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Transmission of linezolid-resistant *Enterococcus* isolates carrying *optrA* and *poxtA* genes in slaughterhouses

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Introduction: The presence of linezolid-resistant enterococci found in animal-derived food has attracted attention for possible transmission to human-derived enterococci through the food chain. Linezolid-resistant enterococci in farms have been widely reported, but enterococci carrying antimicrobial resistance (AMR) genes *poxtA*, *optrA*, or *cfr*(D) in slaughterhouse environments have not been well addressed.

Methods: Enterococcus was isolated from the samples collected from two slaughterhouses in Hangzhou, and the Enterococcus carrying linezolidin-resistant genes was identified by PCR. The minimum inhibitory concentration (MIC) of the Enterococcus carrying linezolidin-resistant genes was determined by microbroth dilution method. Finally, the whole genome of strains carrying two or more linezoline resistance genes was sequenced using the Oxford Nanopore Technology

Results: Here, 291 enterococci strains were isolated from 309 samples (94.17%). A total of 4 *poxtA*-positive enterococci and 42 *optrA*-positive enterococci were identified based on PCR. The antimicrobial susceptibility test showed that the highest rate of florfenicol resistance was 97.82% and the rate of multidrug resistance (MDR) was 95.65%. Two strains carried multiple linezolid resistance genes, among which *Enterococcus casseliflavus* CQFYY22-063 cocarrying *optrA*, *poxtA*, and *cfr*(D) was isolated from the duck cecum, and *Enterococcus faecium* CQFYH22-006 cocarrying *optrA* and *poxtA* was isolated from slaughterhouse sewage for the first time. Furthermore, Oxford Nanopore Technology revealed that the *optrA* gene of strain CQFYY22-063 was located on the Inc18-type plasmid pFYY063-optrA-70K, and the *poxtA* and *cfr*(D) genes were located on the Inc18-type plasmid pFYY063- poxtA-12K. Meanwhile, the *poxtA* gene of strain CQFYH22-006 was located on the Rep3-type plasmid pFYH006-poxtA-25K, and the *optrA* gene was located on the chromosome.

Discussion: Together, linezolid resistance in slaughterhouses deserves extensive attention, indicating the need to strengthen the monitoring of different links in the food production chain within the One Health concept.

KEYWORDS

***Enterococcus*, linezolid, *poxtA*, *optrA*, *Cfr*(D)**

1. Introduction

Enterococci are gram-positive bacteria found in the environment and intestines of animals and humans and are a building block of normal microbes (Staley et al., 2014). Some strains of *Enterococcus*, such as *Enterococcus faecium* EF1, *E. faecium* NCIMB 11181, *E. faecium* NCIMB 10415, *E. faecium* SF68, *E. faecium* M-74 and *E. faecium* NRRL-B 2354 are used as probiotics and feed additives to prevent diarrhea or to improve growth in animals (Franz et al., 2011; Hanchi et al., 2018; Ahmad et al., 2022; Shao et al., 2022). But part of the *E. faecium* and *Enterococcus faecalis* are important pathogens that cause human infections, often causing diseases such as urinary and soft tissue infections, septicemia or meningitis (Bender et al., 2018; Park et al., 2020). Notably, *E. faecium* is on the global list of priority pathogens called ESKAPE (Tacconelli et al., 2018). Antimicrobial resistance (AMR) is recognized as a global public health crisis that urgently needs to be addressed (Li et al., 2017; Guan et al., 2022; Lin et al., 2022; Ma et al., 2022; Tang et al., 2022c). *Enterococcus* has inherent resistance to cephalosporins, anti-staphylococcal penicillin, amtrannan, aminoglycosides, lincoamides, and streptin (Golob et al., 2019). In addition, antimicrobial resistance genes (ARGs) obtained from other sources are transferred and exchanged under different conditions, leading to the emergence of multiple AMR in *Enterococcus*, which increases the prevalence of *Enterococcus* and increases the cost of treatment (Cui et al., 2016; Hao et al., 2019).

Linezolid, the first oxazolidone drug to be approved for sale, has attracted global attention because of its importance as an antibacterial agent of last resort for gram-positive strains (Bender et al., 2018). With the increasing frequency of linezolid use in the clinic, linezolid-resistant *Enterococcus* gradually appeared (Schwarz et al., 2021; Xu et al., 2022). Linezolid resistance can be associated with a point mutation in the 23S rRNA gene or a mobile *cfr*, *optrA*, or *poxtA* resistance gene on the plasmid (Antonelli et al., 2018; Wang et al., 2020). Transferable linezolid resistance genes, including *optrA*, *optrA*, and *cfr,* have been detected in many different species of *Enterococcus* and from different animal sources around the world (Sadowy, 2018; Schwarz et al., 2021). Many studies have shown that most *Enterococcus* (93.3%) exhibit a MDR phenotype (Hu et al., 2019). In addition, more *Enterococcus* carrying *poxtA* were found in environmental samples than in clinical samples, and the prevalence of *E. faecium* was higher than that of *Enterococcus faecalis* (Hao et al., 2019; Huang et al., 2019). The *optrA* gene was found to be more common in *Enterococcus* isolated from livestock than in *Enterococcus* isolated from humans (Yang et al., 2015), the detection rate of the *optrA* gene was 2.0%, and the positive rate of the *optrA* gene in the clinic increased from 0.4% in 2004 to 3.9% in 2014 (Cui et al., 2016; Liu et al., 2020). The presence of plasmids carrying ARGs indicated that *Enterococcus* isolates could transfer between them and promote the further spread of linezolid resistance genes (Cinthi et al., 2022b). Meanwhile, the presence of other ARGs on the plasmids, such as *fexA* and *erm*(A), may contribute to the persistence of linezolid resistance genes (Tang et al., 2020, 2021; Huang et al., 2022). With extensive studies on linezolid resistance genes, various variants have gradually emerged, such as *cfr*(D), *cfr*(B), *poxtA2*, and *optrA*, suggesting that further monitoring of enterococcal resistance is needed (Saavedra et al., 2020; Cinthi et al., 2022a).

The complete genome sequence can effectively analyze the genome characteristics, ARGs, plasmids, and other elements (Wang et al., 2013; Zhu et al., 2015; Peng et al., 2017), and has been an essential means of antimicrobial resistance research in recent years (Tang et al., 2022a,b). The ARGs predicted by the genome have a corresponding good relationship with the AMR phenotype (Zheng et al., 2022; Zhou et al., 2022; Li et al., 2022b; Tang et al., 2022b).

Linezolid resistance gene-mediated enterococcal resistance is widely found in various food animals, among which pigs and chickens are more studied (Osman et al., 2019; Schwarz et al., 2021; Xu et al., 2022). Few studies have been conducted in slaughterhouses and their environment. *Enterococcus* isolates from slaughterhouses and the environment are often multidrug-resistant (MDR) and carry essential risk-resistant genes. It has been reported that most of the *Enterococcus* strains isolated from duck slaughterhouses in Chengdu, China were MDR (90.3%), of which the *optrA* gene (90.7%) was commonly observed (Li et al., 2022a). According to Na′s report, 35.9% of *Enterococcus* isolates from duck feces and carcasses from four slaughterhouses in southern Korea were MDR, 2.3% of which harbor the *optrA* gene (Na et al., 2019). Yu et al. reported that 72.13% of *Enterococcus* isolates from broilers slaughterhouses in Tai'an, China, were MDR, with 18.03% being linezolid-resistant (Yu et al., 2022a). Notably, a vancomycin-resistant *E. faecium* was isolated from a transport crate in a chicken slaughterhouse in Germany (Savin et al., 2020). Previous reports indicate that *Enterococcus* is widespread in slaughterhouses and their environment, posing a potential threat to public health safety. This study focused on monitoring the presence and transmission mechanism of linezolid resistance genes in slaughterhouses and their environment and evaluated the transferability of plasmids carrying *optrA* and *poxtA* resistance genes to *E. faecalis*, providing a solid data reference for theoretical research on AMR.

2. Materials and methods

2.1. Sample acquisition

From May to September 2022, 96 duck cecum samples and 96 environmental samples were collected from a duck slaughterhouse in Fuyang, Zhejiang Province, China, and 33 pig carcass samples and 87 environmental samples were collected from a pig slaughterhouse in Xiaoshan, Zhejiang Province, China. Slaughterhouse sewage was dipped into a 2-ml tube with a sterile cotton swab (HUABAO, Ningbo). Then, all samples were placed at 2–8°C and shipped back to the laboratory for sample pretreatment within 24 h after collection.

2.2. Isolation and identification of enterococci

Samples were mixed with 5 ml of buffered protein water (Landbridge, Beijing) in sterile tubes and incubated at 37°C for 12–18 h. After incubation, each sample (10 μl) was inoculated on *Enterococcus* chromogenic medium (Landbridge, Beijing) and incubated at 37°C for 24–48 h. Purple single colonies were picked on subscoring *Enterococcus* chromogenic plates with Brian Heart Infusion (Landbridge, Beijing) plates and finally identified using MALDI-TOF MS (Bruker, Germany).

2.3. Screening of linezolid resistance genes

PCR screening of all isolated enterococci was performed according to the primers of previously reported linezolid resistance genes *optrA*, *poxtA*, and *cfr* (Supplementary Table S1).

2.4. Antimicrobial susceptibility testing

The microbroth dilution method recommended in the American Committee for Clinical and Laboratory Standards Institute (CLSI) document M100-S27 was used to determine the minimum inhibitory concentration (MIC) of *Enterococcus* to 18 antibiotics (CLSI, 2017; Humphries et al., 2021), including penicillin (PEN), amoxicillin/clavulanate (A/C), erythromycin (ERY), clindamycin (CLI), enrofloxacin (ENR), ofloxacin (OFL), ceftiofur (CEF), cefoxitin (CFX), sulfisoxazole (SF), oxacillin (OXA), vancomycin (VAN), trimethoprim/sulfamethoxazole (SXT), doxycycline (DOX), florfenicol (FFC), tiamulin (TIA), tilmicosin (TIL), gentamicin (GEN), and linezolid (LZD). These antibiotics were purchased from Fosun Diagnostics, Shanghai, China. *Enterococcus faecalis* ATCC 29212 was used as a quality control strain.

2.5. Whole genome sequencing and analysis

Single colonies were selected on BHI plates and inoculated in 5 ml of BHI broth at 37°C with shaking at 220 rpm for 6–7 h. Then, the bacteria were collected with centrifugation at 9,000 rpm for 2 min, and the genome was extracted using a DNA extraction kit (Generay, Shanghai). Strains carrying one of the linezolid resistant genes were sequenced using Illumina Nova sequencing and assembled using CLC Genomics Workbench 12.0. Strains harboring two or more linezolid-resistant genes were further sequenced using the Oxford Nanopore GridION platform and assembled with Unicycler v0.4.4 (Wick et al., 2017). Easyfig 2.2.3 (Sullivan et al., 2011) was used to compare the gene environments, and BRIG was used to map the circles of the plasmids for comparison (Alikhan et al., 2011). Plasmid types were analyzed using the Center for Genomic Epidemiology (CGE, http://www.genomicepidemiology.org/). Finally, heatmaps were generated by TBtools v1.0 (Chen et al., 2020a).

2.6. Statistical analysis

The Chi-square test was used to analyze whether there were differences in *Enterococcus* isolation rates among different sources. A probability (*P*) value<0.05 was considered statistically significant.

3. Results

3.1. Isolation of strains

A total of 291 *Enterococcus* strains were isolated from 309 samples, with an overall isolation rate of 94.17% (291/309; Table 1). Among them, 104 *Enterococcus* strains were isolated (88.89%, 104/117), 33 *Enterococcus* strains were isolated from pig carcass samples, and 71 *Enterococcus* strains were isolated from environmental samples. A total of 187 strains of *Enterococcus* were isolated from the Fuyang duck slaughterhouse, with an isolation rate of 97.39% (187/192). Ninety-five *Enterococcus* strains were isolated from duck cecum samples, and 92 *Enterococcus* strains were isolated from environmental samples. The isolation rate of *Enterococcus* in the pig slaughterhouse was significantly lower than that in the duck slaughterhouse, and significant difference was found between them by chi-square test ($p < 0.05$).

3.2. Screening of linezolid resistance genes

A total of 4 *poxtA*-positive enterococcal strains were screened out of 291 enterococcal strains, among which 2 strains were from pig slaughterhouses and 2 strains were from duck slaughterhouses, with a positive rate of 1.72% (4/291). There were 42 *optrA*-positive enterococci strains, of which 18 were from pig slaughterhouses and 24 were from duck slaughterhouses, with a positivity rate of 16.16% (42/291). No *cfr*-positive enterococci were screened.

The 46 strains of *Enterococcus* that carried linezolid resistance genes belonged to six species: *E. faecalis* (39.13%, $n = 18$), *E. faecium* (30.43%, $n = 14$), *Enterococcus casseliflavus* (13.04%, $n = 6$), *Enterococcus hirae* (6.52%, $n = 3$), *Enterococcus mundtii* (2.17%, $n = 1$), and *Enterococcus gallinarum* (8.70%, $n = 4$). These included 2 *Enterococcus* strains, CQFYY22-063 and CQFYH22-006, from a duck slaughterhouse belonging to *poxtA* and *optrA* co-occurring enterococci.

3.3. Antimicrobial susceptibility testing of enterococci carrying linezolid resistance genes

Thirty-three strains were resistant to linezolid, including 13 *E. faecalis* strains, 11 *E. faecium*, 4 strains of *E. casseliflavus*, 3 strains of *E. gallinarum*, and 1 strain each of *E. hirae* and *E. mundtii* (Figure 1A). Antimicrobial susceptibility testing of 46 *Enterococcus* isolates by microbroth dilution is shown in Figure 1B and Supplementary Figure S1.

In this study, 33 enterococci strains were found to be resistant to linezolid, with linezolid MIC ranged from 8 ~ 16 μg/ml (Supplementary Figure S1). Enterococci were classified into 24

TABLE 1 Information on the samples and isolation rates.

Location		Animal	Samples	Isolates	Isolation rate/%
Fuyang	Slaughterhouse	Ducks	192	187	97.39
Xiaoshan	Slaughterhouse	Pigs	117	104	88.89
Total			309	291	94.17

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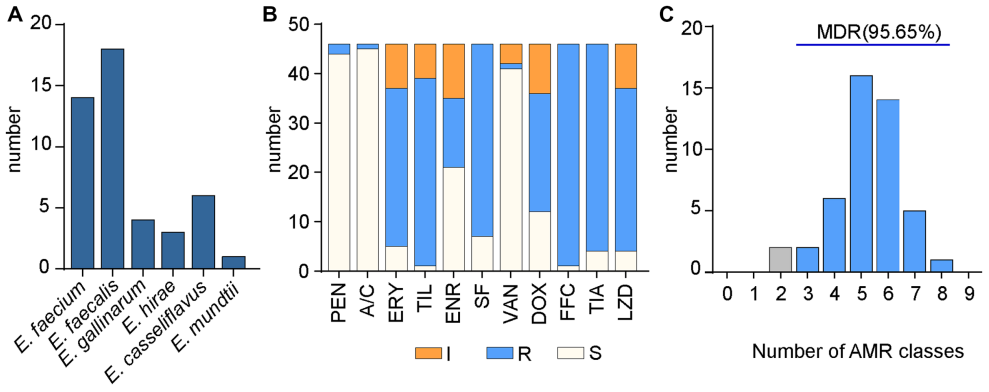

FIGURE 1
Species and AMR of *Enterococcus* isolates in this study. **(A)** Types of *Enterococcus* isolates. **(B)** Resistance of *Enterococcus* to 11 antibiotics. "S" for susceptibility, "I" for mediation, and "R" for resistance. **(C)** The number of *Enterococcus* strains resistant to 9 classes of antibiotics and the proportion of MDR strains.

TABLE 2 AMR patterns of 46 strains of *Enterococcus* carrying *optrA* or *poxtA* genes.

ID	AMR patterns	Number	Percentage (%)
1	ENR-SF	1	2.17
2	ENR-SF-TIL-FFC	1	2.17
3	ENR-TIA-TIL-FFC-LZD	1	2.17
4	ERY-ENR-DOX-TIA-TIL-FFC-LZD	1	2.17
5	ERY-ENR-SF-DOX-TIA-FFC-LZD	1	2.17
6	ERY-ENR-SF-DOX-TIA-TIL-FFC	1	2.17
7	ERY-ENR-SF-DOX-TIA-TIL-FFC-LZD	2	4.35
8	ERY-ENR-SF-TIA-TIL-FFC-LZD	1	2.17
9	ERY-SF-DOX-TIA-TIL-FFC	5	10.87
10	ERY-SF-DOX-TIA-TIL-FFC-LZD	11	23.91
11	ERY-SF-TIA-FFC-LZD	1	2.17
12	ERY-SF-TIA-TIL-FFC	1	2.17
13	ERY-SF-TIA-TIL-FFC-LZD	4	8.70
14	ERY-TIA-TIL-FFC	1	2.17
15	ERY-TIA-TIL-FFC-LZD	1	2.17
16	FFC-LZD	1	2.17
17	PEN-A/C-ERY-ENR-SF-DOX-TIA-TIL-FFC	1	2.17
18	PEN-ERY-ENR-SF-DOX-TIA-TIL-FFC	1	2.17
19	SF-DOX-TIA-FFC-LZD	1	2.17
20	SF-DOX-TIA-TIL-LZD	1	2.17
21	SF-TIA-FFC-LZD	3	6.52
22	SF-TIA-TIL-FFC-LZD	2	4.35
23	SF-TIL-FFC	1	2.17
24	ENR-TIL-TIA-FFC-LZD	2	4.35

different AMR subtypes, of which ERY-SF-DOX-TIA-TIL-FFC-LZD accounted for the largest proportion (23.91%) and were resistant to seven antibiotics. This was followed by ERY-SF-DOX-TIA-TIL-FFC (10.87%; Table 2). Of these, 95.65% were MDR (resistant to three or more antibiotics), mainly to chloramphenicol, tetracyclines and macrolides (Figure 1C).

3.4. Molecular characterization of *optrA* and *poxtA*-positive isolates

Whole genome sequencing was performed on 42 *optrA*-positive strains and 4 *poxtA*-positive strains. There were 35 ARGs for 9 classes of antibiotics detected in all *optrA*-positive or *poxtA*-positive strains. Among them, aminoglycosides had the most resistance genes, with 8. Moreover, there were 2 linezolid genes (*optrA* and *poxtA*), seven lincosamide genes [*lsa*(A), *lsa*(E), *lnu*(A), *lnu*(B), *lnu*(C), *lnu*(D), and *lnu*(G)], three amphenicol genes [*fexA*, *fexB*, *cat*, *cfr*(D), and *cat*(pC221)], one folate pathway antagonist genes (*dfrG*), 5 macrolides gene [*erm*(A), *erm*(B), *mef*(A), *msr*(C) and *msr*(D)], 2 glycopeptide genes (*van*C1XY and *van*C2XY), 1 heat gene (*clpL*) and 2 tetracycline genes [*tet*(L) and *tet*(M); Figure 2]. Meanwhile, plasmid replicons include 6 types of 18 different types, namely, Inc18 type (rep1, rep2, repUS1 and repUS11), Rep1 type (rep22), Rep2 type (repUS52), Rep3 type (rep4a, rep27, rep29, rep33), Rep_trans type (rep7a, rep14b, repUS43), and RepA_N type (rep8b, rep9a, rep9b, rep9c, repUS15; Figure 3B). The virulence genes were *elrA*, *srtA*, *ace*, *agg*, *cCF*10, *cOB*1, *cad*, *camE*, *ebpA*, *ebpB*, *ebpC* (biofilm production), *efaAfs* (cell wall adhesion expressed in serum), *efaAfm*, *fsrB*, *gelE*, *chylA* (hyaluronidase gene), *chylB*, *tpx* (antioxidative stress protection), *cylA*, *cylL*, *cylM*, and *acm* (Figure 3A).

3.5. Genetic environment of *optrA* and *poxtA* copositive isolates

Owing to the presence of both *optrA* and *poxtA* genes in strains CQFYH22-006 and CQFYY22-063, they were selected for nanopore

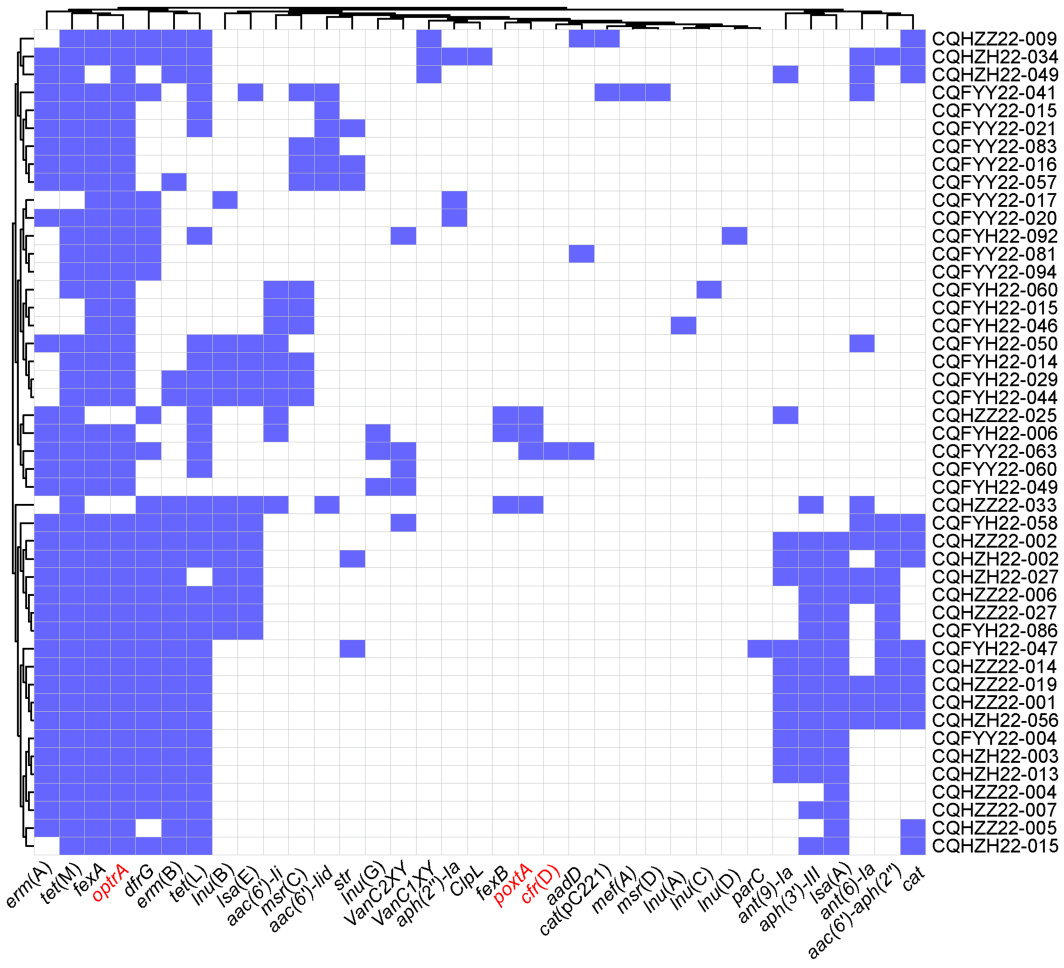

FIGURE 2
Acquired ARGs in *Enterococcus*. Lilac indicates the AMR gene, and white represents the absence of the AMR gene.

sequencing, and their complete genome sequences were obtained. The *optrA* gene in strain CQFYY22-063 is located on pFYY063-optrA-70K (CP116030). pFYY063-optrA-70K was 70,094 bp in length with 35% GC content (Figure 4A). pFYY063-optrA-70K belonged to the Inc18-type plasmid, and by blastn, pFYY063-optrA-70K was less similar to other plasmids carrying *optrA*, with pT17-1-optrA-57k (CP109840) having the highest similarity (63% query coverage and 97.90% homology). The overall backbone of pFYY063-optrA-70K was found to be similar to that of pT17-1-optrA-57k by colinear comparison, but there were differences in the 18,084 bp-sized region of pFYY063-optrA-70K, mainly composed of the proteins "*parA-padR*-IS*L3-spaH-spaA- prgN*," which is consistent with the structure of p_unnamed2 (CP060722; Figure 4B).

The *poxtA* gene of strain CQFYY22-063 is located on pFYY063-poxtA-12 K (CP116031). pFYY063-poxtA-12K is 12,478 bp in length and has a GC content of 36% (Figure 5A). pFYY063-poxtA-12K belongs to the Inc18-type plasmid, while the florfenicol resistance gene *fexA* and the linezolid resistance gene *cfr*(D) are also present in pFYY063-poxtA-12K. The blastn results demonstrated that pFYY063-poxtA-12K was highly similar to other plasmids carrying *poxtA*, with pQZ076-4 (CP098029) having high similarity (99% query coverage and 99.98% homology). A colinear comparison suggested the presence of a transfer element IS*1216E* on the right side of *poxtA* and *cfr*(D) and two transfer elements IS*1216E* in the same direction on both sides of *fexA* (Figure 5B).

The *poxtA* gene of strain CQFYH22-006 is located on pFYH006-poxtA-25 K (CP116025). pFYH006-poxtA-25K is 25,261 bp in length and has a GC content of 35% (Figure 6A). pFYH006-poxtA-25K is a Rep3-type plasmid. Blastn analysis showed that pFYH006-poxtA-25K is highly similar to other plasmids carrying *poxtA*, and colinear comparison suggests the presence of an identically oriented transfer element IS*1216E* on each side of *poxtA* (Figure 6B).

The *optrA* gene of strain CQFYH22-006 was located on the chromosome, and it also carried the ARGs *emr*(A) and *fexA*. The total length of the chromosome was 2,506,224 bp. The selection of regions, including ARGs *optrA*, *emr*(A), and *fexA* for Blastn, was found to be highly similar to that of the *E. faecium* plasmid pP47-61 (CP091102.1). Colinear comparison showed that *optrA*, *emr*(A), and *fexA* had two opposite transfer elements IS*1216* on both sides, and this genetic environment was consistent with pP47-61 (Figure 6C). Therefore, this region in the chromosome might be propagated from the plasmid.

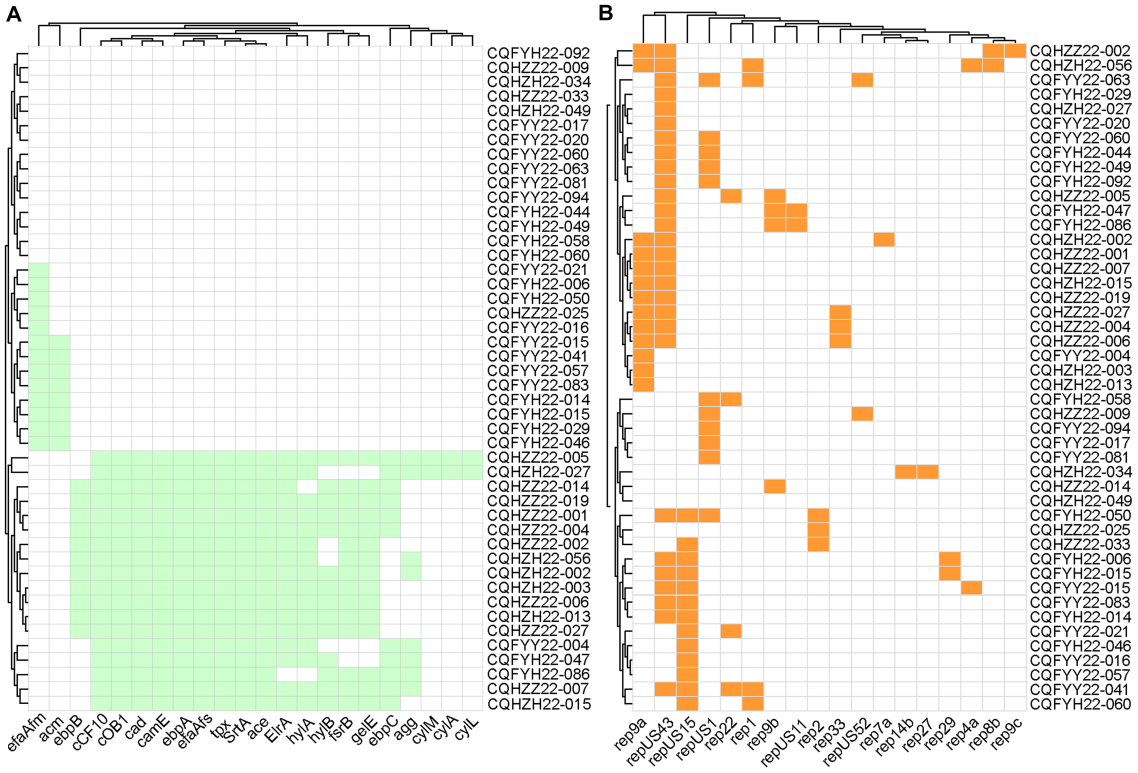

FIGURE 3
(A) Virulence gene in *Enterococcus*. Aqua represents the presence of the virulence gene, and white represents the absence of the virulence gene. **(B)** Plasmid replicon type in *Enterococcus*. Orange represents the plasmid replicon type, and white represents the absence of the plasmid replicon type.

4. Discussion

Enterococci are the main pathogens causing infections in humans and animals (Pillay et al., 2018). Linezolid is considered one of the last lines of defense against methicillin-resistant *Staphylococcus aureus* and VAN-resistant enterococci. However, in recent years, an increasing number of farm animals, humans and the environment around the world have been found to carry multiple linezolid AMR (Chen et al., 2020c; Biggel et al., 2021; Coccitto et al., 2022). Enterococci carrying linezolid resistance genes may pose a potentially serious threat to the health care system and breeding industry (Chen et al., 2020b). Although linezolid is not used in food animals, isolates with linezolid resistance have been reported in China (Lei et al., 2021), Korea (Yoon et al., 2020), the United States (Tyson et al., 2018b), Spain (Ruiz-Ripa et al., 2020) and Tunisia (Elghaieb et al., 2019) from edible meat, animal manure and farming environments. Meanwhile, enterococci carrying *poxtA*–*optrA* or *optrA*-*cfr* genes from swine, human, water and environmental samples have been reported in many countries (Moure et al., 2020; Ruiz-Ripa et al., 2020; Biggel et al., 2021; Nüesch-Inderbinen et al., 2022a). The production chain for livestock and poultry includes the slaughtering process, which is crucial in lowering the prevalence of enterococci. In order to ensure the safety of livestock and poultry products, it is necessary to understand the distribution characteristics of enterococci and ARGs in livestock and poultry slaughterhouses.

In this study, 291 enterococci strains were isolated from two slaughterhouses in Zhejiang Province, with a total isolation rate of 94.17%. The detection rate of *optrA* was 14.43% (42/291), which was lower than the 29.69% (19/64) detection rate reported from Henan Province (Wang et al., 2015). This was much higher than the detection rate of *optrA* reported from 25 large pig farms in Sichuan Province (6/158; 3.80%; Kang et al., 2019). The detection rate of *poxtA* was 1.37% (4/291) lower than the 57.89% (66/114) isolated from two pig farms in Henan Province, China, in 2018 (Hao et al., 2019) and lower than the positive rate in Italian pig farms 4.14% (6/145; Fioriti et al., 2020). To date, there are few reports on the isolation of *E. casseliflavus* from slaughterhouses carrying both *optrA* and *poxtA*. In this study, for the first time, *E. casseliflavus* CQFYY22-063 carrying *optrA*, *poxtA* and *cfr*(D) was isolated from duck cecum (in slaughterhouse), and *E. faecium* CQFYH22-006 carrying *optrA* and *poxtA* was isolated from slaughterhouse sewage.

The phenotype and genotype of lincomycin resistance in enterococci are often not consistent. Rodríguez-Lucas et al. isolated an enterococcus carrying the *optrA* gene from community patients, but it was not resistant to linezolid (Rodríguez-Lucas et al., 2022). Moreover, Nüesch-Inderbinen et al. also reported a pig-derived isolate of *E. faecalis* carrying the *poxtA2* and *cfr*(D) genes but susceptive to linezolid (Nüesch-Inderbinen et al., 2022b). Similar results were also found in this study, 13 *Enterococcus* strains carrying *optrA* or *poxtA* genes were not resistant to linezolid, and the MIC of linezolid was 2–4 μg/ml.

95.65% (44/46) of strains belonged to MDR strains, similar to the MDR rate of enterococci isolated from Polish marketed animal foods (Zarzecka et al., 2022). Among them, the resistance rates of

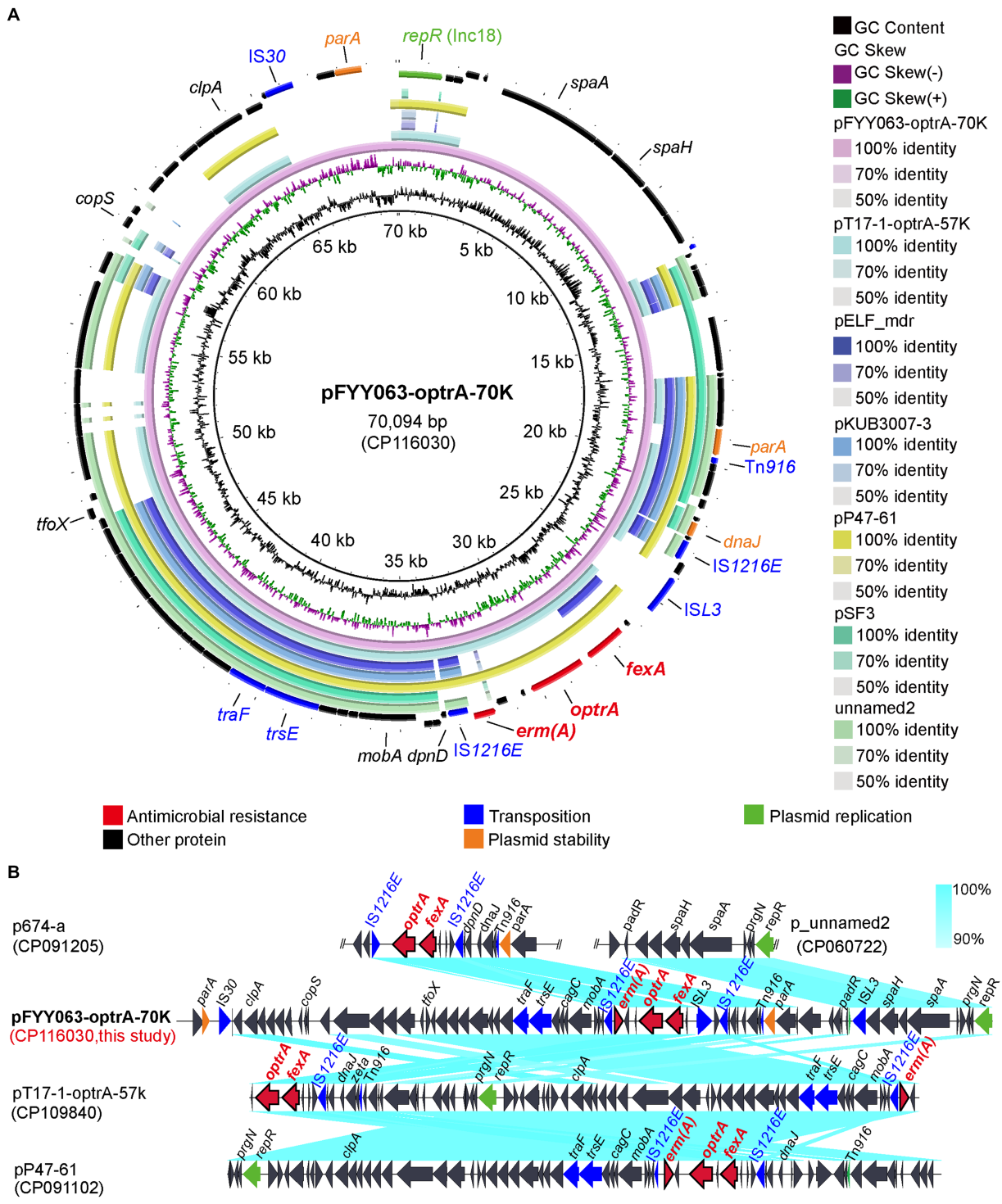

FIGURE 4
Sequence alignment of plasmid pFYY063-optrA-70K and the gene–environment of *optrA* gene. **(A)** Comparison of the circular plasmid sequence between plasmids pFYY063-optrA-70K, pT17-1-optrA-57K, pELF_mdr, pKUB3007-3, pP47-61, pSF3 and unnamed2 of *Enterococcus* strains. **(B)** Linear comparison of the draft genome sequences of *Enterococcus* pFYY063-optrA-70 and *Enterococcus* pT17-1-optrA-57K, pP47-61, p674-a and p_unnamed2. Open arrows indicate coding sequences (red arrows, ARGs; green arrows, plasmid replication; blue arrows, transfer and transfer-related sequences; gray arrows, hypothetical and unclassified) and indicate the direction of transcription.

tilmicosin, sulfisoxazole, tiamulin, florfenicol and linezolid were all above 70%, which was much higher than the resistance rate of *optrA*- or *poxtA*-positive enterococci isolated from retail meat and food-producing animals in Tunisian (Elghaieb et al., 2019). High levels of resistance to tetracyclines, macrolides, and florfenicol, as described above, have also been found in pigs and chickens from Korea (Kwon et al., 2012), the United States (Tyson et al., 2018a), and European countries (de Jong et al., 2018). The widespread prevalence of MDR

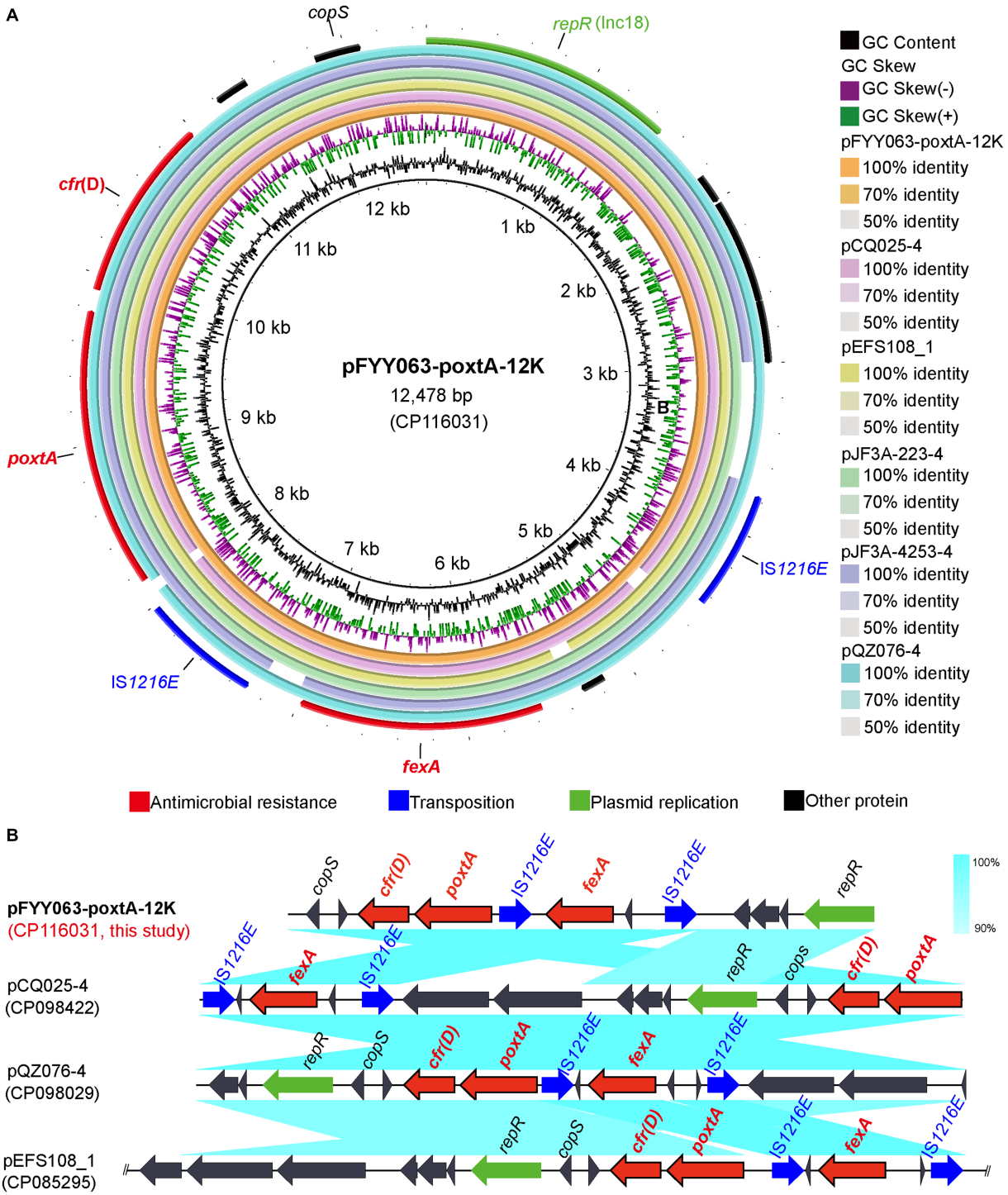

FIGURE 5
Sequence alignment of plasmid pFYY063-poxtA-12K and the gene–environment of *poxtA*. **(A)** Comparison of the circular plasmid sequence between plasmids pFYY063-poxtA-12K, pCQ025-4, pEFS108-1, pJF3A-223-4, pJF3A-2,453-4, and pQZ076-4 of *Enterococcus* strains. **(B)** Linear comparison of the draft genome sequences of *Enterococcus* pFYY063-poxtA-12K and *Enterococcus* pCQ025-4, pQZ076-4 and pEFS108-1. Open arrows indicate coding sequences (red arrows, ARGs; green arrows, plasmid replication; blue arrows, transfer and transfer-related sequences; gray arrows, hypothetical and unclassified) and the direction of transcription.

Enterococcus strains cannot be ignored, so further research on the internal factors mediating the widespread prevalence of *Enterococcus* is necessary.

Mobile genetic elements play an important role in the transmission of ARGs. Plasmids, IS*1216E* and Tn*554* (Murphy et al., 1981), can promote the horizontal transmission of *optrA* (Yu et al., 2022b). Both clonal transmission and horizontal transfer mediated by the Inc18 plasmid and IS*1216E* contributed to the spread of *poxtA* in *Enterococcus* isolates (Lei et al., 2021). It has been reported that IS*1216E*-mediated translocation and

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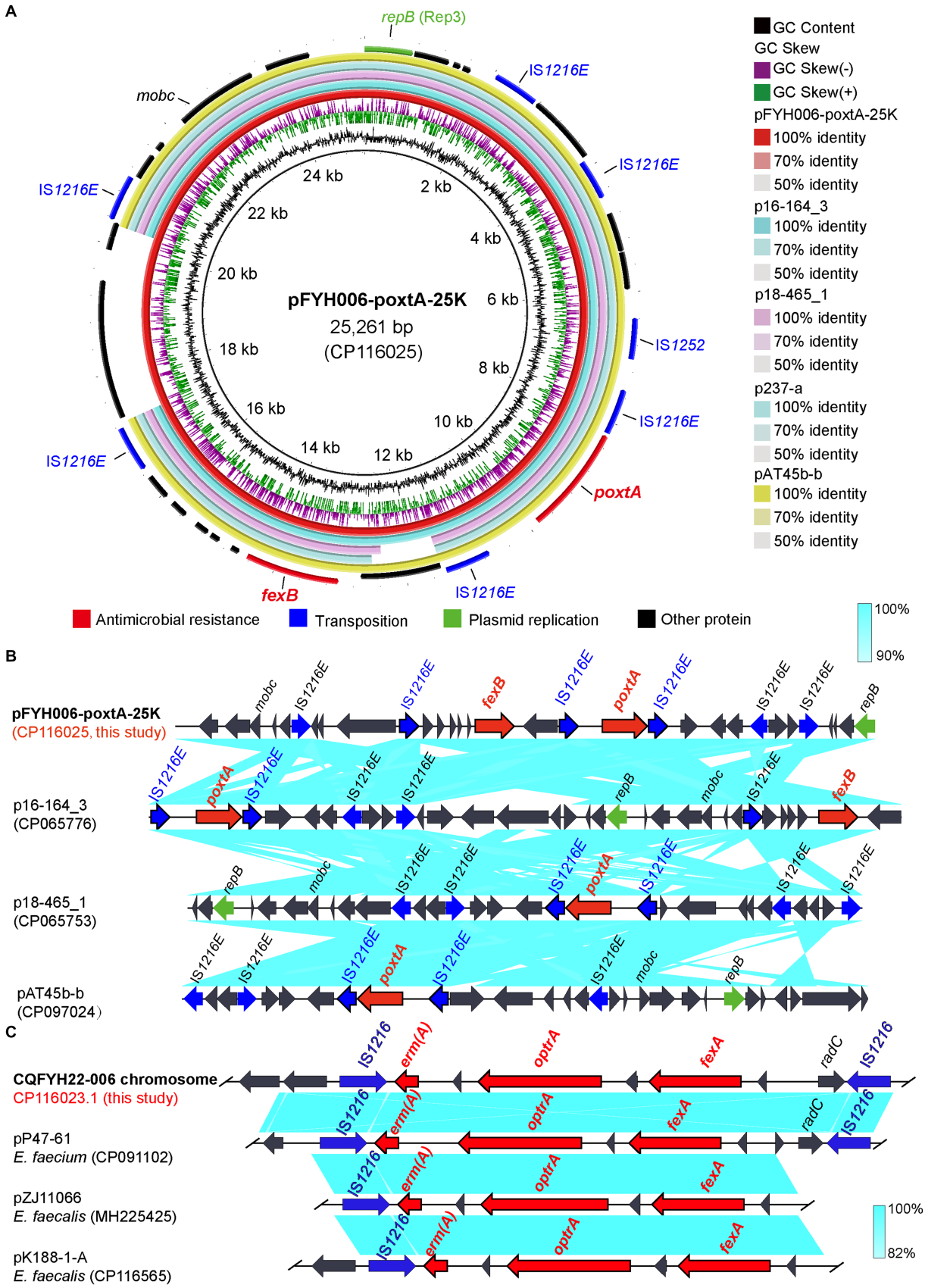

FIGURE 6
Sequence alignment of plasmid pFYH006-poxtA-25K and the gene–environment of *poxtA*. **(A)** Comparison of the circular plasmid sequence between plasmids pFYH006-poxtA-25K, pCQ025-4, pEFS108-1, pJF3A-223-4, pJF3A-2,453-4, and pQZ076-4 of *Enterococcus* strains. **(B)** Linear comparison of

(Continued)

FIGURE 6 (Continued)
the draft genome sequences of *Enterococcus* pFYH006-poxtA-25K and *Enterococcus* pCQ025-4, pQZ076-4 and pEFS108-1. Open arrows indicate coding sequences (red arrows, ARGs; green arrows, plasmid replication; blue arrows, transfer and transfer-related sequences; gray arrows, hypothetical and unclassified) and the direction of transcription. **(C)** The genetic environment of *optrA* on chromosomes. Linear comparison of *optrA* on *Enterococcus* chromosomes with draft genome sequences of *Enterococcus* pP47-61, pZJ11066 and pK188-1-A. Open arrows indicate coding sequences (red arrows, ARGs; green arrows, plasmid replication; blue arrows, transfer and transfer-related sequences; gray arrows, hypothetical and unclassified) and the direction of transcription.

translocation processes promote the spread and persistence of *poxtA* in *Enterococcus* (Shan et al., 2020) and that two parallel oriented IS*1216E* elements on either side have previously been shown to be responsible for horizontal gene transfer of *poxtA* (Egan et al., 2020). The presence of other ARGs (*fexA*, *fexB*) may lead to coselection of *optrA* and *poxtA*. Càmara et al. (2019) reported a high frequency of *optrA* and *fexA* in the same isolate. Furthermore, cotransfer of the *fexA* gene with *optrA* in *E. faecalis* has been demonstrated in many countries, such as China (Wang et al., 2015), Spain (Càmara et al., 2019) and the United States(Wardenburg et al., 2019). In this study, strains also carried the *fexA* gene adjacent to *optrA* or the *fexA* gene adjacent to *poxtA*. In addition, plasmids carrying *poxtA* genes also cocarry other ARGs, such as *cfr*(D). These results suggest that the *poxtA* and *cfr*(D) genes may be transmitted between strains via IS*1216E*.

5. Conclusion

In this study, 291 strains of *Enterococcus* were isolated from two slaughterhouses, and 4 *poxtA*-positive *Enterococcus* strains and 42 *optrA*-positive *Enterococcus* strains were identified. *E. faecium* cocarrying *poxtA* and *optrA* genes was isolated from duck slaughterhouse sewage. In addition, *E. casseliflavus* carrying *optrA*, *poxtA* and *cfr*(D) was isolated from a duck cecum for the first time. This study also proves that *optrA* or *poxtA* genes are widespread in food animals and the slaughter environment. Animal slaughterhouses may act as reservoirs of transferable oxazolidone resistance genes, which can be transmitted through the food chain to humans and would significantly limit treatment of MDR bacteria. Therefore, the prevalence and spread of *poxtA* and *optrA* in *Enterococcus* in animal slaughterhouses should be continuously monitored to reduce potential public health threats.

Data availability statement

The complete genome sequences of strains CQFYH22-006 and CQFYY22-063 were deposited at GenBank under accession numbers CP116023-CP116025 and CP116026-CP116031, respectively.

Author contributions

BT and DP: conceptualization and supervision. BT: funding acquisition. JN, XL, MW, WW, JM, YS, MY, and HY: investigation. JN, XL, WW, and BT: methodology. JN, XL, and MW: visualization. JN, BT, XL, and MW: writing—original draft. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsufs.2023.1179078/full#supplementary-material

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