Transposon-916-like Elements in Clinical Isolates of *Enterococcus faecium*

By HANSEL M. FLETCHER,* LAURA MARRI†
AND LOLITA DANE0-MOORE

Department of Microbiology and Immunology, Temple University School of Medicine,
Philadelphia, PA 19140, USA

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Tetracycline (Tc) resistance was found in nine out of ten clinical isolates of *Enterococcus faecium*. Conjugative transposons, designated Tn5031, Tn5032 and Tn5033, were present in the chromosome of three isolates. The transposons were similar both structurally and functionally to Tn916 containing the *tetM* determinant. A large non-conjugative plasmid found in a fourth isolate contained an element homologous to Tn916. The four isolates containing the element showing homology to Tn916 exhibited a substantially higher level of Tc resistance than the remaining five Tc-resistant isolates. Tc-resistance genes which have not been identified are apparently responsible for the low-level Tc resistance in five clinical isolates.

INTRODUCTION

In the genus *Enterococcus* there has been an increase in the frequency of reports of multiple antibiotic resistance (Finland, 1979). The markers most frequently carried by these strains include those encoding resistance to tetracycline (Tc), penicillin (Pen), erythromycin (Em), kanamycin (Km), chloramphenicol (Cm) and streptomycin (Sm) (Finland, 1979; Le Bouguenec & Horodniceanu, 1982). In this study, we examined clinical isolates of *Enterococcus faecium* that are resistant to a variety of antibiotics for a possible relationship between a putative conjugative element and resistance to several antibiotics. In an earlier study, Le Bouguenec & Horodniceanu (1982) found that six out of ten clinical isolates of *E. faecium* resistant to Pen, Tc, macrolides, Sm and Km transferred Tc and at least one other resistance marker conjugatively. When Tc resistance was transferred alone, no detectable plasmid was seen in the Tc-resistant transconjugants. This phenomenon is strongly suggestive of a chromosomal location for the *tet* resistance-gene, and a conjugative transposon may be implicated in the conjugative process. However, the *tet* gene was not characterized, nor was the conjugative transposon (Le Bouguenec & Horodniceanu, 1982).

There are numerous examples of drug-resistant clinical isolates that lack detectable plasmid DNA, yet in some of these cases the resistance determinant(s) was transferred by a process resembling conjugation (Clewell & Gawron-Burke, 1986). One of the best-studied systems that involve a nonplasmid resistance transfer is that of Tn916, a Tc-resistant transposon originally described in *Enterococcus faecalis* DS16 and containing the *tetM* determinant (Franke & Clewell, 1981; Gawron-Burke *et al.*, 1986). This 16.4 kb conjugative transposon, appears to encode information for its own transfer and transposition functions (Senghas *et al.*, 1988). In addition, Tn916 upon transfer can insert itself in a variety of locations in the chromosome of transconjugants of various species (Gawron-Burke & Clewell, 1982; Gawron-Burke *et al.*, 1986); its transfer is a Rec-independent function and its can transfer from the chromosome to an *E. faecalis* haemolysin plasmid where it inserts within locations that result in hyperexpression or

† Present address: Istituto di Microbiologia, Via Laterina 8, 53100 Siena, Italy.

hypoexpression of the haemolysis gene (Franke & Clewell, 1981; Gawron-Burke & Clewell, 1982).

The initial description of Tn916 ushered in an era where dissemination of similar elements has been demonstrated in other Gram-positive bacteria (Clewell & Gawron-Burke, 1986; Christie *et al.*, 1987; Murray *et al.*, 1988). In this report we describe Tn916-like conjugative Tc-resistant elements in three clinical isolates of *E. faecium*. A fourth isolate contains a Tn916 homologous element on a large non-conjugative plasmid.

METHODS

Bacterial strains, media and reagents. The bacterial strains used in this study are listed in Table 1. Clinical isolates were obtained from Children's Hospital of Philadelphia (CH1, CH2, CH3 and CH4; kindly provided by Dr J. Campos), St Christopher's Hospital of Philadelphia (SC1, SC2, SC3, SC4 and SC5; kindly provided by Dr J. Mortensen) and New York City Hospital (SF8; kindly provided by Dr G. Dunny, Cornell University). All clinical isolates were identified by the Rapid Strep method (API Analytab Products).

Enterococci were grown in Todd-Hewitt broth (THB; Difco) supplemented with L-cysteine (0.1%) and dextrose (2%, w/v). When present in selective agar plates, antibiotics were used at the following concentrations ($\mu\text{g ml}^{-1}$): Tc, 5; Em, 10; rifampin (Rif), 25; fusidic acid (Fa), 25; Sm, 500; spectinomycin (Sp), 500. MICs of antibiotics were determined by the tube dilution method in Antibiotic Medium or Mueller-Hinton broth and/or by the Gram-positive MIC panel (American MicroScan). Antibiotics were obtained from Sigma.

Mating procedures. Filter matings were done essentially as previously described (Franke & Clewell, 1981). An overnight donor culture (0.05 ml) and recipient culture (0.5 ml) were mixed in 4.5 ml fresh THB and cells were collected on a membrane filter (cellulosic white plain 0.45 μm ; Micron Sep). The filters were placed on tryptone soya agar (TSA) plates with 5% (v/v) sheep blood (BBL) and allowed to incubate at 37 °C for 18 to 20 h. The cells were then suspended in 0.5 ml THB, and the suspension was spread on antibiotic-containing plates selective for growth of transconjugants. Plates were examined after 48 h incubation. Donors and recipients were also incubated on antibiotic plates to check for effectiveness of the counterselection. Separate platings where donors alone were plated provided a base for estimating transfer frequency. In experiments using *E. hirae* 9790RF and *E. faecalis* JH2-2 as recipients, the transfer of Tc was not affected by the presence of DNAase ($100 \mu\text{g ml}^{-1}$) and no transfer was detected in broth matings when recipient cells were plated with cell-free supernatants from donor bacteria.

DNA preparation and characterization. Cells were lysed by a modification of the procedure of Le Bouguenec & Horodniceanu (1982). Sedimented cells from a 200 ml 18 h broth culture were suspended in 7.5 ml 25% (w/v) glucose in ET buffer (0.005 M-disodium EDTA, 0.01 M-Tris/HCl, pH 8.5); lysozyme (Boehringer) was then added at a final concentration of 2.5 mg ml⁻¹. After 1 h at 37 °C, pre-digested Pronase (Calbiochem; 1.2 mg ml⁻¹ final concentration) was added and the mixture incubated for 30 min at 37 °C. The protoplast mixture was then treated with RNAase (Millipore) at a final concentration of 0.1 mg ml⁻¹. After 15 min at 37 °C, protoplasts were lysed by the addition of SDS (BDH) at a 1% final concentration. Plasmid-enriched DNA was prepared in the presence of 1 M-NaCl at 4 °C for 16 to 18 h. Macromolecules and some chromosomal DNA was removed by centrifugation at 18000 r.p.m. for 30 min. Isolation of plasmid DNA was by equilibrium centrifugation of SDS lysates in ethidium bromide-CsCl gradients essentially as described by Maniatis *et al.* (1982). Chromosomal DNA was extracted from SDS-lysed cells by precipitation of DNA with cold ethanol and spooling on a glass rod (Rodriguez & Tait, 1983).

Table 1. *Bacterial strains used in this study*

Strain	Relevant chromosome markers	Plasmid content	Source or reference
<i>E. faecium</i>			
19434	None	None	ATCC
19434RF	<i>rif fus</i>	None	This study
<i>E. hirae</i>			
9790		30 kb cryptic plasmid	ATCC
9790RF	<i>rif fus</i>	30 kb cryptic plasmid	This study
9790SS	<i>str spc</i>	30 kb cryptic plasmid	This study
<i>E. faecalis</i>			
JH2-2	<i>rif fus</i>	None	Franke & Clewell (1981)
JH2SS	<i>str spc</i>	None	Tomich <i>et al.</i> (1978)
FA2-2	<i>rif fus</i>	pAM714 (pAD1::Tn917)	Ike & Clewell (1984)
UV202	<i>rif fus</i>	None	Franke & Clewell (1981)

Agarose gel electrophoresis was done in a horizontal gel apparatus (model HE 99; Hoefer Scientific Instruments). Samples were loaded into wells in a 0.7% agarose gel and electrophoresed at 30 V for 14 to 20 h. Electrophoresis was done at room temperature in TBE buffer (0.089 M-Tris, 0.08 M-boric acid, 0.01 M-EDTA, pH 8.0). The gels were stained with a solution of ethidium bromide (1 µg ml⁻¹) for 20 min, and destained with 1 mM-MgSO₄ for 45 min at room temperature.

Restriction endonucleases. DNA was digested with the following enzymes as specified by the manufacturer: *Hind*III, *Hinc*II and *Eco*RI (all from International Biotechnologies Inc.).

DNA-DNA hybridization. DNA blot transfer utilized the Vacu-Blot transfer system (model Vac-1000; American Bionetics) and was done by the method of Southern (1975). Hybridization was done at 68 °C and the blot was washed at 50 °C with 0.1 × SSC (1 × SSC is 0.15 M-NaCl, 0.015 M-sodium citrate, pH 7.0) and 0.1 % SDS. The following probes were used in hybridization experiments: (i) pAM150 (Gawron-Burke & Clewell, 1984), which carries the conjugative transposon Tn916; (ii) the 4.8 kb *Hinc*II fragment of pAM150 which contains the *tetM* determinant of Tn916 (Gawron-Burke *et al.*, 1986); (iii) pMV158 (Burdett *et al.*, 1982), which carries the *tetL* gene. Plasmid DNA labelled with [α -³²P]dCTP (ICN Radiochemicals) was prepared using the Multiprime DNA labelling system (Amersham). Autoradiography was done at -70 °C using a Du Pont Cronex intensifying screen.

RESULTS

Table 2 shows the antimicrobial-susceptibility patterns of 10 clinical isolates of *E. faecium*, and of the type strain ATCC 19434. Eight of ten clinical isolates were resistant to Em and clindamycin (C1). The same eight isolates were resistant to ampicillin (Am) and Pen. All but one isolate were resistant to Tc. Four had a high level of resistance (128 µg ml⁻¹) and five had a low level of resistance (8 to 16 µg ml⁻¹). An apparent, naturally occurring, low-level gentamicin (Gm) resistance was found in all the clinical isolates and in the ATCC strain. All clinical isolates exhibited a high level of resistance to Sm and eight out of ten were resistant to Km.

In streptococci the *tetL* gene confers resistance to Tc but not to minocycline (Mi), whereas the *tetM* gene confers resistance to both antibiotics (Burdett *et al.*, 1982). Table 2 shows that all nine clinical isolates resistant to Tc were also relatively resistant to Mi.

Fig. 1(a) shows that cellular DNA from CH1, CH3 and SF8 contains several similar plasmids smaller than about 10 kb. CH2, CH4 and SC1 contain a limited number of small plasmids, and SC2, SC3, SC4 and SC5 have no detectable small plasmids. Although not necessarily shown in Fig. 1(a) all isolates contain a large plasmid over 30 kb in size. Fig. 1(b) shows the results of a Southern blot of the same material hybridized with a probe consisting of a 4.8 kb *Hinc*II fragment of Tn916 containing the *tetM* determinant (Gawron-Burke *et al.*, 1986). In CH1, CH3 and SF8 the probe hybridized to the chromosome only whereas in SC4 the same probe hybridized to the large plasmid as well as with linear DNA. None of the other isolates showed homology with *tetM*. pMV158 carrying *tetL* did not hybridize with any DNA from the clinical isolates (not shown).

A chromosomal location for the *tetM* determinant in CH1, CH3 and SF8 was confirmed by several additional experiments involving DNA isolated and fractionated by spooling (Rodriguez & Tait, 1983), a procedure that removes all detectable plasmid DNA or CsCl-purified plasmid DNA. The *tetM* probe hybridized to only chromosomal DNA (data not shown). In contrast, in SC4, the *tetM* determinant appeared to be associated with a large plasmid (Fig. 1b).

Cellular DNA from CH1, CH3 and SF8, and plasmid-enriched DNA from SC4 that showed homology with Tn916 was cut with *Hind*III, a restriction enzyme that cuts Tn916 once within the *tetM* determinant (Gawron-Burke & Clewell, 1982). CH1, CH3 and SF8 gave two indistinguishable junction fragments, 10.8 and 14.5 kb in size, whereas SC4 gave two entirely different junction fragments, 3.4 and 17.5 kb in size (Fig. 2). The presence of only two junction fragments in SC4 is inconsistent with the insertion of a *tetM* determinant in both a plasmid and the chromosome. Therefore the linear DNA fragment, hybridizing with *tetM* in Fig. 1(b), lane I', most likely represents linearized plasmid DNA. A *Hind*III/*Eco*RI double digest of DNA from CH1, CH3 and SF8 gave two indistinguishable junction fragments, about 6.8 and 9.3 kb, whereas the *Hind*III/*Eco*RI digest of SC4 gave two junction fragments, 9.2 and 3.4 kb.

Tn916 has five internal *Hinc*II fragments (Yamamoto *et al.*, 1987; Senghas *et al.*, 1988). When

Table 2. MIC determination for strains of *E. faecium*

Antibiotic	ATCC 19434	MIC ($\mu\text{g ml}^{-1}$)									
		CH-1	CH-2	CH-3	CH-4	SF-8	SC-1	SC-2	SC-3	SC-4	SC-5
Erythromycin*	2	>256	4	>256	4	>256	>256	>256	>256	>256	>256
Clindamycin*	≤ 0.25	>256	≤ 0.25	>256	0.5	>256	256	256	>256	64	>256
Ampicillin†	2	128	8	128	4	128	128	128	128	16	32
Penicillin†	4	128	16	128	16	128	128	128	128	64	64
Chloramphenicol†	4	16	4	16	4	16	4	8	8	2	8
Tetracycline†	≤ 1	128	16	128	16	128	16	16	<1	128	8
Minocycline*	<0.5	32	16	32	16	32	8	16	>0.5	16	16
Gentamicin†	>16	16	8	8	8	8	16	>16	16	8	8
Kanamycin*	<125	>8000	<125	>8000	500	>8000	>8000	>8000	>8000	>8000	>8000
Streptomycin*	<125	>8000	>8000	>8000	>8000	>8000	>8000	>8000	>8000	>8000	>8000

* MICs determined by serial dilution in Mueller-Hinton Broth.

† MICs determined using the commercial Gram-positive MIC panels and procedures from American MicroScan.

Fig. 1. (a) Agarose gel (0.7%) electrophoresis of cellular DNA isolated from clinical isolates of *E. faecium*. Lanes A to D show CH1, CH2, CH3 and CH4 respectively. Lane E shows SF8 and lanes F to J show SC1, SC2, SC3, SC4 and SC5 respectively. (b) Autoradiogram obtained after Southern transfer of DNA from the agarose gel in panel (a), followed by hybridization with the ^{32}P -labelled 4.8 kb *HincII* fragment of pAM150 (see Methods).

HincII-digested DNA of CH1, CH3, SF8 and SC4 was probed with pAM150, homology was detected in CH1, CH3 and SF8 on the fragments which were the same size as the five internal *HincII* fragments of Tn916 (Fig. 3). For SC4, homology was detected on only four such fragments. Further, a 4.8 kb fragment was missing, but a new 7.3 and a 2.1 kb band was seen. An external 3.2 kb fragment not present in Tn916 was seen in CH1, CH3 and SF8 (Fig. 3). When the 4.8 kb *HincII* fragment of pAM150, which contains the *tetM* determinant, was used as a probe, homology was detected on the 4.8 kb *HincII* fragment in CH1, CH3 and SF8, in contrast to the 7.3 kb *HincII* fragment in SC4 (data not shown). Thus CH1, CH3 and SF8 showed the same *HincII* profile. It is to be noted that CH1 and CH3 were isolated from the same hospital, but two years apart from each other, whereas SF8 was isolated in New York City several years previously. Nevertheless, the data from the *HindIII*, *HindIII/EcoRI* and *HincII* digestions indicate that the determinant similar to Tn916 occupies apparently the same location in the chromosome of CH1, CH3 and SF8.

That CH1, CH3 and SF8 are not re-isolates of the same strain was demonstrated by restriction polymorphism. Digestion of cellular DNA from the three isolates using *AvaI* yielded a 6.8 kb band evident only in CH1. A *HindIII* digest of the same DNA yielded a 2.7 kb band present only in CH1 and CH3. Further, an *EcoRI/HindIII* digest gave a 7.5 kb fragment unique only to CH3 (data not shown).

All 10 clinical isolates were used in conjugation experiments by the filter mating procedure. Recipients were *E. faecalis* JH2-2 and *E. hirae* 9790RF, both resistant to Rif and Fa (Table 1). Those antibiotics and Tc, Em and Pen were used for counterselection. Table 3 is typical of the

Fig. 2. Autoradiogram obtained from filter blot hybridization analyses of *Hind*III-digested DNA from clinical isolates of *E. faecium*. Lanes A, B and C show cellular DNA of CH1, CH3 and SF8 respectively. Lane D shows plasmid DNA of SC4. The probe was the ^{32}P -labelled 4.8 kb *Hinc*II fragment of pAM150.

results obtained with either *E. faecalis* or *E. hirae*. CH1, CH3 and SF8 transferred only the *tet* marker at a frequency of 7.3×10^{-6} , 8.6×10^{-8} and 1.1×10^{-7} , respectively. None transferred *pen*. In some experiments *E. faecium* 19434RF (Table 1) was used as the recipient but no transfer was observed when CH1, CH3 or SF8 were used as donors. However, Tc resistance could be transferred conjugatively from an *E. hirae* transconjugant of CH1 to the Tc-susceptible *E. faecium* SC3. In each instance conjugal transfer was checked by determining the antimicrobial susceptibility pattern of a recipient as well as the recipient species.

Since all three isolates (CH1, CH3 and SF8) contained a *tetM*-like determinant in an apparently identical chromosomal location, transconjugants were examined for location of the *tet* determinant, and for the effects of the position of the *tet* determinant on the ability to transfer the marker in a second round of matings. Fig. 4 shows the hybridization pattern of transconjugants from CH1 into *E. hirae*. The *tetM*-like determinants occur on more than one

Fig. 3. Autoradiogram obtained from filter blot hybridization analysis of *HincII*-digested DNA from CH1, CH3, SF8, SC4 and pAM150. Lanes A to C show cellular DNA of CH1, CH3 and SF8 respectively. Lane D shows plasmid DNA of SC4. Lane E shows pAM150. The sizes (kb) of the internal *HincII* fragments of Tn916 are as follows: 5.6, 4.8, 1.6, 1.1 and 0.4. The 0.4 kb band is evident on prolonged exposure. The probe used was the ^{32}P -labelled pAM150.

Table 3. *Transfer of tetracycline-resistance from clinical isolates of E. faecium by conjugation*

The transfer experiments involved overnight filter matings (see text)

Donor	Recipient	Selection	Transfer frequency/ donor cell	Phenotype of transconjugants
CH1	9790RF	Rif, Fa, Tc	7.3×10^{-6}	Rif ^r Fa ^r Tc ^r
CH2	9790RF	Rif, Fa, Tc	$< 2.8 \times 10^{-9}$	—
CH3	9790RF	Rif, Fa, Tc	8.6×10^{-8}	Rif ^r Fa ^r Tc ^r
CH4	9790RF	Rif, Fa, Tc	$< 2.7 \times 10^{-8}$	—
SF8	9790RF	Rif, Fa, Tc	1.1×10^{-7}	Rif ^r Fa ^r Tc ^r
SC1	9790RF	Rif, Fa, Tc	$< 2.2 \times 10^{-8}$	—
SC2	9790RF	Rif, Fa, Tc	$< 2.2 \times 10^{-9}$	—
SC4	9790RF	Rif, Fa, Tc	$< 1.3 \times 10^{-8}$	—
SC5	9790RF	Rif, Fa, Tc	$< 1.2 \times 10^{-9}$	—

Fig. 4. Autoradiogram obtained from filter blot hybridization of cellular DNA from Tc-resistant transconjugants (Table 3). Lanes A to J show *Hind*III-digested DNA from transconjugants which were obtained using CH1 (Fig. 2) as donor. The probe used was the 32 P-labelled 4.8 kb *Hinc*II fragment of pAM150.

chromosomal location and in some cases differ from each other. Five transconjugants (Fig. 4, lanes A, E, H, I and J) gave rise to two chromosome-transposon junction fragments, whereas five other transconjugants (Fig. 4, lanes B, C, D, F and G) gave rise to four or more bands. Similar results were obtained when CH1 was mated with *E. faecalis*. When an *E. hirae* transconjugant containing a Tn916-like element obtained from CH1 was used as donor in a conjugation with *E. faecium* SC3, the marker was also transferred to the chromosome in a variety of locations (data not shown). Consequently, the *tetM*-like determinant that is transferred to recipients in filter matings can insert into several chromosomal locations and can occur in multiple copies in *E. hirae*, *E. faecium* and *E. faecalis*. Similar results were obtained for CH3 and SF8.

When 20 *E. hirae* Tc-resistant transconjugants were examined for ability to conjugate further, 14 did and 6 did not, at least at detectable frequencies (data not shown). Those that did conjugate had conjugation frequencies (10^{-6} to 10^{-8}) similar to the original donor strains (Table 3). The location of the *tetM*-like determinant (as indicated by the sizes of *Hind*III-cut junction fragments hybridizing with *tetM*) did not appear to be specific for transconjugants capable or incapable of further matings. However, of six of the transconjugants that were capable of a second round mating five had between two and three insertions (see for example lanes B, C, D, F and G, in Fig. 4). In contrast, of six transconjugants that were incapable of a second round mating none had multiple insertions (see for example lanes A, E, H, I and J, in Fig. 4).

CH1, CH3 and SF8 were used as donors in overnight filter matings with the recombination-deficient strain UV202, a derivative of *E. faecalis* JH2-2 (Franke & Clewell, 1981). Tc-resistance was transferred to UV202 and to JH2-2 with comparable frequencies.

Table 4. Transfer of tetracycline resistance from derivatives of either CH1, CH3 or SF8 to JH2SS by conjugation

The transfer experiments involved overnight filter matings. JH2SS is a plasmid-free Sm^rSp^r strain (see text)

Donor strain*	Plasmid in donor	Transfer frequency/ donor cell	Haemolysin† (%)
HF14	pAM714	7.2×10^{-7}	+ ^H (3) + (79) - (18)
HF21	pAM714	2.6×10^{-6}	+ ^H (4) + (72) - (24)
HF22	pAM714	2.9×10^{-6}	+ ^H (3) + (73) - (24)

* HF14, HF21 and HF22 are tetracycline-resistant transconjugants from conjugation of CH1, CH3 and SF8, respectively, with 9790RF.

† -, Non-haemolytic; +, haemolytic on 5% (w/v) sheep-blood agar plates; +^H, hyperhaemolytic.

The Tn916-like determinant derived from CH1, CH3 and SF8 was examined for ability to affect expression of the haemolysin gene of pAM714 (pAD1::Tn917) (Ike & Clewell, 1984). pAM714 was introduced into *E. hirae* 9790RF containing the Tn916-like element from CH1, CH3 or SF8. Each transconjugant was used as a donor in conjugations with JH2SS. Tc-resistant transconjugants were obtained with frequencies of 10^{-6} to 10^{-7} per donor cell (Table 4). Of these 3 to 4% were hyperhaemolytic. Some 18 to 24% of the Tc-resistant transconjugants showed loss of haemolysis (Table 4). An *Eco*RI restriction analysis of plasmid DNA in a hyperhaemolytic and a non-haemolytic transconjugant demonstrated the presence of a 16.5 kb insertion equivalent in size to Tn916. Further, in the hyperhaemolytic transconjugant the insertion was in the D fragment of the haemolysin gene (Ike & Clewell, 1984).

DISCUSSION

Of the ten clinical isolates of *E. faecium* studied nine showed resistance to Tc and Mi. Four isolates had high-level resistance to both antibiotics and carried a Tn916-like element. Thus there is a correlation between high-level resistance to Tc and the presence of a Tn916-like element. This is consistent with previous reports on the level of resistance conferred by the *tetM* gene (Burdett *et al.*, 1982). It is noteworthy that none of the isolates showed similarity with the *tetL* determinant. In a study in *E. faecalis*, Pepper *et al.* (1987) found homology with Tn916 in all eight isolates (two on plasmids, six in the chromosome). In addition, three of the eight contained an element homologous to a *tetL* probe. The *E. faecalis* isolates had MICs for Tc of 32 to 128 µg ml⁻¹. The low-level resistance seen here in five *E. faecium* isolates may be due to an as yet undescribed Tc-resistance determinant in enterococci.

Three of the four *tetM*-like elements examined here were clearly conjugative. They were localized in the chromosome and conjugated at a low frequency. One *tetM*-like element was found on a large non-conjugative plasmid. Further, this same plasmid contained the *tet* determinant on a 7.3 kb *Hinc*II fragment, in contrast to a 4.8 kb *Hinc*II fragment in the other three isolates and Tn916. Although fragments which were the same size as the internal *Hinc*II fragments of Tn916 were detected, no transposon function has yet been demonstrated. Tn916-like elements in enterococci seem to occur mostly in the chromosome (reviewed in Clewell & Gawron-Burke, 1986), but in rare cases they have been found on plasmids. The latter have been reported only in *E. faecalis* [pCF10 (Christie *et al.*, 1987); pIP1440 (Pepper *et al.*, 1987)].

We demonstrated that the Tn916-like element is a transposon in three ways: (1) it can be found in a variety of locations and frequently in more than one copy in transconjugants; (2) it

transfers equally well in Rec⁺ and Rec⁻ enterococcal strains; (3) it can transfer from the chromosome to a haemolysin plasmid where it inserts within locations that results in hyperexpression or hypoeexpression of the haemolysin gene.

To our knowledge, this report is the first to provide rigorous evidence of transposons with conjugative properties in *E. faecium*; hence we designate these transposons Tn5031, Tn5032 and Tn5033. The other conjugative transposons so far identified are Tn918 in *E. faecalis* (Clewell *et al.*, 1985), Tn919 in *Streptococcus sanguis* (Fitzgerald & Clewell, 1985), Tn920 and Tn925 in *E. faecalis* (Murray *et al.*, 1988; Christie *et al.*, 1987). All code for Tc-resistance and share homology with Tn916, a 16.4 kb element (Gawron-Burke & Clewell, 1982; Franke & Clewell, 1981).

Although the three chromosomal inserts appear to be in the same chromosomal location, this is not so in the transconjugants. We were puzzled to find a transposon in apparently the same location in three clinical isolates which show restriction polymorphism and were obtained at different times from different places. In *Streptococcus pneumoniae* and other streptococcal strains large transposon-like elements have been shown to contain several antibiotic resistance markers (Clewell & Gawron-Burke, 1986). In some cases conjugative transfer of the resistance traits occurs *en bloc*, while in other cases only *tet* is transferred. Perhaps here only the Tn916-like element can conjugate, but the other resistant markers are present, providing common adjacent restriction sequences. Clewell *et al.* (1988) reported data suggestive of a non-replicative insertion mechanism where Tn916 contains a sequence partially homologous with the target sequence, which facilitates insertion of a circular intermediate of the transposon via a reciprocal recombination. Possibly a site in the clinical isolates contained homologous sequences facilitating insertion. However, our data indicate that in second round conjugations no specific sites were selected for transposon insertion even when an *E. faecium* strain was used as the recipient.

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