AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy®

MECHANISMS OF RESISTANCE

Tn*6674* Is a Novel Enterococcal *optrA*-Carrying Multiresistance Transposon of the Tn*554* Family

Dexi Li,[a] Xing-Yun Li,[a] Stefan Schwarz,[b] Mengyan Yang,[a] Su-Mei Zhang,[a] Wenbo Hao,[a] Xiang-Dang Du[a]

[a]College of Animal Science and Veterinary Medicine, Henan Agricultural University, Zhengzhou, People's Republic of China
[b]Institute of Microbiology and Epizootics, Centre for Infection Medicine, Department of Veterinary Medicine, Freie Universität Berlin, Berlin, Germany

ABSTRACT The novel 12,932-bp nonconjugative multiresistance transposon Tn*6674* was identified in the chromosomal DNA of a porcine *Enterococcus faecalis* strain. Tn*6674* belongs to the Tn*554* family of transposons. It shares the same arrangement of the transposase genes *tnpA*, *tnpB,* and *tnpC* with Tn*554*. However, in addition to the Tn*554*-associated resistance genes *spc* and *erm*(A), Tn*6674* harbored the resistance genes *fexA* and *optrA*. Circular forms of Tn*6674* were detected and suggest the functional activity of this transposon.

KEYWORDS *Enterococcus faecalis*, *optrA*, oxazolidinones, phenicols, transposon

Oxazolidinones, such as linezolid and tedizolid, are often used for the treatment of infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE) in humans (1). Phenicols, such as florfenicol, are approved for the control of respiratory tract infections in food-producing animals (2). The gene *optrA*, which confers combined resistance to oxazolidinones and phenicols, has emerged during recent years (3) and poses a serious therapeutic challenge to both human and veterinary medicine. Although the *optrA* gene has been originally described to encode an ABC transporter, a recent study confirmed that OptrA does not mediate the efflux of oxazolidinones and phenicols (4). Meanwhile, OptrA is considered to be an ABC-F protein which acts by protecting the bacterial ribosome (5).

Since the first description of the *optrA* gene in *Enterococcus faecalis* and *Enterococcus faecium* (3), this gene has been identified on various plasmids of different sizes or in the chromosomal DNA of enterococci from humans and food-producing animals (1). In some cases, the *optrA* gene was also present in combination with *cfr*, *cfr*(B), or *poxtA* on the same strain or plasmid (6–8). In addition, it was also found in *Staphylococcus sciuri*, together with *cfr* and other resistance genes on the same plasmid, and in *Streptococcus suis* and *Streptococcus gallolyticus*. In *S. suis*, it was shown to be part of integrative and conjugative elements (ICEs) (9–11). In the present study, we identified a novel chromosome-borne transposon of the Tn*554* family, designated Tn*6674*, which carried the *optrA* gene together with the resistance genes *spc*, *erm*(A), and *fexA* in an *E. faecalis* strain.

During a routine survey on antimicrobial resistance in bacteria from pigs, the florfenicol- and linezolid-resistant *E. faecalis* strain E1731 was isolated from a fecal sample of a pig in Henan Province, China, in 2018. Antimicrobial susceptibility testing (AST) was performed by broth microdilution according to the recommendations given in document M100 (28th ed) of the Clinical and Laboratory Standards Institute (CLSI) (12). *S. aureus* ATCC 29213 served as the quality control strain in AST. The AST results are shown in Table 1. Multilocus sequence typing (MLST) revealed that it belonged to sequence type 691 (ST691). The presence of the *optrA* gene was detected by PCR using the primers *optrA*-fw (5′-GCA CCAGACCAATACGATACAA-3′) and *optrA*-rev (5′-TCCTTCTTAACCTTCTCCTTCTCA-3′), with an annealing temperature of 59°C and amplicon size of 794 bp.

Citation Li D, Li X-Y, Schwarz S, Yang M, Zhang S-M, Hao W, Du X-D. 2019. Tn*6674* is a novel enterococcal *optrA*-carrying multiresistance transposon of the Tn*554* family. Antimicrob Agents Chemother 63:e00809-19. https://doi.org/10.1128/AAC.00809-19.

Address correspondence to Xiang-Dang Du, xddu@henau.edu.cn.

Received 16 April 2019
Returned for modification 27 May 2019
Accepted 11 June 2019
Accepted manuscript posted online 17 June 2019
Published 23 August 2019

TABLE 1 Resistance phenotype and genotype of *E. faecalis* E1731

Antimicrobial agent	MIC (mg/liter)	Associated resistance gene(s) or mutation(s)
Florfenicol	128	*optrA*, *fexA*
Linezolid	8	*optrA*
Erythromycin	512	*erm*(A), *erm*(B)
Gentamicin	>512	*aac*(A)-*aph*(D)
Lincomycin	512	*erm*(B)
Ciprofloxacin	64	GyrA S83R, ParC S80I
Tetracycline	64	*tet*(M), *tet*(L)
Spectinomycin	64	*spc*
Vancomycin	2	None

To investigate the transferability of the *optrA* gene, conjugation and transformation experiments were performed as previously described using *E. faecalis* E1731 as the donor and *E. faecalis* JH2-2 (rifampin resistant [Rif^r]) as the recipient (3, 8). For the screening of the transconjugants and transformants, brain heart infusion (BHI) agar was supplemented with 10 mg/liter florfenicol and 50 mg/liter rifampin. However, the conjugation experiments repeatedly failed. The plasmids were then extracted from the donor *E. faecalis* E1731, and transfer into *E. faecalis* JH2-2 by electrotransformation was attempted. The transformation experiments also repeatedly failed.

To gain information about the genetic environment of the *optrA* gene, the whole-genomic DNA of *E. faecalis* E1731 was sequenced by the PacBio RS II and Illumina MiSeq platforms (Shanghai Personal Biotechnology Co., Ltd., China). The sequences from the Illumina MiSeq platform were assembled using Newbler version 2.8 (454 Life Sciences, Branford, CT, USA), and PacBio sequencing reads were *de novo* assembled. The prediction of open reading frames (ORFs) and their annotations were performed using Glimmer 3.0. The analysis of regions flanking the *optrA* gene revealed the presence of a Tn*554* family transposon-like element of 12,932 bp, designated Tn*6674* according to the nomenclature of transposons (https://transposon.lstmed.ac.uk/). Tn*6674* consisted of seven ORFs of more than 150 amino acids (aa). The first three ORFs were indistinguishable from the genes *tnpA*, *tnpB,* and *tnpC*, whose products are involved in the transposition of *Staphylococcus aureus* transposon Tn*554* (13). However, the remaining four ORFs represented the resistance genes *spc* (resistance to spectinomycin), *erm*(A) (resistance to macrolides, lincosamides, and streptogramin B antibiotics), *fexA* (resistance to phenicols), and *optrA* and replaced its counterpart in Tn*554*, consisting of only *spc* and *erm*(A) (Fig. 1). Sequence analysis also identified several antimicrobial resistance genes or mutations related to the respective phenotypes observed in *E. faecalis* E1731 (Table 1).

An NCBI BLASTN search with the Tn*6674* sequence as a query sequence showed that it displayed 99% nucleotide sequence identity (query cover, 100%) with sequences deposited in GenBank (*E. faecalis* A101, accession no. MH018572.1; *E. faecalis* TZ2, accession no. MH225421.1) (14), 99% nucleotide sequence identity (query cover, 93%) with that deposited in GenBank (*E. faecalis* 743142, accession no. MF443377.1) (15), and 99% nucleotide sequence identity (query cover, 100%) with those of whole-genome shotgun contigs (*E. faecalis* EF294, accession no. QDDM01000007.1; *E. faecalis* 33710, accession no. QNHF01000012.1) (16, 17). In addition, a similar structure was also described in an *optrA*-carrying ST16 *E. faecalis* strains (18).

The analysis of the regions flanking Tn*6674* revealed that Tn*6674* inserted into the gene *radC* gene, which encodes a DNA repair protein (Fig. 1). The *radC* gene had been described to be a common integration site for transposons of the Tn*554* family, such as Tn*554*, Tn*5406*, Tn*558*, Tn*559*, Tn*6133*, Tn*6188,* and Tn*6260* in various Gram-positive bacteria (19–25). In *E. faecalis* E1731, Tn*6674* displayed the hexanucleotide sequence 5′-AATCCG-3′ at the left-end junction and the sequence 5′-GATCCT-3′ at the right-end junction, which marked the boundaries of the integrated segment. This is similar to that in the *Staphylococcus lentus* transposon Tn*558* (21). Transposons of the Tn*554* family,

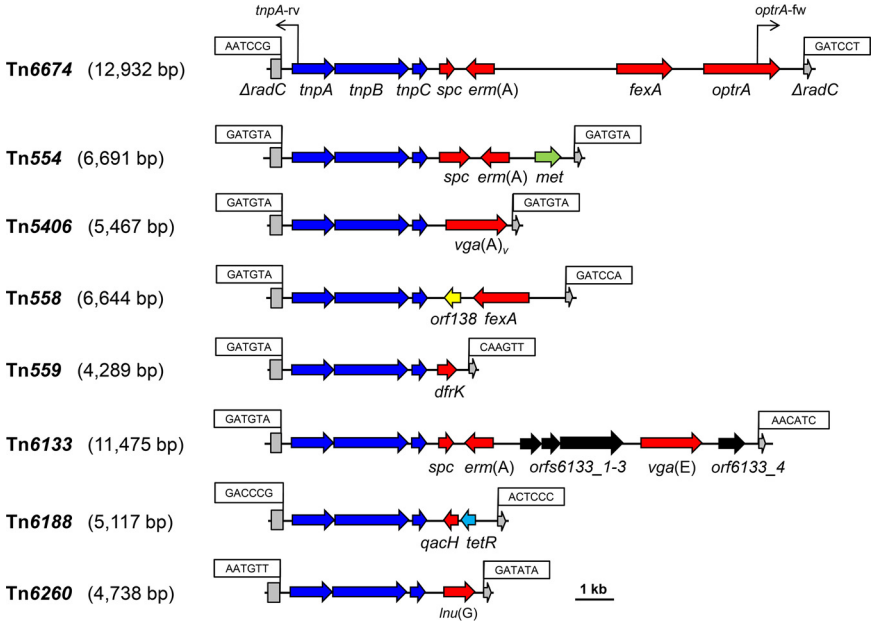

FIG 1 Structural comparison of *E. faecalis* transposon Tn*6674* with other related Tn*554* family transposons. Accession numbers are as follows: Tn*554*, X03216; Tn*5406*, AF186237; Tn*558*, AJ715531; Tn*559*, FN677369; Tn*6133*, FR772051; Tn*6188*, HF565366; Tn*6260*, KX470419; and Tn*6674*, MK737778. A distance scale in kilobases is given. The transposase genes (*tnpA*, *tnpB*, and *tnpC*), antimicrobial and disinfectant resistance genes, and genes that code for other functions are shown in blue, red, and black, respectively. The 6-bp nucleotide sequences at the transposon junctions are shown in boxes. The disrupted *radC* genes are indicated by asterisks. The positions of primers circ-fw/circ-rev used to detect the presence of the circular intermediates are shown by black arrows.

including Tn*6674*, do not contain inverted repeats at their termini and also do not generate a duplication of the target sequence at the integration site. Instead, studies on serial transposition of Tn*554* into primary and secondary target sites revealed that the sequences at the junctions of Tn*554* varied with respect to the target sites, as follows: with each new transposition event, the sequence originally present in the target site is found at the left terminus of Tn*554*, whereas the former left-end junction is now found at the right terminus, and the former right-end junction is lost (21, 26).

Tn*6674* has an overall structure similar to that of other members of the Tn*554* family of transposons. However, all so-far-known Tn*554*-like transposons differ distinctly in their resistance gene regions. These are composed of *spc* and *erm*(A) in Tn*554* from *S. aureus* (13), a *vga*(A) variant in Tn*5406* from *S. aureus* (20), *fexA* in Tn*558* from *S. lentus* (21), *dfrK* in Tn*559* from *S. aureus* (22), *spc*, *erm*(A), and *vga*(E) in Tn*6133* from *S. aureus* (23), *lnu*(G) in Tn*6260* from *E. faecalis* (25), and *spc*, *erm*(A), *fexA,* and *optrA* in Tn*6674* from *E. faecalis*.

Previous studies have shown that the active transposons, such as Tn*5406*, Tn*558*, and Tn*559*, can excise from their host DNA and form circular intermediates which precede their integration into a new target site (20–22). Thus, an inverse PCR assay was set up to detect whether these circular intermediates were present in *E. faecalis* E1731. For this, the pair of primers circ-fw (5′-TAGATGAACCGACAAACC-3′) and circ-rev (5′-TCAA CCAACCTACGAAGT-3′), with an annealing temperature of 47°C and amplicon size of 1,445 bp, was used. Sequence analysis of this amplicon consisted of 380 bp, which included part of *tnpA* and its upstream region at the left end of Tn*6674*, whereas the remaining 1,065 bp of the amplicon represented the right end of Tn*6674*. The presence of circular Tn*6674* forms suggested that this transposon was active in *E. faecalis* E1731.

On the one hand, the location of *optrA* as part of transposon Tn*6674* suggests a novel mechanism for the dissemination of *optrA* in enterococci, which is different from IS*1216*-mediated recombination that was previously described in other studies (27, 28). On the other hand, its location as an integral part of a functionally active transposon

will likely accelerate the dissemination of the *optrA* gene. Since the *optrA* gene confers combined resistance to oxazolidinones used in human medicine (1) and phenicols used in food-producing animals (3, 8, 9), the prudent use of both classes of antimicrobial agents in their respective fields is urgently needed.

Data availability. The complete sequence of Tn*6674* determined in this study has been deposited in GenBank under accession number MK737778.

ACKNOWLEDGMENTS

We thank Don B. Clewell (University of Michigan) for providing *E. faecalis* reference strain JH2-2.

This work was supported by grants from the National Natural Science Foundation of China (grant U1604103), the Program for Innovative Research Team (in Science and Technology) in University of Henan Province (grant 18IRTSTHN020), and the German Federal Ministry of Education and Research (BMBF) under project number 01KI1727D as part of the Research Network Zoonotic Infectious Diseases.

REFERENCES

1. Sadowy E. 2018. Linezolid resistance genes and genetic elements enhancing their dissemination in enterococci and streptococci. Plasmid 99:89–98. https://doi.org/10.1016/j.plasmid.2018.09.011.
2. Priebe S, Schwarz S. 2003. In vitro activities of florfenicol against bovine and porcine respiratory tract pathogens. Antimicrob Agents Chemother 47:2703–2705. https://doi.org/10.1128/AAC.47.8.2703-2705.2003.
3. Wang Y, Lv Y, Cai J, Schwarz S, Cui L, Hu Z, Zhang R, Li J, Zhao Q, He T, Wang D, Wang Z, Shen Y, Li Y, Feßler AT, Wu C, Yu H, Deng X, Xia X, Shen J. 2015. A novel gene, *optrA*, that confers transferable resistance to oxazolidinones and phenicols and its presence in *Enterococcus faecalis* and *Enterococcus faecium* of human and animal origin. J Antimicrob Chemother 70:2182–2190. https://doi.org/10.1093/jac/dkv116.
4. Wang Y, Li X, Wang Y, Schwarz S, Shen J, Xia X. 2018. Intracellular accumulation of linezolid and florfenicol in optrA-producing *Enterococcus faecalis* and *Staphylococcus aureus*. Molecules 23:3195. https://doi.org/10.3390/molecules23123195.
5. Sharkey LK, Edwards TA, O'Neill AJ. 2016. ABC-F proteins mediate antibiotic resistance through ribosomal protection. mBio 7:e01975-15. https://doi.org/10.1128/mBio.01975-15.
6. Lazaris A, Coleman DC, Kearns AM, Pichon B, Kinnevey PM, Earls MR, Boyle B, O'Connell B, Brennan GI, Shore AC. 2017. Novel multiresistance *cfr* plasmids in linezolid-resistant methicillin-resistant *Staphylococcus epidermidis* and vancomycin-resistant *Enterococcus faecium* (VRE) from a hospital outbreak: co-location of *cfr* and *optrA* in VRE. J Antimicrob Chemother 72:3252–3257. https://doi.org/10.1093/jac/dkx292.
7. Kuroda M, Sekizuka T, Matsui H, Suzuki K, Seki H, Saito M, Hanaki H. 2018. Complete genome sequence and characterization of linezolid-resistant *Enterococcus faecalis* clinical isolate KUB3006 carrying a *cfr*(B)-transposon on its chromosome and *optrA*-plasmid. Front Microbiol 9:2576. https://doi.org/10.3389/fmicb.2018.02576.
8. Hao W, Shan X, Li D, Schwarz S, Zhang SM, Li XS, Du XD. 2019. Analysis of a *poxtA*- and *optrA*-co-carrying conjugative multiresistance plasmid from *Enterococcus faecalis*. J Antimicrob Chemother 74:1771–1775. https://doi.org/10.1093/jac/dkz109.
9. Li D, Wang Y, Schwarz S, Cai J, Fan R, Li J, Feßler AT, Zhang R, Wu C, Shen J. 2016. Co-location of the oxazolidinone resistance genes *optrA* and *cfr* on a multiresistance plasmid from *Staphylococcus sciuri*. J Antimicrob Chemother 71:1474–1478. https://doi.org/10.1093/jac/dkw040.
10. Huang J, Chen L, Wu Z, Wang L. 2017. Retrospective analysis of genome sequences revealed the wide dissemination of *optrA* in Gram-positive bacteria. J Antimicrob Chemother 72:614–616. https://doi.org/10.1093/jac/dkw488.
11. Mendes RE, Deshpande L, Streit JM, Sader HS, Castanheira M, Hogan PA, Flamm RK. 2018. ZAAPS programme results for 2016: an activity and spectrum analysis of linezolid using clinical isolates from medical centres in 42 countries. J Antimicrob Chemother 73:1880–1887. https://doi.org/10.1093/jac/dky099.
12. Clinical and Laboratory Standards Institute. 2018. Performance standards for antimicrobial susceptibility testing, 28th ed. CLSI supplement M100. Clinical and Laboratory Standards Institute, Wayne, PA.
13. Murphy E, Huwyler L, de Freire Bastos MDC. 1985. Transposon Tn*554*: complete nucleotide sequence and isolation of transposition-defective and antibiotic-sensitive mutants. EMBO J 4:3357–3365. https://doi.org/10.1002/j.1460-2075.1985.tb04089.x.
14. Cai J, Schwarz S, Chi D, Wang Z, Zhang R, Wang Y. 2019. Faecal carriage of *optrA*-positive enterococci in asymptomatic healthy humans in Hangzhou, China. Clin Microbiol Infect 25:630.e1–630.e6. https://doi.org/10.1016/j.cmi.2018.07.025.
15. Deshpande LM, Castanheira M, Flamm RK, Mendes RE. 2018. Evolving oxazolidinone resistance mechanisms in a worldwide collection of enterococcal clinical isolates: results from the SENTRY Antimicrobial Surveillance Program. J Antimicrob Chemother 73:2314–2322. https://doi.org/10.1093/jac/dky188.
16. Zhou W, Gao S, Xu H, Zhang Z, Chen F, Shen H, Zhang C. 2019. Distribution of the *optrA* gene in *Enterococcus* isolates at a tertiary care hospital in China. J Glob Antimicrob Resist 17:180–186. https://doi.org/10.1016/j.jgar.2019.01.001.
17. Chen M, Pan H, Lou Y, Wu Z, Zhang J, Huang Y, Yu W, Qiu Y. 2018. Epidemiological characteristics and genetic structure of linezolid-resistant *Enterococcus faecalis*. Infect Drug Resist 11:2397–2409. https://doi.org/10.2147/IDR.S181339.
18. Tsilipounidaki K, Gerontopoulos A, Papagiannitsis C, Petinaki E. 2019. First detection of an *optrA*-positive, linezolid-resistant ST16 *Enterococcus faecalis* from human in Greece. New Microbes New Infect 29:100515. https://doi.org/10.1016/j.nmni.2019.01.010.
19. Murphy E, Reinheimer E, Huwyler L. 1991. Mutational analysis of *att554*, the target of the site-specific transposon Tn*554*. Plasmid 26:20–29. https://doi.org/10.1016/0147-619X(91)90033-S.
20. Haroche J, Allignet J, El Solh N. 2002. Tn*5406*, a new staphylococcal transposon conferring resistance to streptogramin A and related compounds including dalfopristin. Antimicrob Agents Chemother 46:2337–2343. https://doi.org/10.1128/AAC.46.8.2337-2343.2002.
21. Kehrenberg C, Schwarz S. 2005. Florfenicol-chloramphenicol exporter gene *fexA* is part of the novel transposon Tn*558*. Antimicrob Agents Chemother 49:813–815. https://doi.org/10.1128/AAC.49.2.813-815.2005.
22. Kadlec K, Schwarz S. 2010. Identification of the novel *dfrK*-carrying transposon Tn*559* in a porcine methicillin-susceptible *Staphylococcus aureus* ST398 strain. Antimicrob Agents Chemother 54:3475–3477. https://doi.org/10.1128/AAC.00464-10.
23. Schwendener S, Perreten V. 2011. New transposon Tn*6133* in methicillin-resistant *Staphylococcus aureus* ST398 contains *vga*(E), a novel streptogramin A, pleuromutilin, and lincosamide resistance gene. Antimicrob Agents Chemother 55:4900–4904. https://doi.org/10.1128/AAC.00528-11.
24. Müller A, Rychli K, Muhterem-Uyar M, Zaiser A, Stessl B, Guinane CM, Cotter PD, Wagner M, Schmitz-Esser S. 2013. Tn*6188*-a novel transposon in *Listeria monocytogenes* responsible for tolerance to benzalkonium chloride. PLoS One 8:e76835. https://doi.org/10.1371/journal.pone.0076835.
25. Zhu XQ, Wang XM, Li H, Shang YH, Pan YS, Wu CM, Wang Y, Du XD, Shen JZ. 2017. Novel *lnu*(G) gene conferring resistance to lincomycin

by nucleotidylation, located on Tn*6260* from *Enterococcus faecalis* E531. J Antimicrob Chemother 72:993–997. https://doi.org/10.1093/jac/dkw549.
26. Murphy E, Lofdahl S. 1984. Transposition of Tn*554* does not generate a target duplication. Nature 307:292–294. https://doi.org/10.1038/307292a0.
27. He T, Shen Y, Schwarz S, Cai J, Lv Y, Li J, Feßler AT, Zhang R, Wu C, Shen J, Wang Y. 2016. Genetic environment of the transferable oxazolidinone/phenicol resistance gene *optrA* in *Enterococcus faecalis* isolates of human and animal origin. J Antimicrob Chemother 71:1466–1473. https://doi.org/10.1093/jac/dkw016.
28. Morroni G, Brenciani A, Antonelli A, D'Andrea MM, Di Pilato V, Fioriti S, Mingoia M, Vignaroli C, Cirioni O, Biavasco F, Varaldo PE, Rossolini GM, Giovanetti E. 2018. Characterization of a multiresistance plasmid carrying the *optrA* and *cfr* resistance genes from an *Enterococcus faecium* clinical isolate. Front Microbiol 9:2189. https://doi.org/10.3389/fmicb.2018.02189.