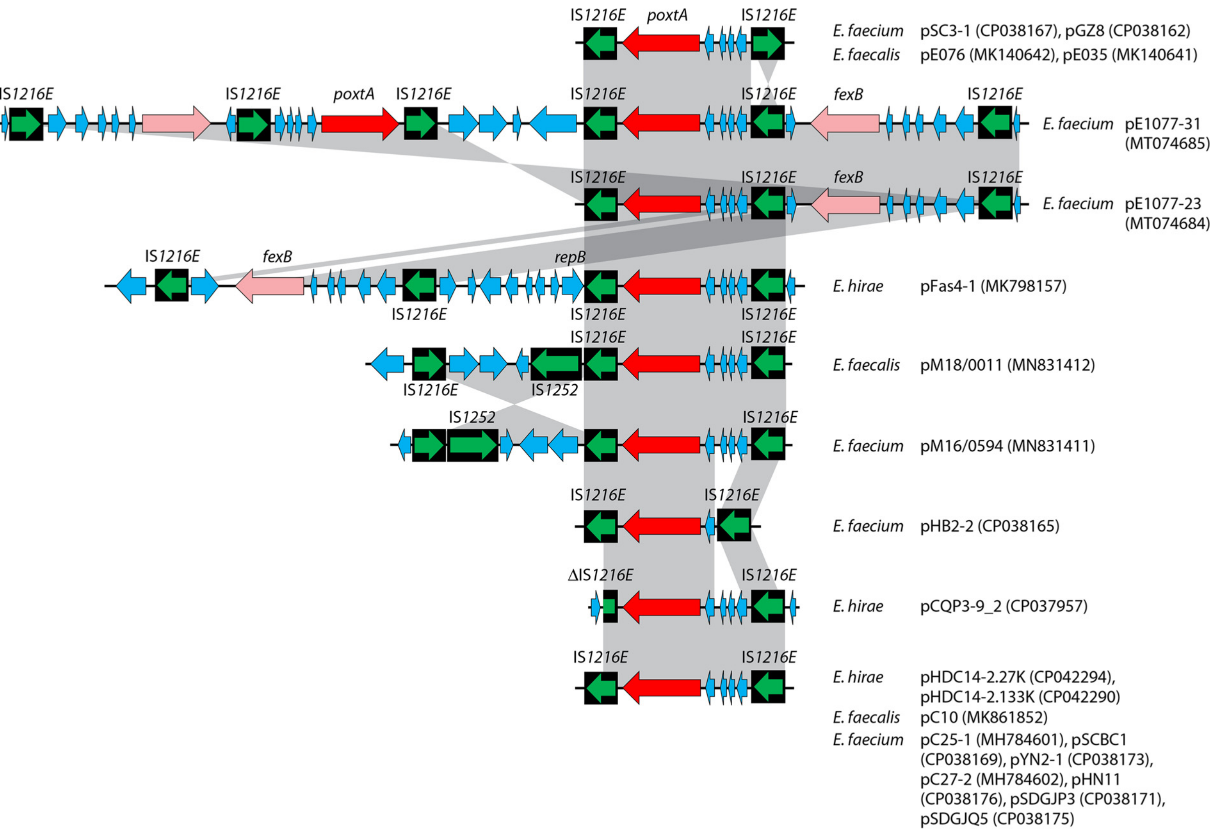

FIG 14 Insertion sequences flanking the *poxtA* gene. The insertion sequences IS*1216E* and IS*1252* are displayed as black boxes, with the green arrow inside symbolizing the respective transposase gene. The *poxtA* gene is shown as a red arrow. The additional resistance gene *fexB* is displayed as a rose arrow, while other genes are shown as blue arrows. In all cases, the arrowhead indicates the direction of transcription. The gray-shaded area indicates >99% nucleotide sequence identity. For each specific IS-*cfr*-IS arrangement, the bacterial species, the plasmid on which it is located, and the database accession number (in brackets) are given on the righthand side.

ORFs for hypothetical proteins bracketed by IS*1216E* elements in the same orientation. This arrangement was seen in all *poxtA*-carrying plasmids listed in Table 7, except in the *E. faecium* plasmid pHB2-2, where only one ORF for a hypothetical protein was present, and in the *E. hirae* plasmid pCQP3-9_2, where the IS*1216E* downstream of *poxtA* was truncated. This IS*1216E-poxtA-hp-hp-hp-hp*-IS*1216E* arrangement was also part of larger *poxtA* genetic environments, where IS*1216E*-bounded *fexB* genes were identified upstream (pE1077-23 from *E. faecium*) or downstream (pFas4-1 from *E. hirae*) of the *poxtA* region. In plasmid pT-E1077-31 from porcine *E. faecium* isolates, the entire *poxtA-fexB* region was duplicated and present in opposite orientations. In four of the plasmids, namely, pSC3-1 and pGZ8 from *E. faecium* and pE076 and pE035 from *E. faecalis*, the IS*1216E-poxtA-hp-hp-hp-hp*-IS*1216E* was also present, albeit with the two IS*1216E* elements in opposite orientations (Fig. 14). It should also be noted that the *poxtA* gene located in the whole-genome sequence of *P. acidilactici* BCC1 was bracketed by IS*1216E* elements.

The formation of a TU by the recombination of the IS*1216E* elements was confirmed for plasmid pFas4-1. Here, a TU of 3,321 bp that included the gene *poxtA* and one copy of IS*1216E* was detected (209). During conjugation experiments using *E. faecium* isolate

FIG 13 Legend (Continued)

ring. The *poxtA* gene is indicated in red. The innermost circle provides a size scale, while the next innermost circle shows the GC content. Other plasmids used for comparison are indicated by color-coded rings, with the reference plasmid representing the innermost colored ring. (A) pSDGJP3, pYN2-1, pSCBC1, pSC3-1, pGZ8, and pHB2-2, and (B) pHN11, pC25-1, and pC27-2, as well as (C) pFas4-2, pCQP3-9_2, pC10, and pM16/0594.

E1077 as a donor, a novel 31,742-bp plasmid, designated pT-E1077-31, was identified in a transconjugant. Sequence analysis indicated that pT-E1077-31 was formed by the integration of a Tn*6657*-derived, IS*1216E*-based *fexB*- and *poxtA*-carrying TU into a copy of plasmid pE1077-23 (302). These observations suggested that IS*1216E* might play a relevant role in the persistence and the dissemination of the *poxtA* gene among enterococci.

Chromosomal *poxtA* Genes

Although plasmids are the predominant *poxtA* gene carriers in enterococci, this gene has also been identified in the chromosomal DNA of the MRSA isolate AOUC-0915 of human origin in Italy (214). In this MRSA isolate, the *poxtA* gene was part of the 48-kbp transposon Tn*6349*, which was inserted into a chromosomal ΦN315-like prophage (214).

COLOCATED RESISTANCE GENES AND THEIR ROLE IN THE DISSEMINATION OF OXAZOLIDINONE RESISTANCE GENES

Since oxazolidinones are exclusively approved for therapeutic use in humans and are strictly forbidden for use in food-producing animals, the direct selection pressure imposed by the use of oxazolidinones in animals is negligible. However, all three groups of mobile oxazolidinone resistance genes, including the various *cfr* genes, *optrA*, and *poxtA*, also confer phenicol resistance. Chloramphenicol has been banned since 1994 from use in food-producing animals in the European Union (303). Other countries followed this example, and according to the FAO, most countries had banned chloramphenicol for use in food animal production by 2002 (http://www.fao.org/asiapacific/news/detail-events/fr/c/47419/). The reason for this ban was to protect consumers from possible adverse effects caused by chloramphenicol residues in food animal carcasses or products, as chloramphenicol is able to provoke an irreversible, dose-independent aplastic anemia in humans (303). In 1995, florfenicol, a fluorinated thiamphenicol derivative which does not have this side effect, was approved in the European Union for use in cattle (303). In 2000, it was also approved for use in pigs (303). In other countries, including China, florfenicol was also approved for use in fish and poultry. Thus far, florfenicol or derivatives thereof have not been approved for use in human medicine worldwide. The widespread use of florfenicol in farm animals may select for florfenicol-resistant bacteria, which also include those that carry *cfr* genes, *optrA*, and/or *poxtA*. A recent study from China showed that the presence of florfenicol residues is associated with the abundance of oxazolidinone resistance genes in livestock manures (304). Interestingly, the antimicrobial resistance genes, frequently colocated with either *cfr* genes, *optrA*, or *poxtA*, are the phenicol exporter genes *fexA* in staphylococci, *fexB* in enterococci, and *floR* in Gram-negative bacteria. The presence of two phenicol resistance genes may account for higher phenicol MICs, especially when both genes are located on the same plasmid, as shown for *cfr* and *fexA* (117).

In Tables 2 to 5 and Table 7, resistance genes that can colocate with *cfr* genes, *optrA*, or *poxtA* on plasmids are listed. These tables clearly showed that, besides phenicol resistance genes, genes coding for resistance to other frequently used classes of antimicrobial agents are also often present on plasmids carrying oxazolidinone resistance genes. In Gram-positive bacteria, these genes include (i) the MLS_B resistance genes *erm*(A), *erm*(B), *erm*(C), *erm*(T), and *erm*(33), (ii) the aminoglycoside resistance genes *aacA-aphD*, *aadD*, *aphA3*, *aadE*, and *aadY*, (iii) the tetracycline resistance genes *tet*(L), *tet*(M), *tet*(S), and *tet*(O/W/32/O), (iv) the spectinomycin resistance genes *spc* and *spw*, (v) the lincosamide resistance genes *lnu*(A), *lnu*(B), *lnu*(G), *lnu*(P), and *lsa*(E), (vi) the macrolide resistance genes *msr*(A) and *mef*(E), (vii) the streptothricin resistance gene *sat4* and the bacitracin resistance operon *bcrABR*, (viii) the vancomycin resistance *vanA* gene cluster, (ix) the bleomycin resistance gene *ble*, (x) the trimethoprim resistance gene *dfrG*, and (xi) the streptogramin A resistance gene *vat*(E). In addition, a copper resistance operon or the gene *czcD* for resistance to cobalt, zinc, and cadmium was occasionally detected on *cfr*-, *optrA*-, or *poxtA*-carrying plasmids. In Gram-negative bacteria, the colocated resistance genes

included, besides *floR*, (i) the β-lactam resistance genes $bla_{CTX-M-14b}$, bla_{OXA-10}, bla_{TEM-1}, and $bla_{TEM-176}$, (ii) the tetracycline resistance genes *tet*(A), *tet*(B), and *tet*(M), (iii) the aminoglycoside resistance genes *aacC4*, *aadA1*, *aadA2*, *aphA1*, *hph*, *strA*, and *strB*, (iv) the colistin resistance gene *mcr-1.1*, (v) the macrolide resistance genes *msr*(E) and *mph*(E), (vi) the lincosamide resistance gene *lnu*(F), (vii) the MLS_B resistance gene *erm*(B), (viii) the sulfonamide resistance genes *sul1* and *sul2*, (ix) the trimethoprim resistance gene *dfrA12*, (x) the chloramphenicol resistance gene *catB3*, (xi) the bleomycin resistance gene *ble*, and (xii) the quinolone resistance gene *qnrS1*. Occasionally, the quaternary ammonium compound resistance gene *qacE*Δ*1*, the mercury resistance operon *mer*, and the arsenic resistance operon *ars* were also detected.

The listing of all these colocated antimicrobial, biocide, and heavy metal resistance genes shows that there are manifold options for coselection of the oxazolidinone resistance genes. Several of these antimicrobial classes, such as the tetracyclines, penicillins, macrolides, sulfonamides, and trimethoprim, as well as aminoglycosides, are approved for and are widely used in veterinary medicine worldwide. The examples presented (Tables 2 to 5 and Table 7) showed that oxazolidinone resistance genes are often colocated with genes conferring resistance to antimicrobial agents, biocides and metals on the same plasmid and that *cfr*-, *optrA*-, and *poxtA*-carrying multiresistance plasmids are widespread among bacteria of animal and human origin. The same is true for transposons, ICEs, and prophages that carry oxazolidinone resistance genes. When such multiresistance MGEs are transferred to new host bacteria, all of their colocated resistance genes are transferred, too. It is important to understand that the selective pressure by the use of one selecting agent is sufficient to ensure that the bacterium does not lose the respective multiresistance MGE (305).

In summary, not only the direct selection pressure imposed by the use of florfenicol in animals and oxazolidinones in humans, but also the indirect selection pressure imposed by the use of any of the other aforementioned non-oxazolidinone antimicrobial agents in humans and animals as well as the use of heavy metals or biocides play important roles in the coselection and persistence of mobile oxazolidinone resistance genes. The concept of indirect selective pressure imposed by the use of non-oxazolidinone antibiotics is important in our understanding of how oxazolidinone resistance genes disseminate and finally end up in bacterial lineages of humans and animals. The results of monitoring and surveillance studies in combination with a detailed analysis of the respective bacteria and individual antimicrobial consumption data will tell whether the prevalence of oxazolidinone resistance genes in clinical isolates will increase in the coming years due to coselection.

MOLECULAR AND PHENOTYPIC DETECTION OF OXAZOLIDINONE RESISTANCE GENES

When a new mobile oxazolidinone resistance gene was identified, a PCR assay to specifically detect this gene was usually described as well. This was true for *cfr* (117), *cfr*(B) (48), *cfr*(C) (50), *cfr*(D) (238), *optrA* (54), and *poxtA* (298). In the meantime, numerous other PCR primers and conditions have been described for most of the aforementioned oxazolidinone resistance genes (e.g., see references 212, 259, 262, 281, 296, 298, 306, 307). Bender and coworkers developed a multiplex PCR to simultaneously detect the mobile oxazolidinone resistance genes *cfr*, *optrA*, and *poxtA* in enterococcal isolates of clinical origin (308). Hasman and colleagues developed a web tool, LRE-Finder (where LRE stands for linezolid-resistant enterococci), for the detection of the most common 23S rRNA mutations, G2576T and G2505A, and the mobile oxazolidinone resistance genes *optrA*, *cfr*, *cfr*(B), and *poxtA* in whole-genome sequences from enterococci (309). The LRE-Finder tool was validated against 21 LRE isolates and 1,473 non-LRE isolates. It showed 100% agreement with the results of phenotypic susceptibility testing (309). LRE-Finder version 1.0 is available at https://cge.cbs.dtu.dk/services/LRE-finder/.

Mobile oxazolidinone resistance genes can also be identified in whole-genome or whole-plasmid sequences when referring to the five most common antimicrobial resistance databases, including ResFinder (https://cge.cbs.dtu.dk/services/ResFinder/)

(310), the Comprehensive Antibiotic Resistance Database (CARD; https://card.mcmaster.ca/) (311), AMRFinder at the National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/pathogens/antimicrobial-resistance/AMRFinder/) (312), ARG-ANNOT (http://backup.mediterranee-infection.com/article.php?laref=282&titre=arg-annot) (313), and MEGARes (https://megares.meglab.org/) (314). All seven mobile oxazolidinone resistance genes known to date could only be identified using the ResFinder and the AMRFinder tools. CARD did not identify *cfr*(C) and *cfr*(E), while ARG-ANNOT and MEGARes missed the genes *cfr*(C), *cfr*(D), and *cfr*(E).

For phenotypic detection of linezolid- or tedizolid-resistant staphylococci, streptococci, enterococci, and corynebacteria, the clinical breakpoints from CLSI or EUCAST might be used. However, mobile oxazolidinone resistance genes also occurred in other bacteria, including the genera *Bacillus*, *Campylobacter*, *Clostridioides*, *Clostridium*, *Escherichia*, *Listeria*, *Morganella*, *Pediococcus*, *Providencia*, and *Proteus*, among others. Thus far, no clinical breakpoints have been available to reliably assess resistance or susceptibility to linezolid and tedizolid in these bacteria in phenotypic assays.

Dejoies and coworkers comparatively investigated 20 *E. faecalis* and 80 *E. faecium* isolates (including one *optrA*/*poxtA*-, 17 *poxtA*-, and 20 *optrA*-carrying isolates) for their linezolid susceptibility by broth microdilution and seven commercial methods (agar disc diffusion [Bio-Rad] and Etest [bioMérieux], two nonautomated broth assays [Sensititre and UMIC], and three automated broth assays [MicroScan WalkAway, Phoenix, and Vitek 2]) (315). Results were read after 18 and 42 h and interpreted according to CLSI and EUCAST breakpoints. Substantial variations in the results obtained with the different AST methods were found; in particular, the automated systems Phoenix and Vitek 2 did not detect several isolates classified as resistant by broth microdilution. In general, the nonautomated methods (UMIC and Sensititre) and, to a lesser extent, Etest exhibited an acceptable correlation with the broth microdilution reference method for the detection of isolates with low linezolid MICs after expanded incubation. Another two comparative studies for the detection of linezolid susceptibility provided, in part, different results (316, 317).

In a study from South Korea, 27 MRSA isolates from 14 patients were investigated for their linezolid susceptibility using the automated system Vitek 2 (bioMérieux, Marcy-l'Étoile, France) and broth microdilution according to CLSI (318). Only four isolates from the same patient were identified as resistant (MICs $\geq 8\,\mu$g/ml) by both methods, while the remaining 23 isolates were classified as resistant by Vitek 2 and as susceptible by broth microdilution (318). Molecular analysis of the 27 isolates identified the 23S rRNA mutations (T2500A in two of the five rRNA operons) only in the four resistant isolates, while none of the isolates carried the genes *cfr*, *cfr*(B), or *optrA* (318).

As screening of bacterial isolates for linezolid resistance becomes increasingly important in microbiological diagnostic laboratories, Werner and colleagues validated a screening agar for the detection of linezolid-resistant enterococci (319). The authors recommended the use of an enterococcal selective agar (e.g., Enterococcosel agar) supplemented with 2 mg/liter linezolid and an incubation period of 48 h. SuperLinezolid agar was developed and validated by Nordmann and coworkers (320). This selective culture medium was intended to screen for linezolid-resistant Gram-positive bacteria of the genera *Staphylococcus* and *Enterococcus* and contains 1.5 mg/liter linezolid as the threshold concentration. This medium can accurately detect linezolid-resistant staphylococci and enterococci after 24 h of incubation.

REVERSAL AND INHIBITION OF OXAZOLIDINONE RESISTANCE GENES

The development of inhibitors of resistance-mediating proteins, which are often small-molecular compounds that by binding to the targets destroy or block the activity of resistance-mediating proteins, is a promising approach to reverse the efficacy of antimicrobial agents in the treatment of infections caused by multidrug-resistant bacteria. For instance, the inhibitors for β-lactamases, clavulanic acid and sulbactam, could significantly prolong the life span and extend the application of β-lactams (321, 322). However, no inhibitor has been used

to reverse the resistance to oxazolidinones conferred by Cfr or OptrA. Mechanism explorations, especially structural and biochemical studies, can provide hundreds of new targets and opportunities for future drug discovery. Given that ATP hydrolysis is a characteristic requirement for ABC-F proteins, including OptrA and PoxtA, to confer resistance, inhibitors targeting ATP hydrolysis at nucleotide binding domains should be considered (323). Recently, Zhong et al. found a novel and specific inhibitor of OptrA, CP1, which suppressed the ATPase activity of OptrA *in vitro* by 30% (324). A hydrogen bond formed between the 8-position phenylcyclic cyano group in CP1, and the amino acid residue Lys-271 allows CP1 to form a stable complex with the OptrA protein (324), which impaired the ribosome protection function of OptrA. This study provided a theoretical basis for the further optimization of the inhibitor structure to obtain inhibitors with higher efficiencies. Besides, it also provided a possibility to develop inhibitors that target the ATPase centers of either ABC-F proteins or ABC efflux pumps to counteract antimicrobial resistance conferred by them (324). To date, there is no report about an inhibitor for Cfr-mediated oxazolidinone resistance, but several studies found that some *E. faecalis* (171, 172) and *S. haemolyticus* (153) isolates, were, for yet unknown reasons, susceptible to linezolid despite the fact that they harbored a complete *cfr* gene including its promoter (153, 171, 172). In one case, the authors could also show that the Cfr protein was produced and was detectable by Western blotting. In addition, the Cfr-specific methylation of A2503 was also shown, suggesting that there must be factors that are responsible for the non-PhLOPS$_A$ phenotype (171). In another study, a *cfr*(B) gene from either *C. difficile* or *E. faecium* was unable to confer linezolid resistance when cloned and transferred into different *E. faecalis* recipient strains (232). Thus, unraveling and using the mechanism of Cfr and Cfr(B) failure to confer resistance to linezolid could be an opportunity to overcome the Cfr-/Cfr(B)-mediated oxazolidinone resistance.

CONCLUSIONS AND OUTLOOK

Oxazolidinones are important antimicrobial agents for the treatment of infections caused by multidrug-resistant Gram-positive bacteria. Thus, it is of utmost relevance to preserve their efficacy for the future. During the first 20 years after introduction of linezolid into clinical use, only very low numbers of resistant bacteria have been identified in respective monitoring programs. However, despite this overall very favorable situation, resistant bacteria have been identified occasionally in samples from humans and animals. Even worse, some of these resistant bacteria harbor transferable oxazolidinone resistance genes, of which at least seven different ones have been identified. They are spread all over the world and have been identified in numerous Gram-positive, but also Gram-negative, bacteria. Surprisingly, all oxazolidinone resistance genes known so far not only confer resistance to oxazolidinones, but also to phenicols (*cfr* and its variants, as well as *optrA* and *poxtA*) and tetracyclines (*poxtA*) or lincosamides, pleuromutilins, and streptogramin A antibiotics (*cfr* and its variants). This situation offers manifold opportunities for coselection by the use of the respective antimicrobial agents. Oxazolidinones are used in human medicine, but only very rarely in companion animals and not at all in food-producing animals. In contrast, phenicols, such as chloramphenicol, play only a minor role in human medicine, whereas florfenicol is widely and exclusively used for therapeutic purposes in livestock animals worldwide. Moreover, tetracyclines are the most and second most frequently used group of antimicrobial agents—after β-lactams—in human and veterinary medicine, respectively (325, 326). As the aforementioned oxazolidinone resistance genes are circulating among and between bacteria of human, animal, environmental, and food origin, a One Health approach is needed for monitoring the emergence and transmission of these genes and the bacteria which harbor them.

In addition, the oxazolidinone resistance genes have been found on a variety of MGEs, including plasmids, transposons, ICEs, prophages, and genomic islands, in various bacteria. These MGEs, but also IS-mediated, oxazolidinone resistance gene-carrying TUs, which can integrate into the aforementioned MGEs, play an important role in the spread of *cfr*, *optrA*, and *poxtA* genes across not only strain, species, and genus, but also family and order borders. In addition, these MGEs often carry additional resistance genes which support the coselection and persistence of the oxazolidinone resistance genes. The most important measure to reduce

the dissemination of resistant bacteria is to decrease the selection pressure. This is usually achieved by a reduced application of the respective selecting or coselecting antimicrobial agents. However, due to the numerous colocated antimicrobial resistance genes (as visible from Tables 2 to 5 and Table 7), it will be a difficult task to avoid the coselection of oxazolidinone resistance genes in the different bacteria. Encouragingly, the Chinese Ministry of Agriculture and Rural Affairs (CMARA) has issued a pilot project entitled "Action of Reduction of Antimicrobial Agents used in Veterinary Practice," which aims at maintaining zero increase in the use of antimicrobial agents in farm animals over the period from 2018 to 2021 (http://www.moa.gov.cn/govpublic/SYJ/201804/t20180420_6140711.htm). Moreover, the CMARA further issued a strict withdrawal policy which included the instruction that all antimicrobial agents were to be prohibited as growth promoters from 1 July 2020 onward (http://www.moa.gov.cn/govpublic/xmsyj/201907/t20190710_6320678.htm). In other countries/regions of the world, similar regulations are in place. In the European Union, the use of antimicrobial growth promoters has been prohibited since 2006 (https://ec.europa.eu/commission/presscorner/detail/en/IP_05_1687), and numerous countries have started attempts to decrease the use of antimicrobial agents in human and veterinary medicine. As an example, the sales figures of veterinary antimicrobial agents in Germany have dropped by more than 60% between 2011 and 2019 (https://www.bft-online.de/fileadmin/bft/publikationen/Blickpunkt/BP_94/Blickpunkt_94.pdf). The future will show whether the reduction of the selective pressure by a lesser use of coselecting antimicrobial agents (e.g., tetracyclines, phenicols, lincosamides, and pleuromutilins) will have a positive impact on the dissemination of oxazolidinone resistance genes in bacteria of both animal and human origin.

The costs of antimicrobial agents can also regulate, in a way, the quantity of use. The original linezolid-containing Zyvox was a high-cost antimicrobial agent that was only prescribed and used when there was no less expensive option. In 2017, linezolid has become a generic drug that is no longer protected by a patent. As a consequence, several generic linezolid-containing medicinal products have become available, and their costs are distinctly lower than that of Zyvox. The future will show whether these lower costs will lead to an increased use of linezolid, accompanied by a higher selection pressure and an increase in the frequency of resistant isolates. In this regard, monitoring and surveillance programs need to include oxazolidinones (if not done yet) to early detect a rise in oxazolidinone resistance and—if one is detected—to rapidly implement counteractive measures. In addition, newly developed approaches such as inhibitors of linezolid resistance determinants, phages/phage lysins, and also compounds of traditional Chinese medicine are promising weapons and alternative ways to combat oxazolidinone-resistant pathogens.

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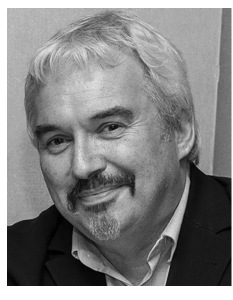

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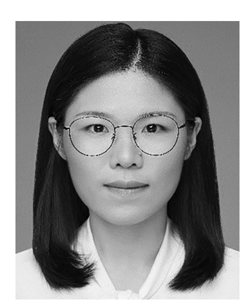

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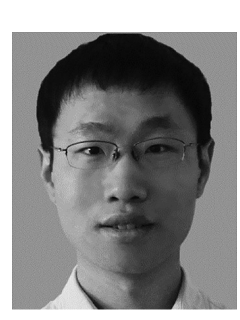

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