J Antimicrob Chemother 2016; **71**: 1466–1473
doi:10.1093/jac/dkw016 Advance Access publication 21 February 2016

Journal of Antimicrobial Chemotherapy

Genetic environment of the transferable oxazolidinone/phenicol resistance gene *optrA* in *Enterococcus faecalis* isolates of human and animal origin

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Received 18 October 2015; returned 4 January 2016; revised 10 January 2016; accepted 11 January 2016

Objectives: Aim of this study was to analyse 17 non-related *Enterococcus faecalis* isolates of human and animal origin for the genetic environment of the novel oxazolidinone/phenicol resistance gene *optrA*.

Methods: WGS and *de novo* assembly were conducted to analyse the flanking sequences of the *optrA* gene in the 17 *E. faecalis* isolates. When *optrA* was located on a plasmid, conjugation assays were performed to check whether the plasmids are conjugative and to confirm the resistance phenotype associated with these plasmids.

Results: All nine *optrA*-carrying plasmids were conjugated into *E. faecalis* JH2-2 and the transconjugants exhibited the *optrA*-associated phenotype. In these plasmids, an IS*1216E* element was detected either upstream and/or downstream of the *optrA* gene. In eight plasmids, the phenicol exporter gene *fexA* was found upstream of *optrA* and in six plasmids, a novel *erm*(A)-related gene for macrolide–lincosamide–streptogramin B resistance was detected downstream of *optrA*. When located in the chromosomal DNA, the *optrA* gene was found downstream of the transcriptional regulator gene *araC* in four isolates, or downstream of the *fexA* gene in another four isolates. Integration of the *optrA* region into a Tn*558*-Tn*554* hybrid, located in the chromosomal *radC* gene, was seen in two isolates.

Conclusions: The findings of the present study extend the current knowledge about the genetic environment of *optrA* and suggest that IS*1216E* elements play an important role in the dissemination of *optrA* among different types of enterococcal plasmids. The mechanism underlying the integration of *optrA* into the chromosomal DNA requires further investigation.

Introduction

Linezolid, the first oxazolidinone introduced into clinical use, has demonstrated antimicrobial activity against the most clinically relevant pathogens, such as MRSA, VRE and penicillin-resistant pneumococci.[1] In addition to mutations in the central loop of domain V of the 23S rRNA, two genes, *cfr* and *optrA*, that mediate transferable resistance to linezolid, have been identified in enterococci.[2–4] The multiresistance gene *cfr* encodes a 23S rRNA methyltransferase that mediates resistance to five chemically unrelated antimicrobial agents (phenicols, lincosamides, oxazolidinones, pleuromutilins and streptogramin A) and decreases susceptibility to the 16-membered macrolides spiramycin and josamycin.[3,5] In contrast to *cfr*, the ABC transporter gene *optrA*, recently identified in *Enterococcus faecalis* and *Enterococcus faecium* of human and animal origin,[4] also confers resistance to tedizolid, a novel oxazolidinone that was approved in June 2014 by the US FDA for the management of acute bacterial skin and skin-structure infections in adults.[6] The *optrA* gene also confers resistance to fluorinated and non-fluorinated phenicols.[4] As enterococcal infections have become one of the major treatment problems,[7] the emergence and spread of the oxazolidinone/phenicol resistance gene *optrA* in enterococci represent serious challenges to clinical treatment.

Our initial study revealed that the *optrA* gene was located on a plasmid in 16 *E. faecalis* isolates and in the chromosomal DNA of another 14 *E. faecalis* isolates.[4] One of these plasmids, pE349 from *E. faecalis* E349 of human origin, was sequenced completely

and sequence analysis identified the *optrA* gene in close proximity to the phenicol resistance gene *fexA*. ISs were not detected on this plasmid.[4] No information is currently available on the genetic environment of the *optrA* gene in the remaining 29 *E. faecalis* isolates that carried this gene on plasmids or in the chromosomal DNA.

For the present study, 17 epidemiologically unrelated *E. faecalis* isolates were chosen from the aforementioned collection. These isolates differed by their origin, S1-PFGE patterns, MLST types and antimicrobial resistance profiles.[4] Nine isolates carried the *optrA* gene on plasmids while eight isolates harboured it in their chromosomal DNA. The aim of this study was to investigate these 17 isolates for the genetic environment of *optrA* to gain insight into elements that may play a role in the interplasmid dissemination and the chromosomal integration of the *optrA* gene.

Materials and methods

Conjugation and susceptibility testing

Conjugation by filter mating was performed as previously described using the nine isolates, that carried the *optrA* gene on plasmids, as donors and the rifampicin-resistant *E. faecalis* JH2-2 as recipient.[8] Transconjugants were selected on medium supplemented with 1 mg/L linezolid and 25 mg/L rifampicin. The transconjugants were confirmed by PCR detection of the *optrA* gene and investigated for their MICs of chloramphenicol, florfenicol, linezolid, tedizolid, erythromycin, vancomycin, doxycycline and gentamicin by broth microdilution according to the recommendations given in the CLSI documents VET01-S2 (2013) and M100-S25 (2015).[9,10] The reference strain *E. faecalis* ATCC 29212 served as a quality control strain in MIC determinations.

WGS and analysis

Whole-cell DNA of all 17 isolates was prepared using the Wizard Genomic DNA Purification Kit (Promega, Beijing, China), following the manufacturer's instructions, and then submitted to WGS, preceded by library construction, on a HiSeq 2500, which produced 150 bp paired-end reads (Berry Genomics Company, Beijing, China). A draft assembly of the sequences was conducted using the CLC Genomics Workbench 5 (CLC Bio, Aarhus, Denmark); the assembly algorithm works by using de Bruijn graphs.[11] All contigs with the average coverage of >100-fold were searched for the *optrA* gene using BLAST analysis. The regions flanking the *optrA* gene were identified using *de novo* assembly as earlier described and a primer walking strategy.[12] Sequence analysis was conducted using the ORF Finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html) and BLAST functions (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

To determine whether recombination between two IS*1216E* elements can result in the formation of *optrA*-carrying minicircles, inverse PCR assays were conducted using outward primers (primer 1: 5′-TTAGATGAACCGACAAAC-3′; primer 2: 5′-CATTCGCTTAGAACAAGA-3′; annealing temperature 55°C). When detected, minicircles were sequenced using the aforementioned primers. The formation of Tn*558*-*optrA* circular intermediates from the chromosomal DNA of *E. faecalis* isolates E016 and G20 was tested using inverse PCRs with the following primers (primer 1: 5′-ATGAACCGACAAACCACA-3′; primer 2: 5′-GCATCCCATCCACTTCTA-3′; annealing temperature 55°C).

The sequences of the *optrA*-carrying regions of 11 *E. faecalis* isolates have been deposited in GenBank under the following accession numbers KT862775 (p10-2-2), KT862776 (pE121), KT862777 (pE419), KT862778 (pFX13), KT862779 (pSF35), KT862780 (pXY17), KT862781 (E016), KT862782 (E079), KT862783 (E147), KT862784 (G20) and KT862785 (LY4). The sequences of the *optrA* regions in the remaining six isolates were indistinguishable from the corresponding region in one or the other of the aforementioned 11 isolates as listed in Figures 2 and 3.

Results and discussion

Susceptibility profiles of the transconjugants

Conjugation assays were successfully performed with all nine *optrA*-carrying plasmids and the corresponding transconjugants exhibited resistance or reduced susceptibility to florfenicol (MICs 64–128 mg/L), chloramphenicol (MICs 32–128 mg/L), linezolid (MICs 2–16 mg/L) and tedizolid (MICs 1–2 mg/L) (Table 1). In addition, seven transconjugants were resistant to erythromycin (MICs 8 to ≥256 mg/L). All but one transconjugant were susceptible to doxycycline (≤0.5 mg/L) (Table 1).

Tetracycline and phenicol resistance genes located on optrA-carrying plasmids

Plasmid p10-2-2 also conferred resistance to tetracyclines as confirmed by its doxycycline MIC of 64 mg/L (Table 1). Two complete *tet* genes, *tet*(L) encoding a 458 amino acid efflux protein of the major facilitator superfamily and *tet*(M) encoding a 639 amino acid ribosome protective protein, were identified on a 31 kb contig of the transconjugant that carries plasmid p10-2-2. Both *tet* genes were separated by a 196 bp spacer, which also included the *tet*(L) translational attenuator. The reading frame for a 20 amino acid peptide located in the *tet*(L)-associated translational attenuator[13] was truncated by a T to A exchange, which turned the third codon (TGT) into an early stop codon (TGA). A cat_{pC223} gene encoding a 215 amino acid chloramphenicol acetyltransferase was detected 1044 bp downstream of the *tet*(L) stop codon. This *cat* gene was also preceded by a translational attenuator for inducible *cat* gene expression.[14] The entire *tet*(M)-*tet*(L)-cat_{pC223} segment was part of a 18731 bp relic of the 25963 bp transposon Tn*6248* of *E. faecium* strain E506 (GenBank accession no. KP834592). In this transposon relic, the genes responsible for transposition, as well as insertion and excision of Tn*6248*, were missing.

The gene *fexA*, which mediates resistance to fluorinated and non-fluorinated phenicols,[15] was detected upstream of the *optrA* gene in eight of the nine plasmids. No relics of the transposon Tn*558*, on which *fexA* has been detected in staphylococci,[16] were detectable in the vicinity of the *fexA* gene in any of these seven plasmids. Both, *E. faecalis* XY17 and its transconjugant, exhibited an erythromycin MIC of 8 mg/L. This MIC classifies the isolate and the transconjugant as borderline resistant. However, no macrolide resistance gene could be detected in the contigs of XY17 that are available.

A novel erm gene located on optrA-carrying plasmids

In six plasmids (Table 1), a novel type of the *erm* gene was detected downstream of the *optrA* gene. This *erm* gene encoded an rRNA methylase of 244 amino acids, which showed 82.7% amino acid identity to the erythromycin resistance methylase Erm(A) (formerly known as ErmTR) of *Streptococcus pyogenes* (GenBank accession no. AF002716)[17] and 85.2% amino acid identity to the Erm(A) protein from transposon Tn*554* of *Staphylococcus aureus* (GenBank accession no. X03216).[18] As *E. faecalis* is intrinsically resistant to lincosamides and no streptogramin B compound is commercially available for susceptibility

Table 1. Background information on the 17 *E. faecalis* isolates and susceptibility profiles of 9 *optrA*-positive transconjugants

Isolate	Host	Location (city)	PFGE pattern	MLST	Location of *optrA* (~kb)	Co-located resistance genes	MICs of transconjugants (mg/L)							
							FFC	CHL	LZD	TZD	ERY	VAN	DOX	GEN
FX13	pig	Shanghai	P	ST622	plasmid (34)	*erm*(A)-like	64	64	4	1	≥256	1	≤0.25	≤500
SF35	chicken	Henan	J	ST330	plasmid (65)	*fexA*, *erm*(A)-like	128	128	8	2	≥256	2	≤0.25	≤500
G22	pig	Tibet	A2	ST116	plasmid (55)	*fexA*, *erm*(A)-like	128	64	8	2	≥256	2	≤0.25	≤500
E419	human	Hangzhou	X	ST480	plasmid (80)	*fexA*, *erm*(A)-like	128	128	2	1	128	1	0.5	≤500
10-2-2	pig	Guangzhou	D	ST59	plasmid (60)	*fexA*, *erm*(A)-like, *tet*(M), *tet*(L), cat_{pC223}	128	128	4	1	≥256	2	64	≤500
E121	human	Shangrao	F1	ST480	plasmid (80)	*fexA*, *erm*(A)-like	128	64	4	1	128	2	≤0.25	≤500
XY17	pig	Henan	E	ST27	plasmid (30)	*fexA*	128	64	8	2	8	2	≤0.25	≤500
E161	human	Hangzhou	AC	ST585	plasmid (60)	*fexA*	64	32	16	2	≤0.25	2	0.5	≤500
E071	human	Hangzhou	F2	ST480	plasmid (60)	*fexA*	64	32	16	2	≤0.25	2	0.5	≤500
JH2-2	—	—	—	—	—	—	2	4	2	0.5	≤0.25	2	0.5	≤500
E016	human	Hangzhou	M	ST16	chromosome	*fexA*, Δ*erm*(A)-like	NA							
G20	pig	Tibet	B	ST93	chromosome	*fexA*	NA							
E079	human	Jinhua	W	ST27	chromosome	—	NA							
LY4	chicken	Shangdong	S	ST21	chromosome	*erm*(A)-like	NA							
E147	human	Hangzhou	H	ST476	chromosome	*fexA*	NA							
5-7	pig	Tibet	C	ST619	chromosome	*fexA*	NA							
10-120	pig	Guangzhou	Q1	ST403	chromosome	*fexA*	NA							
E381	human	Taizhou	Y2	ST256	chromosome	*fexA*	NA							

FFC, florfenicol; CHL, chloramphenicol; LZD, linezolid; TZD, tedizolid; ERY, erythromycin; VAN, vancomycin; DOX, doxycycline; GEN, gentamicin; NA, not applicable.

testing, the high erythromycin MICs of at least 128 mg/L in the various transconjugants (Table 1) indicate the functional activity of this gene. Since both previously known *erm*(A) gene variants are inducibly expressed via translational attenuation, the regulatory region located upstream of each *erm*(A)-like gene was analysed for the presence of inverted repeated sequences and reading frames for the short peptides of 15 amino acids and 19 amino acids known to be present in the *erm*(A) translational attenuator from Tn*554*.[18] Although the isolates in the present study originated from humans, pigs and chickens in different provinces of China, all six plasmid-borne and the two chromosomal *erm*(A)-like genes harboured exactly the same 116 bp deletion in the translational attenuator (Figure 1a). This deletion comprised the reading frame for the 19 amino acid peptide and the inverted repeats IR1, IR2 and IR3. Based on the current knowledge about structural alterations in the translational attenuators of the *erm*(A), *erm*(C) and *erm*(T) genes,[19–21] short regions of homology are sufficient to allow recombinations that result in deletions of different sizes. Thus, it is likely that the observed 116 bp deletion was also due to a recombination (Figure 1b).

Despite the structural differences between the *erm*(A) gene from Tn*554*, the *erm*(A) gene formerly known as *erm*(TR) and the *erm*(A)-related gene identified in this study, the nomenclature centre for MLS_B resistance genes (http://faculty.washington.edu/marilynr/) requests to use the same designation for all three genes.[22] The identity cut-off at <80% on protein level for a new *erm* gene designation is debatable. This cut-off value is from a time when hybridization was often used to identify resistance genes and is based on the observation that two genes, whose products have <80% amino acid sequence identity, usually provided enough variability in their nucleotide sequences to permit distinct probes to be designed.[22] In the era of WGS, this concept needs reconsideration. The use of the same gene designation might be misleading, particularly when the variant genes occur in completely different genetic contexts as compared with the original gene. A recent proposal suggests using a cut-off of ≥2% difference in the protein and/or the DNA sequences as a realistic point for assigning a new gene designation.[23] Using this cut-off, all three *erm*(A) genes mentioned would be considered as individual *erm* genes and might receive a unique *erm* gene designation.

Genetic environment of optrA on plasmids

The nine *optrA*-carrying plasmids ranged in their approximate sizes between 30 and 80 kb.[4] The *E. faecalis* isolates, from which these plasmids originated, exhibited different PFGE patterns and MLST profiles, and the *optrA* gene regions of the plasmids showed varying degrees of similarity to one another and to the originally reported *optrA*-carrying plasmid pE349 (Figure 2a). The *optrA* upstream regions were highly similar in plasmids p10-2-2 and pXY17 (both of porcine origin), pE121, pE161 and pE071 (all of human origin) and in the original *optrA*-carrying plasmid pE349 (Figure 2a). The *optrA* downstream regions were virtually indistinguishable in six plasmids, including pSF35 of chicken origin, p10-2-2, pG22 and pFX13 of porcine origin and pE419 and pE121 of human origin (Figure 2a).

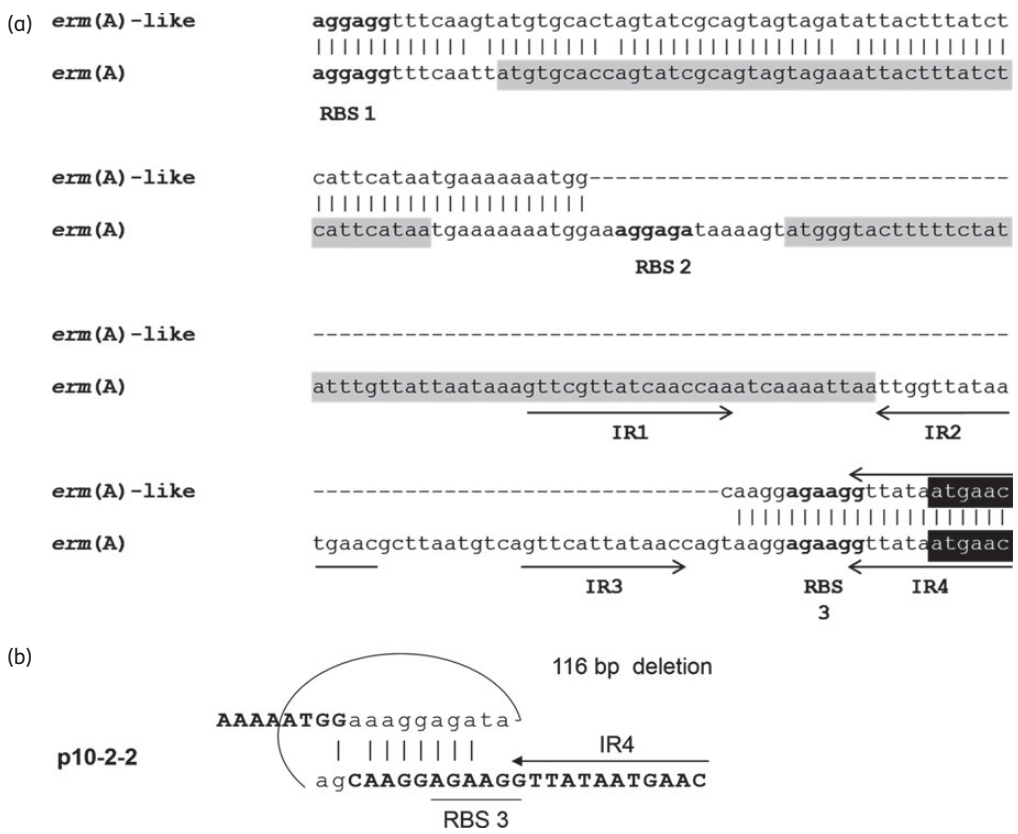

Figure 1. (a) Comparison of the complete regulatory regions of the *erm*(A) gene from Tn*554* and the *erm*(A)-like gene identified in this study in plasmid p10-2-2. The 116 bp deletion in p10-2-2 is indicated by dashes. Vertical bars indicate identical bases in the two sequences. Two pairs of inverted repeated sequences IR1:IR2 and IR3:IR4 are marked by arrows. Reading frames for the 15 amino acid and 19 amino acid peptides are highlighted by grey shading. 5′-terminal parts of the *erm*(A) and *erm*(A)-like genes are shown by white letters on a black background. Ribosomal binding site (RBS) 1, RBS 2 and RBS 3 are shown in bold. (b) Possible site for recombination in the translational attenuator of the *erm*(A)-like gene resulting in the observed 116 bp deletion. Sequence present in the p10-2-2-associated translational attenuator is shown in bold. Lower case letters represent the beginning and end of the deleted sequence. Important structural elements with respect to Figure 1(a), such as RBS 1, as well as the IR4 sequence, are indicated. Vertical bars mark identical bases in the sequences most likely involved in the recombinational event.

ISs of the type IS*1216E* were found upstream and/or downstream of the *optrA* gene in all nine plasmids. When two copies of IS*1216E* were present and bracketed the *optrA*-carrying central region, these IS*1216E* copies were located in the opposite orientation on plasmids pSF35, pG22, pE419 and pE121, whereas they were in the same orientation on plasmids pFX13 and p10-2-2 (Figure 2a). When located in the same orientation, recombination between the two IS*1216E* copies can lead to the formation of minicircles that contain the bracketed region plus one copy of the IS*1216E* element. Inverse PCR confirmed that minicircles of plasmids pFX13 and p10-2-2 were detectable, which contained the central region and one intact IS*1216E* (data not shown). This observation might indicate that the IS*1216E*-flanked segment was unstable in these two plasmids and could be excised via IS*1216E*-mediated recombination. In plasmids pXY17, pE161 and pE071, only one copy of IS*1216E* was identified in the *optrA* downstream region. These three plasmids shared the same *optrA* genetic environment (Figure 2a).

IS*1216E* belongs to the IS*6* family and such IS elements (IS*1216*, IS*1216E* and IS*1216V*) show >98% nucleotide identity and have been associated with the vancomycin resistance gene *vanA* in *E. faecium*, the multidrug resistance gene *cfr* in *E. faecalis*, the macrolide–lincosamide–streptogramin B resistance genes *erm*(B) (*ermAM*) and *erm*(T) in Enterococcus *hirae* and *Streptococcus gallolyticus* subsp. *pasteurianus*, respectively, and the tetracycline resistance gene *tet*(S) in *Streptococcus infantis*.[12,24–27] Thus, the *optrA* gene may be disseminated among different species of enterococci via IS*1216*-mediated recombination events. Moreover, BlastP analysis revealed that proteins, which showed 99% amino acid identity to OptrA from enterococci, are present in *Streptococcus suis* (GenBank accession no. WP_050571857) and *Staphylococcus sciuri* (GenBank accession no. ALI92811). Thus, the *optrA* gene may have already been transferred to other Gram-positive bacteria.

Genetic environment of optrA in the chromosomal DNA

Contigs of different sizes (6088–29141 bp) that harboured the *optrA* gene were obtained from the whole-genome sequences of the eight *E. faecalis* isolates that carried the *optrA* gene in their chromosomal DNA. The flanking regions of *optrA* in the chromosomal DNA differed distinctly from those on plasmids (Figure 2b). In the three isolates G20, E016 and E079, the putative transcriptional regulator gene *araC* was located immediately upstream of the *optrA* gene (Figures 2b and 3a).

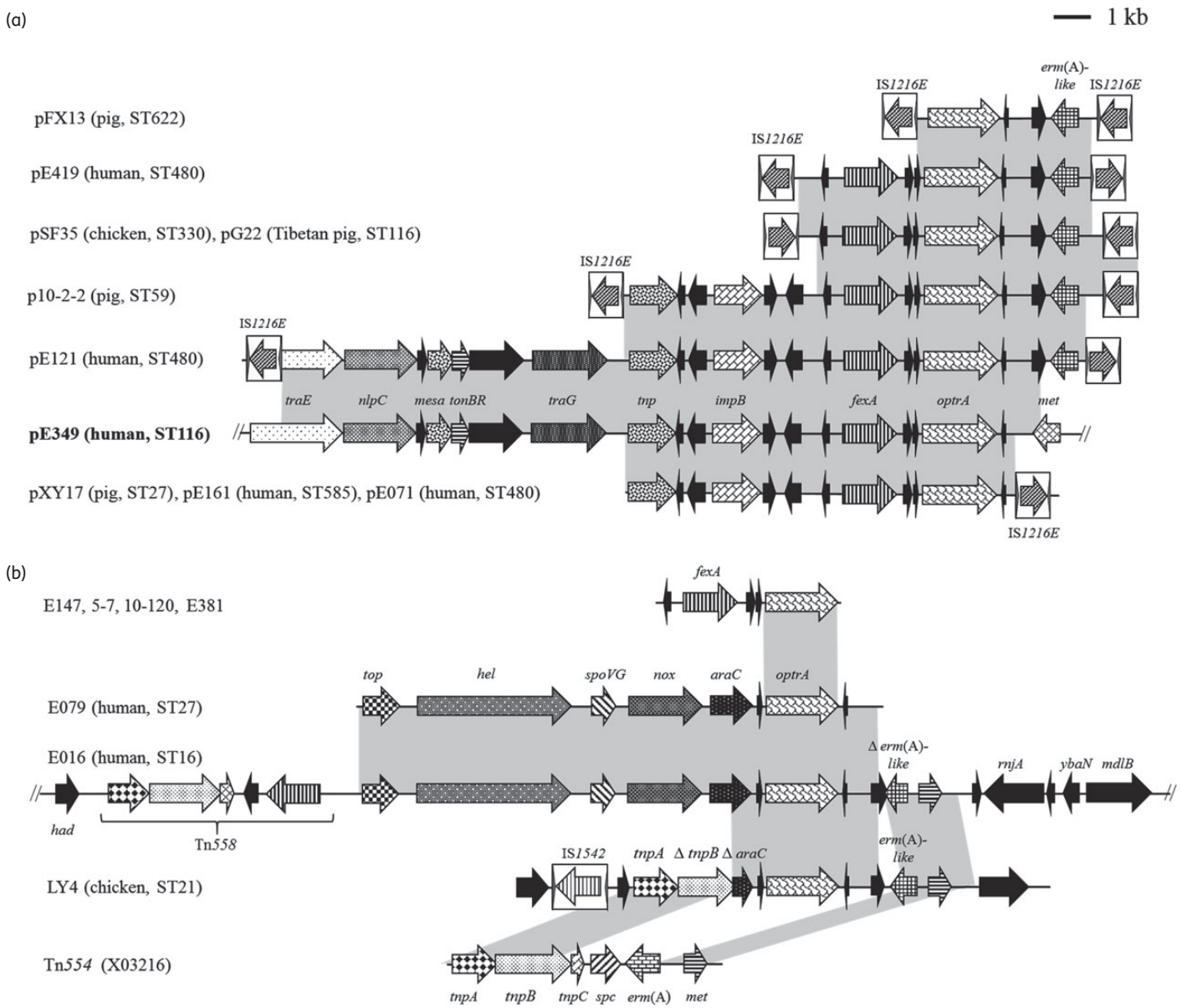

Figure 2. (a) Schematic presentation and comparison of the genetic environment of *optrA* in the nine *E. faecalis* plasmids investigated in this study. Plasmid pE349 (shown in bold) is the original plasmid on which the *optrA* was first identified and has been included for comparative reasons. (b) Comparison of chromosomal *optrA*-containing regions in seven *E. faecalis* isolates. Arrows indicate the positions and directions of transcription of the different genes. Genes with known functions are displayed in different textures, whereas genes with unknown functions are shown in black. ISs are indicated as boxes with the arrow inside the box showing the transposase gene. Regions of ≥98% nucleotide sequence identity are shaded grey. Δ symbol indicates a truncated gene.

In the human *E. faecalis* ST16 isolate E016, from which the largest contig was obtained, four ORFs, which encoded a topoisomerase, a helicase, the septation protein SpoVG and a putative NADH oxidase, respectively, were observed upstream of the *araC-optrA* segment. Further upstream, a complete copy of the transposon Tn*558*, including *fexA*, *orf138* and the three transposase genes *tnpA*, *tnpB* and *tnpC*, was identified (Figures 2b and 3a). Downstream of *optrA*, two genes for hypothetical proteins, a truncated *erm*(A)-like gene and an *S*-adenosylmethionine (SAM)-dependent methyltransferase gene (*met*) were detected. The 22 720 bp segment comprised at the 5′ terminus a complete Tn*558* of 6639 bp (99.8% identity to Tn*558*[15]) and at the 3′ terminus 1279 bp of the terminal part of Tn*554* (98.4% nucleotide sequence identity to Tn*554* from *S. aureus*).[18]

In the *E. faecalis* ST93 isolate G20 from a Tibetan pig, a similar *optrA*-containing segment was detected. This segment was only 11 083 bp in size and comprised at its 5′ terminus a complete Tn*558* of 6639 bp (99.9% identity to Tn*558*[15]) and at its 3′ terminus 329 bp, which are identical to those in Tn*554*.[18] Within these 329 bp, a truncated *met* gene, of which only the terminal

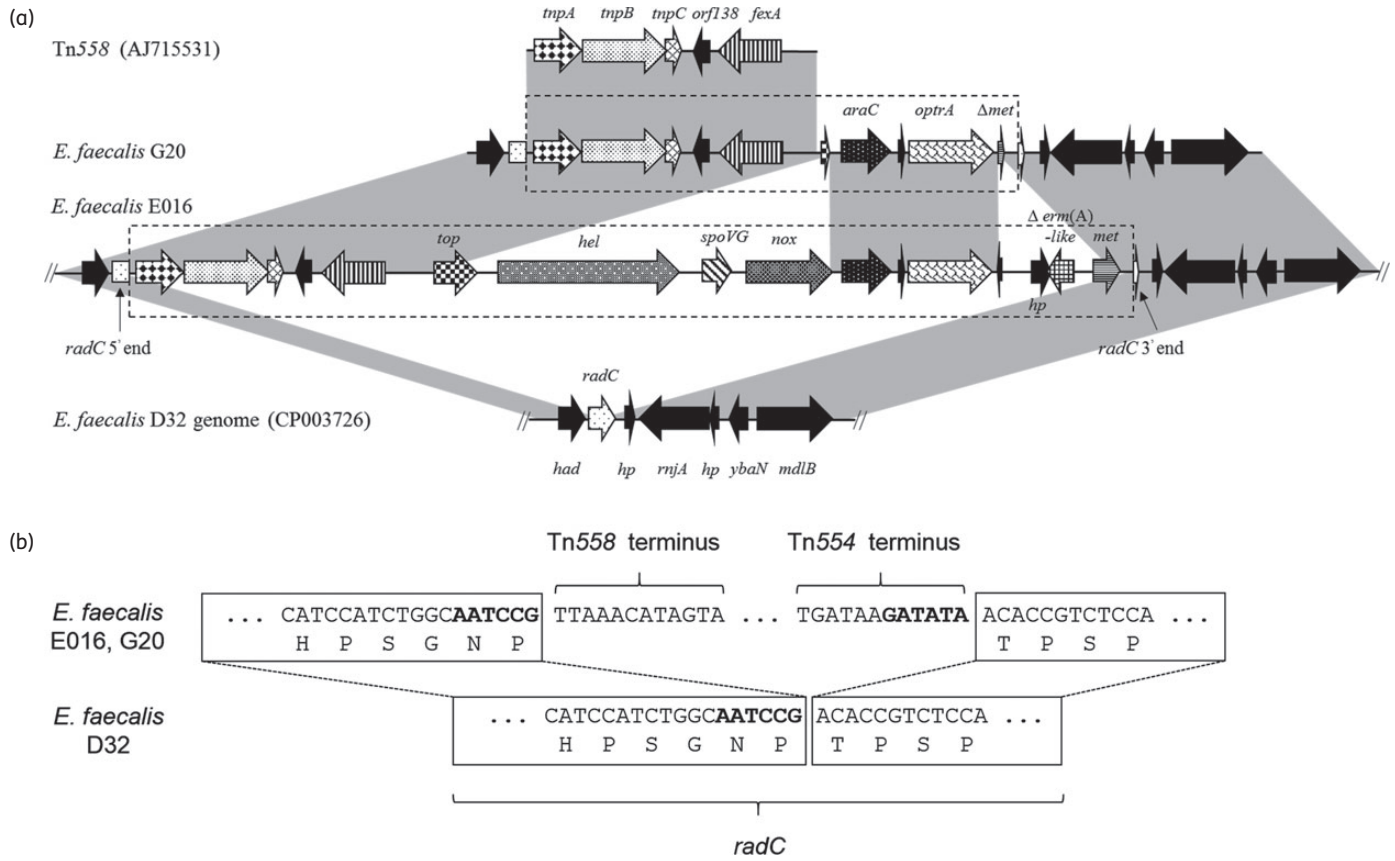

Figure 3. (a) Integration of larger *optrA*-carrying segments (boxed with a broken line) into the chromosomal *radC* gene in *E. faecalis* isolates E016 and G20. Arrows indicate the positions and directions of transcription of the different genes. Genes with known functions are displayed in different textures, whereas genes with unknown functions are shown in black. Regions of ≥98% nucleotide sequence identity are shaded grey. Δ symbol indicates a truncated gene. (b) Detailed presentation of the boundaries of the inserted segments in comparison with the *radC* sequence of *E. faecalis* D32.[34] Amino acids are given using the single-letter code. The 6 bp core nucleotide sequences at the transposon junctions are shown in bold.

95 bp were present, was detected. Except for the initial 5 bp of *top* and the terminal 63 bp of *nox*, the entire segment from *top* to *nox* present in isolate E016 was lost in isolate G20. Moreover, a segment of 2594 bp, including the Δ*erm*(A)-like gene, was also deleted in the part downstream of *optrA* in isolate G20 (Figure 3a). Thus, the *optrA* segments in isolates E016 and G20 are embedded between a complete Tn*558* and Tn*554* relics of different sizes, which were inserted into the gene *radC* encoding a DNA repair protein (Figure 3a). The *radC* gene is known to be the common integration site for transposons of the Tn*554* family, such as Tn*5406*, Tn*554*, Tn*558* and Tn*559* in staphylococci and enterococci.[16,18,28–31] Previous studies have shown that the functionally active Tn*5406*, Tn*554*, Tn*558* and Tn*559* could excise from their host DNA and form circular intermediates that precede their re-insertion into their target site.[16,28–30] Inverse PCRs to detect such circular forms in isolates E016 and G20 failed in repeated experiments suggesting that the Tn*558* elements in both *E. faecalis* isolates could not transpose.

Transposons of the Tn*554* family do not contain inverted repeats at their termini and do not generate a duplication of the target sequence at the integration site.[16] Studies on serial transposition of the best-studied transposon of this family, Tn*554*, into different target sites showed that the sequences at the Tn*554* junctions varied with respect to the target sites. With each new transposition event, the sequence originally present in the target site is found at the left end of the transposon, whereas the former left-end junction is then found at the right end and the former right-end junction is lost.[16,29,32] In *E. faecalis* isolates E016 and G20, the integration of the Tn*558*/Tn*554* flanked segment occurred within the *radC* coding sequence as depicted in Figure 3(b). The hexanucleotide sequence 5′-AATCCG-3′ at the left-end junction of Tn*558* and the sequence 5′-GATGTA-3′ at the right-end junction of the Tn*554* relic were seen in both isolates E016 and G20 and indicated the boundaries of the integrated segment. A comparison of the so far seen target sites of transposons of the Tn*554* family in staphylococci and enterococci with the one identified in this study is presented in Figure 4.

In *E. faecalis* LY4 of chicken origin, the region including the *optrA* and the *erm*(A)-like gene showed >98% nucleotide sequence identity to the corresponding regions detected in all *erm*(A)-like-positive plasmids characterized in this study. The remaining parts of the *optrA* flanking regions, however, differed from those in the other isolates. Upstream of the *optrA* in LY4, an intact IS*1542* element was detected, which was 81% homologous to IS*256* and has been detected in the genomes of 26 of 28 *vanA*-positive enterococci isolated from poultry in the UK and Ireland.[33]

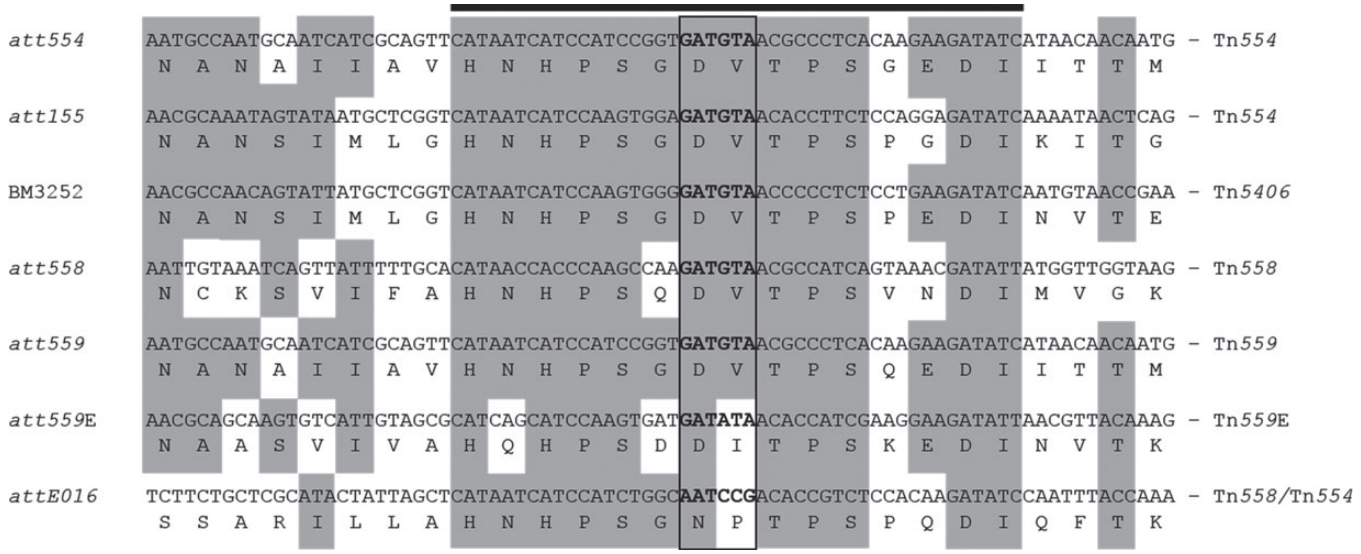

Figure 4. Nucleotide and amino acid sequence alignment of the attachment sites *att554* of Tn*554* in *S. aureus* N3159 and *att155* in *Staphylococcus epidermidis*, that of Tn*5406* in *S. aureus* strain BM3252, *att558* of Tn*558* in plasmid pSCFS2 from *Staphylococcus lentus*, *att559* in *S. aureus*, *att559*E in *E. faecium* and *attE016* in *E. faecalis*. Comparative sequences were taken from a previous study.[31] Grey shading indicates identical amino acids found in at least four of the seven aligned sequences. Hexanucleotide core sequences of the integration sites are framed. Black bar above the *att554* sequence indicates the minimum sequence required for transposition into this site as determined by deletion analysis using Tn*554*.[29]

In the remaining four *E. faecalis* isolates (E147, 5-7, 10-120 and E381), only the segment containing *optrA* and *fexA* was detected. Although this segment showed a high level of homology to the corresponding segments of the *optrA*-positive plasmids, the flanking regions of the plasmid-borne *fexA-optrA* segment were not detected in the chromosomal DNA of these four isolates. Moreover, no relics of mobile genetic elements, such as IS*1216*, were observed in these flanking regions. It is unknown how the *fexA-optrA* segment was integrated in these cases into the chromosomal DNA.

In conclusion, the present study provided insight into the structural diversity of the regions flanking the *optrA* gene when located on plasmids or in the chromosomal DNA of unrelated *E. faecalis* isolates of human and food animal origin. The observation that IS*1216E* elements are in close vicinity of the *optrA* gene and that *optrA*-carrying segments can be 'looped out' via IS*1216E*-mediated recombination, points towards the role of this IS element in dissemination of the *optrA* gene. Moreover, the fact that the *optrA* gene is co-located with other resistance genes on the same conjugative plasmids also opens options for the transfer of such multiresistance plasmids and their co-selection and persistence under the selective pressure of antimicrobial agents other than phenicols and oxazolidinones. Little is known about the mechanisms of chromosomal integration of the *optrA* gene although the close proximity to Tn*558* and Tn*554* suggests an involvement of these transposons. Further studies are needed to identify the ways by which *optrA* was integrated into the chromosomal DNA and plasmids, particularly when no IS elements and other mobile genetic elements seem involved.

Funding

This study was financially supported by grants from the National Natural Science Foundation of China (31370046), the National Basic Research Program of China (2013CB127200), the German Federal Ministry of Education and Research (BMBF) through the German Aerospace Center (DLR), grant number 01KI1301D (MedVet-Staph 2), the National Natural Science Foundation of China (81501774, 81572033) and the Chinese Universities Scientific Fund (2015DY004).

Transparency declarations

None to declare.

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