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Antibiotic and antiseptic resistance genes are linked on a novel mobile genetic element: Tn*6087*

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Objectives: Tn*916*-like elements are one of the most common types of integrative and conjugative element (ICE). In this study we aimed to determine whether novel accessory genes, i.e. genes whose products are not involved in mobility or regulation, were present on a Tn*916*-like element (Tn*6087*) isolated from *Streptococcus oralis* from the human oral cavity.

Methods: A minocycline-resistant isolate was analysed using restriction fragment length polymorphism (RFLP) analysis on amplicons derived from Tn*916* and DNA sequencing to determine whether there were genetic differences in Tn*6087* compared with Tn*916*. Mutational analysis was used to determine whether the novel accessory gene found was responsible for an observed extra phenotype.

Results: A novel Tn*916*-like element, Tn*6087*, is described that encodes both antibiotic and antiseptic resistance. The antiseptic resistance protein is encoded by a novel small multidrug resistance gene, designated *qrg*, that was shown to encode resistance to cetyltrimethylammonium bromide (CTAB), also known as cetrimide bromide.

Conclusions: This is the first Tn*916*-like element described that confers both antibiotic and antiseptic resistance, suggesting that selection of either antibiotic or antiseptic resistance will also select for the other and further highlights the need for prudent use of both types of compound.

Keywords: ICEs, CTAB, tetracycline resistance, IS*1216*, Tn*916*, conjugative transposon

Introduction

The Tn*916*/Tn*1545* family is the most widespread of the conjugative transposons. Tn*916* was first discovered in the late 1970s when tetracycline resistance was transferred from a resistant to a susceptible strain of *Enterococcus faecalis* in the absence of a detectable plasmid.[1] Subsequently a variety of Tn*916*-like elements have been described[2,3] with elements containing different recombinases and resistance genes. These elements comprise four functional modules: conjugation, regulation, recombination and accessory genes, the latter are often antibiotic resistance genes.[3] Most Tn*916*-like elements possess the tetracycline resistance gene *tet*(M), located within the regulatory module, and some contain macrolide resistance genes *erm*(B) (e.g. Tn*1545*, Tn*6002* and Tn*6003*)[4,5] and *mef*(A) (e.g. Tn*2009* and Tn*2017*),[6,7] as well as kanamycin (*aphA-3*) (Tn*1545*)[5] and mercury (*mer*) (Tn*6009*)[8] resistance genes. Tn*916*-like elements have been

found in >30 bacterial genera and are common among the human oral commensal bacteria, which act as a reservoir for resistance genes.[9]

The aim of the current study was to determine whether a Tn*916*-like element found in a minocycline-resistant oral isolate, *Streptococcus oralis* F.MI.5, contained any additional accessory resistance genes. An element, designated Tn*6087*, which contains *tet*(M) and a quaternary ammonium compound (QAC) resistance gene (*qrg*) was identified. Qrg is a protein that confers resistance to cetyltrimethylammonium bromide (CTAB), a cationic antiseptic belonging to the QACs. The co-localization of antiseptic and antibiotic resistance has important clinical ramifications, as the selection of one will lead to the selection of the other. Linkage between antibiotic and antiseptic resistance has been described previously in some *Staphylococcus* species;[10,11] however, this is the first described member of the Tn*916* family of elements encoding antiseptic resistance.

Materials and methods

Sampling, bacterial strains and plasmids

Saliva was obtained from 10 healthy volunteers who had not received antibiotics in the previous 3 months. Ethics approval for the project was obtained from the University College London (UCL) Ethics Committee (project no. 1364/001). Pooled saliva was cultured anaerobically on fastidious anaerobe agar (Oxoid) and at 5% CO_2 on Columbia blood agar (Oxoid) at 37°C. All media contained 2 mg/L minocycline and were incubated for 48 h. Bacterial strains and plasmids used in this study are listed in Table S1 (available as Supplementary data at *JAC* Online).

Molecular biology

DNA was extracted using the ArchivePure DNA Yeast and Gram Positive Bacteria Kit (5Prime) according to manufacturer's instructions. Mutanolysin at a final concentration of 15 μg/mL and lysozyme at a final concentration of 3 μg/mL were added at the cell lysis step of the protocol. DNA was resuspended in molecular-grade water (Sigma-Aldrich) and stored at −20°C.

The sequences of all primers used in the study are shown in Table S2 (available as Supplementary data at *JAC* Online). Standard PCR was carried out using BioMix™ Red (Bioline). Long PCR (using O1F and CT25R; CTn9824F and PW01R spanning almost the entire Tn*916*) was carried out with TaKaRa Ex Taq™ (Lonza) according to the manufacturer's instructions.

Single specific primer (ssp)-PCR was performed in order to find the target sequence for the insertion of Tn*6087*. Genomic DNA was digested with HindIII and EcoRI and ligated into HindIII/EcoRI digested and dephosphorylated pUC19. A primer specific to the left end or right end (LEO, REO, respectively) of the transposon and/or M13F or M13R were used to amplify the end of the transposon and the flanking sequence. LEO and REO were used in a PCR to amplify the joint of the excised Tn*6087* in circular form. DNA sequencing was carried out at the UCL Wolfson Institute DNA sequencing facility.

Southern blot hybridization was performed on HincII- and HindIII-digested genomic DNA of the strain containing Tn*6087* using a probe derived from the Tn*916 int* gene (U09422, 16 704–17 725 bp) and the IS*1216* sequence (HQ663849, 9139–9857 bp and 11 456–12 174 bp) from within Tn*6087*.

Genotypic characterization of the novel transposon

The entire Tn*6087* element was sequenced using primers O1F, CTn670F, CTn1320F, CTn1960F, CT14F, CT14-19 W1F, CTn4028F, CTn4470F, CT14-19 W3F, CTn5109F, CT20F, CTn6352F, CTn6990F, O7F, CTn8286F, CTn9824F, CTn10462F, CTn10593R, CTn11122F, O11F, CTn12353F, RT1-4 W2F, CTn13562F, CTn14122F, CTn14749F, CTn15401F, CTn16068F, CTn16707F, PW01F and PW01R resulting in at least triple coverage. The transposon name was designated by the transposon registry (http://www.ucl.ac.uk/eastman/tn/).[12]

Primers (W1F, Tn6087 IS1R, Tn6087 IS2F, W1R) were used in PCR to investigate if the excision of the IS*1216* elements could be detected in *S. oralis* F.MI.5. Primers SMR-01 and SMR-02 were used to detect *qrg* in metagenomic DNA isolated from pooled saliva and faecal samples taken from Norway, Finland, France and Italy.[13] Each sample (either saliva or faecal) from each country is a pooled sample derived from 20 healthy individuals.

Mutation of qrg

A construct with identical sequence (~550 bp upstream of *qrg* and 400 bp downstream) to the regions flanking *qrg*, but with the chloramphenicol resistance gene *cat*(P) (derived from the plasmid pMTL900)[14] inserted in its place, was used in an allelic replacement experiment to knock out *qrg*. The construct was cloned into pGEM T-Easy vector, linearized and transformed into *S. oralis* F.MI.5. For transformation, the recipient strain was grown to exponential phase; at this point 1 mg of the construct was mixed with the cells and the mix was incubated for 3 h at 37°C prior to spreading on selective plates. Chloramphenicol-resistant (10 mg/L) colonies were then selected for further analysis. One strain with the correct mutation was designated *S. oralis* F.MI.5Δ*qrg*. For complementation, a construct containing *qrg* and flanking regions was inserted into the BamHI site of the shuttle vector pVA838[15] and transformed into F.MI.5Δ*qrg*. The complemented strain was selected on chloramphenicol (10 mg/L) and CTAB (32 mg/L). The complemented strain was designated *S. oralis* F.MI.5Δ*qrg* pVA838::*qrg*.

Transfer studies

Mating experiments were performed using *S. oralis* F.MI.5 as a donor and both *Enterococcus faecalis* JH2-2 and *Streptococcus pneumoniae* DP1004 as recipients using two previously described methods.[16,17] As these experiments yielded no transconjugants, transformation experiments were performed. *S. oralis* F.MI.5 genomic DNA (10 μg) was added to minocycline-susceptible *Streptococcus australis* FRStet12 (Table S1) while competent during the exponential growth phase. Transformants were selected on media containing 4 mg/L minocycline.

MICs

MICs were investigated according to the BSAC guidelines (http://www.bsac.org.uk/) using tetracycline, minocycline, CTAB, acriflavine, Acridine Orange and ethidium bromide. These were carried out using *S. oralis* F.MI.5, *S. australis* FRStet12 and *S. australis* FRStet12::Tn*6087*. Iso-sensitest agar containing 5% horse blood was used and plates were incubated at 37°C for 24 h. Antibiotics and antiseptics were used at concentrations ranging from 0 to 128 mg/L. All cultures were used at a 1/10 dilution of the 0.5 McFarland standard. MICs for CTAB were also investigated for *S. oralis* F.MI.5Δ*qrg* and F.MI.5Δ*qrg* pVA838s::*qrg*.

Results

Isolation of Tn6087 and identification of qrg

Minocycline-resistant *S. oralis* F.MI.5 was isolated from pooled saliva. Partial 16S rRNA gene sequencing showed that the strain had 99.7% identity to *S. oralis* strain VS2971S (AY944234).

To identify the gene responsible for minocycline resistance, degenerate primers (RPP-F and RPP-R) (Table S2), which amplify ribosome protection genes, were used to amplify DNA from *S. oralis* F.MI.5. Sequencing of the PCR products showed that *tet*(M) was present, and further PCR showed that it was located on a Tn*916*-like element. Two long PCRs spanning almost the entire length of Tn*916* then indicated that the conjugation module was ~3 kb longer than that from wild-type Tn*916* in *Bacillus subtilis* BS34A (Tables S1 and S3; available as Supplementary data at *JAC* Online). The entire element was subsequently sequenced, and designated Tn*6087* (Figure 1). Excluding the insertion in the conjugation region, Tn*6087* is 99.03% identical to Tn*916* (U09422) at the nucleotide level. The base pair differences result in some changes to the open reading frames (ORFs) of the element. O*rf24* contains a stop codon at 263 bp, resulting in a 16 amino acid truncation in the polypeptide. Also, *orf20* contains a premature stop codon at
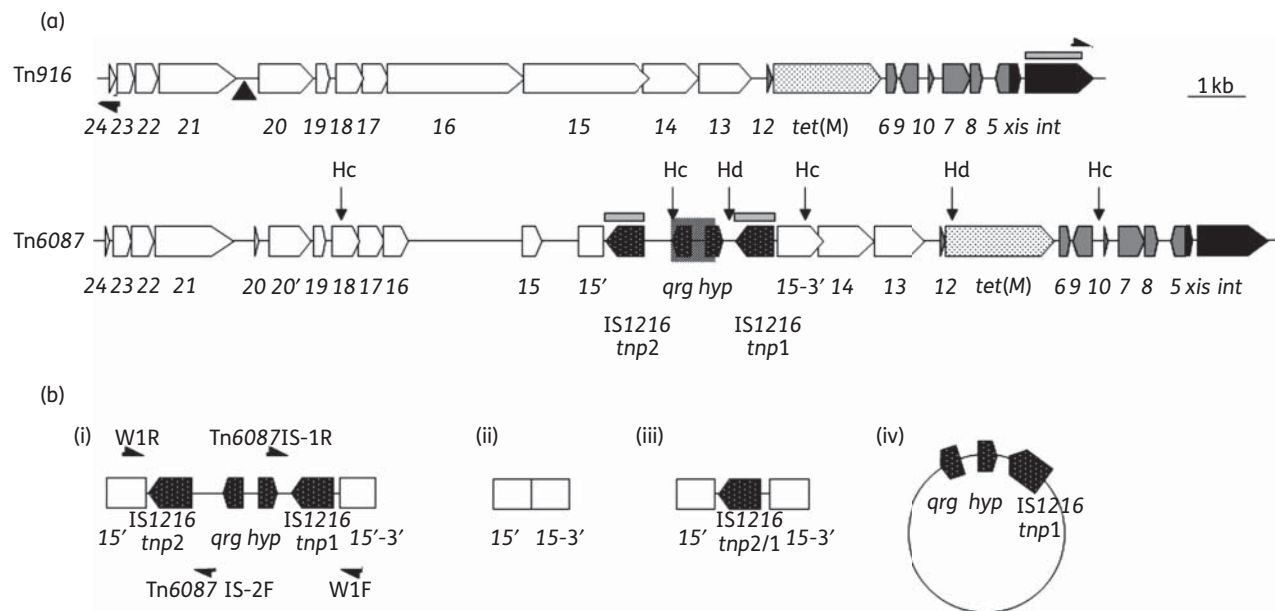
Figure 1. (a) A schematic representation of Tn*916* and Tn*6087* showing the functional modules (conjugation in white; regulation in light grey; the accessory gene in white with black dots; and recombination in dark grey). The position of the primers LEO (at the left end) and REO (at the right end) are shown on Tn*916*. A black triangle indicates the position of the origin of transfer, *oriT*, of Tn*916*. The IS*1216* flanked insertion of Tn*6087* is shown in black with white dots. The shaded region between the IS*1216* sequences represents the sequence similar to *S. gallolyticus* strain UCN34 (FN597254). HindIII and HincII (denoted Hd and Hc) restriction sites are shown; the *int* and IS*1216* probes are shown as grey rectangles appearing above the relevant genes. (b) The four forms of the *qrg* gene and IS*1216* insertion obtained using PCR analysis of *S. oralis* F.MI.5 with the primers shown in Figure 1b(i). (i) The entire 3124 bp insertion within *orf15* (primers W1R, Tn*6087* IS-2F, Tn*6087* IS-1R, W1F are shown from left to right); (ii) the entire insertion excised from *orf15*; (iii) only a chimeric form of the two IS*1216* sequences remaining; and (iv) a circular molecule consisting of the *qrg* gene, the hypothetical protein and the IS*1216* 1 sequence.

2906 bp, resulting in a 314 amino acid truncation; *orf16* contains a stop codon at 5621 bp, resulting in a 673 amino acid truncation; and *orf15* contains a stop codon at 7997 bp, resulting in truncated protein of 119 amino acids. The Tn*6087* *tet*(M) is 99.7% identical to *tet*(M) from the *E. faecalis* Tn*916*-like conjugative transposon (DQ223248), and is 93.85% identical to *tet*(M) of Tn*916* (U09422).

A 3124 bp composite transposon-like structure was found inserted in *orf15* at 9099 bp, which contained ORFs predicted to encode a small multidrug resistance protein and a hypothetical protein, flanked by IS*1216* elements (Figure 1). The two IS*1216* elements were 99.38% identical to each other at the nucleotide level. Approximately half (812 bp) of the region between the two IS*1216* sequences, including the small multidrug resistance gene *qrg* (Figure 1), showed 95% identity to genomic sequence from a *Streptococcus gallolyticus* strain UCN34 (FN597254).

Southern blots probed with Tn*916* *int* and IS*1216* confirmed that *S. oralis* F.MI.5 contained only one copy of the *int* gene and two IS*1216* elements, suggesting that the strain possessed a single copy of Tn*6087* (results not shown).

MICs of minocycline, tetracycline, CTAB, acriflavine, Acridine Orange and ethidium bromide were carried out on *S. oralis* F.MI.5, *S. australis* FRStet12 and *S. australis* FRStet12::Tn*6087*. The results showed that the presence of Tn*6087* increased resistance to minocycline, tetracycline and CTAB (16-fold, 32-fold and 16-fold, respectively; Table 1). A 2-fold increase in resistance to acriflavine was also detected; however, there was no difference

Table 1. MICs of antibiotics and QACs (mg/L)

	TET	MIN	EB	AO	AF	CTAB
S. oralis F.MI.5 Tn*6087*	32	8	8	8	4	64
S. oralis F.MI.5 Tn*6087*Δ*qrg*	32	8	8	8	4	16
S. oralis F.MI.5 Tn*6087*Δ*qrg* pVA838::*qrg*	32	8	8	8	4	32
S. australis FRStet12	4	0.25	8	16	4	4
S. australis FRStet12::Tn*6087*	128	4	8	16	8	64

TET, tetracycline; MIN, minocycline, EB, ethidium bromide; AO, Acridine Orange; AF, acriflavine; CTAB, cetyltrimethylammonium bromide.

seen in resistance to Acridine Orange or ethidium bromide. A 16-fold increase in resistance to CTAB suggested that Qrg, encoded for by Tn*6087,* provided resistance to some QACs. To prove this, *qrg* was replaced by the chloramphenicol resistance gene *cat*(P) in *S. oralis* F.MI.5, generating *S. oralis* F.MI.5Δ*qrg*. MICs for CTAB were performed on these strains and on a complemented F.MI.5Δ*qrg* designated *S. oralis* F.MI.5Δ*qrg* pVA838::*qrg*. The results showed a 4-fold decrease in CTAB resistance in F.MI.5Δ*qrg* and an increase of resistance to CTAB in F.MI.5Δ*qrg* pVA838::*qrg* (Table 1), confirming that *qrg* was responsible for CTAB resistance.

To investigate if *qrg* is present in other populations, PCR analysis of oral and faecal metagenomic DNA taken from healthy volunteers from four European countries was performed. All

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(a)  CTTACTAGCTGGTTTTTTT  AAGATA  AAAAAAGACGAGAAA
(b)  CTTACTAGCTGGTTTTTTT  TATATA  AAAATAGCATAAAAA
(c)  AGAAATTTATACTTTGTTT  AAGATA  AAAAAAGACGAGAAA
(d)  AGAAATTTATACTTTGTTT  AAGATA  AAAATAGCATAAAAA
                         TATATA
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Figure 2. (a) The Tn*6087* target site within *S. oralis* F.MI.5. (b) The left end and (c) right end of Tn*6087* are shown in bold. (d) The joint of the circular intermediate of Tn*6087* is shown. The transposon's coupling sequences are shown in bold italics. As can be seen in (d), the coupling sequence at the joint of the circular form is a heteroduplex.

samples (eight samples in total; pooled saliva and pooled faecal samples from four countries) contained amplicons of the expected size. Subsequent sequence analysis of the PCR products amplified from saliva samples were shown to have an identity of at least 97.81% to *qrg* from Tn*6087*.

Tn*6087* could be transferred by transformation to *S. australis* FRStet12

Excision and circularization of Tn*6087* was detected using PCR for the joint of the circular form in strain *S. oralis* F.MI.5. However, no transconjugants could be isolated following mating experiments with both *E. faecalis* JH2-2 and *S. pneumoniae* DP1004 as recipients (numbers of donor cells varied from 1.6×10^7 to 1×10^{10} and numbers of recipient cells varied from 1.5×10^7 to 2.6×10^{10}). Tn*6087* was transferred by transformation using *S. oralis* F.MI.5 genomic DNA and the minocycline-susceptible recipient *S. australis* FRStet12 at a frequency of $\sim 1.2 \times 10^{-8}$ transformants/µg of DNA. A minocycline-resistant transformant was isolated and PCR analysis confirmed that the transformant possessed the entire Tn*6087* element. Sequencing of the *sod*(A) gene confirmed transfer, as the sequence in the recipient was identical to that of the transformant but different from that of the donor (99.32% identity). The presence of a circular intermediate of Tn*6087* was confirmed in the transformant by performing a PCR with primers LEO and REO (Table S2). The target sites of Tn*6087* were investigated using ssp-PCR in the transformant and were identical to *S. oralis* F.MI.5 (Figure 2).

The region containing IS*1216* undergoes genetic rearrangement

In order to investigate the plasticity of the insertion within *orf15* of Tn*6087*, a series of PCRs were carried out to detect recombination and/or transposition products using *S. oralis* F.MI.5 genomic DNA. PCR analysis showed at least two different rearrangements were present in the region of the transposon containing IS*1216* (Figure 1b). The first was a complete deletion of the IS elements and the DNA between them [Figure 1b(ii)]. In the second, a single chimeric copy of the IS element remained [Figure 1b(iii)], and in addition a circular form of the element containing one IS element was detected [Figure 1b(iv)]. It is likely that the sequences seen in Figure 1[b(iii) and b(iv)] arose by separate recombination events between the directly repeated IS elements. One reaction deletes the DNA between the ISs, resulting in one chimeric copy of IS*1216* within Tn*6087*, and the other reaction results in a circular molecule containing a copy of IS*1216* and *qrg*, as observed.

Discussion

This is the first reported Tn*916*-like element that encodes both antibiotic and antiseptic resistance to tetracyclines and CTAB, respectively. Plasmids encoding both antiseptic and antibiotic resistance have been described previously.[10,11,18,19] The gene combinations found on plasmids have usually been *qacA/B/C* and *blaZ* in *Staphylococcus* sp., and these are often associated with IS elements such as IS*256* and IS*257*.[10,20,21] In one case a plasmid-located *qacB* was found adjacent to the incomplete and non-functional β-lactamase-encoding transposon Tn*552*.[10] Additionally, a putative *qacF* was found on a transposon present on a plasmid belonging to the IncP-1β family.[18]

IS*1216* elements have been associated with transposons, e.g. Tn*1546*, which encodes resistance to vancomycin,[22,23] and the partially sequenced Tn*5397*-like *erm*(B) containing transposon Tn*1116*.[24] Additionally, a composite transposon present on a plasmid comprising a benzalkonium chloride (a QAC) resistance cassette (ORFs *bcrABC*) flanked by IS*1216* elements was recently reported.[25] In another example, IS*1216* elements have been involved in the evolution of the multiresistant plasmid pK214.[26] This 30 kb plasmid encodes resistance to tetracyclines, macrolides, chloramphenicol and streptomycin and contains three copies of IS*1216*. In this case the IS*1216* sequences seem to have been involved in the insertion of a set of *Staphylococcus*-derived genes including a chloramphenicol acetyltransferase and a streptomycin adenyltransferase, as well as the insertion of a group of Tn*916*-like genes including *tet*(S) and spanning part of the regulation module.

In addition to the novel architecture, Tn*6087* also represents the first proven QAC resistance gene identified in oral bacteria. The oral environment is home to a rich and complex community of bacteria that have ample opportunity to exchange genes by multiple mechanisms.[9] It was not possible to show transfer by conjugation to the proven recipient strains *E. faecalis* JH2-2 or *S. pneumoniae* DP1004; however, this is likely to be due to the insertion of the IS*1216*-like element containing *qrg* into the region thought to be involved in conjugation, as well as the truncation of a number of other conjugation genes. It is also possible that Tn*6087* might be able to transfer by conjugation if the conjugation functions lost are provided *in trans* by another mobile element. However, the element could transfer by transformation, a mechanism of genetic exchange likely to be important in the oral cavity due to the large number of naturally competent streptococci. The *qrg* gene was shown by PCR to be present in metagenomic oral and faecal DNA isolated from other European countries, demonstrating that the gene is likely to be distributed throughout Europe.

This work has resulted in the detection of a novel small multidrug resistance gene, *qrg*, that encodes resistance to CTAB. It is located on a composite transposon consisting of two IS*1216* elements flanking *qrg*, inserted in a Tn*916*-like element capable of transfer by transformation to another streptococcal strain.

Transparency declarations

None to declare.

Supplementary data

Tables S1–S3 are available as Supplementary data at *JAC* Online (http://jac.oxfordjournals.org).

References

1 Franke AE, Clewell DB. Evidence for a chromosome-borne resistance transposon (Tn*916*) in *Streptococcus faecalis* that is capable of conjugal transfer in the absence of a conjugative plasmid. *J Bacteriol* 1981; **145**: 494–502.

2 Flannagan SE, Zitzow LA, Su YA *et al*. Nucleotide-sequence of the 18-Kb conjugative transposon Tn*916* from *Enterococcus faecalis*. *Plasmid* 1994; **32**: 350–4.

3 Roberts AP, Mullany P. A modular master on the move: the Tn*916* family of mobile genetic elements. *Trends Microbiol* 2009; **17**: 251–8.

4 Warburton PJ, Palmer RM, Munson MA *et al*. Demonstration of *in vivo* transfer of doxycycline resistance mediated by a novel transposon. *J Antimicrob Chemother* 2007; **60**: 973–80.

5 Cochetti I, Tili E, Mingoia M *et al*. *erm*(B)-carrying elements in tetracycline-resistant pneumococci and correspondence between Tn*1545* and Tn*6003*. *Antimicrob Agents Chemother* 2008; **52**: 1285–90.

6 Del Grosso M, Camilli R, Libisch B *et al*. New composite genetic element of the Tn*916* family with dual macrolide resistance genes in a *Streptococcus pneumoniae* isolate belonging to clonal complex 271. *Antimicrob Agents Chemother* 2009; **53**: 1293–4.

7 Del Grosso M, d'Abusco AS, Iannelli F *et al*. Tn*2009*, a Tn*916*-like element containing *mef*(E) in *Streptococcus pneumoniae*. *Antimicrob Agents Chemother* 2004; **48**: 2037–42.

8 Soge OO, Beck NK, White TM *et al*. A novel transposon, Tn*6009*, composed of a Tn*916* element linked with a *Staphylococcus aureus mer* operon. *J Antimicrob Chemoth* 2008; **62**: 674–80.

9 Roberts AP, Mullany P. Oral biofilms: a reservoir of transferable, bacterial, antimicrobial resistance. *Expert Rev Anti Infect Ther* 2010; **8**: 1441–50.

10 Sidhu MS, Heir E, Sorum H *et al*. Genetic linkage between resistance to quaternary ammonium compounds and beta-lactam antibiotics in food-related *Staphylococcus* spp. *Microb Drug Resist* 2001; **7**: 363–71.

11 Bjorland J, Steinum T, Kvitle B *et al*. Widespread distribution of disinfectant resistance genes among staphylococci of bovine and caprine origin in Norway. *J Clin Microbiol* 2005; **43**: 4363–8.

12 Roberts AP, Chandler M, Courvalin P *et al*. Revised nomenclature for transposable genetic elements. *Plasmid* 2008; **60**: 167–73.

13 Seville LA, Patterson AJ, Scott KP *et al*. Distribution of tetracycline and erythromycin resistance genes among human oral and fecal metagenomic DNA. *Microb Drug Resist* 2009; **15**: 159–66.

14 Roberts AP, Hennequin C, Elmore M *et al*. Development of an integrative vector for the expression of antisense RNA in *Clostridium difficile*. *J Microbiol Methods* 2003; **55**: 617–24.

15 Macrina FL, Tobian JA, Jones KR *et al*. A cloning vector able to replicate in *Escherichia coli* and *Streptococcus sanguis*. *Gene* 1982; **19**: 345–53.

16 Roberts AP, Mullany P, Wilson M. Gene transfer in bacterial biofilms. *Microbial Growth in Biofilms Pt A* 2001; **336**: 60–5.

17 Santoro F, Oggioni MR, Pozzi G *et al*. Nucleotide sequence and functional analysis of the *tet*(M)-carrying conjugative transposon Tn*5251* of *Streptococcus pneumoniae*. *FEMS Microbiol Lett* 2010; **308**: 150–8.

18 Schluter A, Heuer H, Szczepanowski R *et al*. Plasmid pB8 is closely related to the prototype IncP-1 beta plasmid R751 but transfers poorly to *Escherichia coli* and carries a new transposon encoding a small multidrug resistance efflux protein. *Plasmid* 2005; **54**: 135–48.

19 Bjorland J, Sunde M, Waage S. Plasmid-borne *smr* gene causes resistance to quaternary ammonium compounds in bovine *Staphylococcus aureus*. *J Clin Microbiol* 2001; **39**: 3999–4004.

20 Alam MM, Kobayashi N, Uehara N *et al*. Analysis on distribution and genomic diversity of high-level antiseptic resistance genes *qac*A and *qac*B in human clinical isolates of *Staphylococcus aureus*. *Microb Drug Resist* 2003; **9**: 109–21.

21 Berg T, Firth N, Apisiridej S *et al*. Complete nucleotide sequence of pSK41: evolution of staphylococcal conjugative multiresistance plasmids. *J Bacteriol* 1998; **180**: 4350–9.

22 Jensen LB. Internal size variations in Tn*1546*-like elements due to the presence of IS*1216V*. *FEMS Microbiol Lett* 1998; **169**: 349–54.

23 Sletvold H, Johnsen PJ, Wikmark OG *et al*. Tn*1546* is part of a larger plasmid-encoded genetic unit horizontally disseminated among clonal *Enterococcus faecium* lineages. *J Antimicrob Chemother* 2010; **65**: 1894–906.

24 Brenciani A, Bacciaglia A, Vecchi M *et al*. Genetic elements carrying *erm*(B) in *Streptococcus pyogenes* and association with *tet*(M) tetracycline resistance gene. *Antimicrob Agents Chemother* 2007; **51**: 1209–16.

25 Elhanafi D, Dutta V, Kathariou S. Genetic characterization of plasmid-associated benzalkonium chloride resistance determinants in a *Listeria monocytogenes* strain from the 1998–1999 Outbreak. *Appl Environ Microbiol* 2010; **76**: 8231–8.

26 Perreten V, Schwarz F, Cresta L *et al*. Antibiotic resistance spread in food. *Nature* 1997; **389**: 801–2.