Demonstration of in vivo transfer of doxycycline resistance mediated by a novel transposon

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Received 21 March 2007; returned 10 May 2007; revised 6 August 2007; accepted 7 August 2007

Objectives: The aim of this study was to investigate the transfer of bacterial doxycycline resistance between oral bacteria in subjects receiving systemic doxycycline for the treatment of periodontitis.

Patients and methods: Streptococci were cultured before and after treatment from the subgingival plaque of two patients with periodontitis, genotyped and investigated for the presence of antimicrobial resistance determinants and conjugative transposons.

Results: In one subject, a strain of Streptococcus sanguinis resistant to doxycycline was a minor component of the pre-treatment streptococcal flora but dominated post-treatment. In a second subject, a strain of Streptococcus cristatus, which was sensitive to doxycycline before treatment, was found to have acquired a novel conjugative transposon during treatment, rendering it resistant to doxycycline and erythromycin. The novel transposon, named CTn6002, was sequenced and found to be a complex element derived in part from Tn916, and an unknown element which included the erythromycin resistance gene erm(B). A strain of Streptococcus oralis isolated from this subject pre-treatment was found to harbour CTn6002 and was therefore implicated as the donor.

Conclusions: This is the first direct demonstration of transfer of antimicrobial resistance carried on a conjugative transposon between oral bacteria during systemic antimicrobial treatment of periodontitis in humans.

Keywords: transposon, tetracycline, antimicrobial resistance, periodontitis

Introduction

Bacterial resistance to antimicrobials is the primary obstacle to the successful chemotherapy of infectious diseases and is a growing problem.1 Resistance can be spread among bacterial strains in a variety of ways with conjugative transposons (CTn) among the most important.2

Periodontitis is an inflammatory condition of the structures supporting the teeth caused by dental plaque bacteria in susceptible hosts.3 It is normally treated by removal of the bacterial biofilm on the affected root surface in the periodontal pockets formed as a result of the disease. However, in some individuals, including those who have a form of the disease refractory to routine mechanical treatment, additional antimicrobial therapy is indicated, administered either systemically or by local application.4 One of the most commonly prescribed classes of antimicrobials for the treatment of periodontitis is the tetracycline group,5 primarily for their broad-spectrum activity but also because they possess anti-collagenolytic activity, which is of value in protecting the connective tissue of the periodontal ligament from attack by bacterial and host proteases.6

Antibiotic resistance to tetracyclines is widespread, with most bacteria acquiring resistance through the acquisition of mobile genetic elements harbouring genes encoding resistance determinants.7 Thirty-eight such genes have been described to date, 23 of which encode efflux transporters.8 Eleven genes encode ribosomal protection proteins (RPPs) that alter the structure of the bacterial ribosome thus preventing tetracycline activity.9 RPP genes are typically found on transposons that integrate into the bacterial chromosome.10

RPPs are the commonest tetracycline resistance determinants among oral bacteria11,12 with Tet(M) widespread among
commensal organisms. The mouth is therefore a reservoir of resistance genes which could potentially be transferred to pathogens found in the upper respiratory tract, such as *Streptococcus pneumoniae*. It has been hypothesized that bacteria acquire tetracycline resistance through the transfer of conjugative transposons that include these RPP genes. Such transfer has been demonstrated in *vitro*, but definitive evidence that this occurs *in vivo* is lacking.

Resistance to tetracycline among periodontal bacteria has been shown to increase during tetracycline treatment for periodontitis administered both systemically and topically, although the proportion of resistant organisms reverts to baseline levels quickly after the cessation of treatment.

The aim of this study was to determine the mechanisms responsible for the increased proportion of the periodontal microbiota resistant to doxycycline during doxycycline treatment for periodontitis.

### Materials and methods

#### Subjects and samples

Ethics approval for the collection of plaque samples from patients with periodontitis was given by the Guy’s Hospital Research Ethics Committee (ref: 96/9/9). Two patients undergoing routine treatment for generalized severe periodontitis, characterized by subgingival infection affecting the majority of their teeth, gave their informed consent for the collection of subgingival plaque before and 1 week after a course of systemic doxycycline consisting of a 200 mg loading dose, followed by 100 mg per day for 7 days. Subgingival plaque samples were collected using a sterile curette from the diseased root surface beneath the detached gum margin.

#### Bacterial culture

Subgingival plaque samples were serially diluted in reduced transport medium and appropriate dilutions plated out in triplicate onto Blood Agar (BA, LabM, Bury, UK) supplemented with 5% horse blood, Fastidious Anaerobe Agar (FAA, LabM) supplemented with 5% horse blood, Tryptone, Yeast, Cystine agar (TYC) and BA, FAA and TYC supplemented with 3 mg/L doxycycline (BAD, FAAD and TYCD). The BA and TYC plates, with and without doxycycline, were incubated in air + 5% CO₂ for 4 days at 37°C. The FAA plates, with and without doxycycline, were incubated anaerobically (80% N₂, 10% H₂ and 10% CO₂) for 7 days at 37°C. All plates were counted after incubation and the total anaerobic counts and proportion of doxycycline-resistant organisms in the samples were calculated. Approximately 200 colonies were subcultured from the pre-treatment TYC plates and ~100 isolates from the post-treatment TYCD plates. In addition, representative colonies of each morphological type of doxycycline-resistant organism grown on the BAD and FAAD plates were subcultured. This was done to enable the identification of possible source organisms for genes encoding resistance determinants.

#### DNA extraction

Bacterial cells were suspended in TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0) and treated with lysozyme (500 mg/L) for 30 min at 37°C followed by protease K (500 mg/L) and Sarkosyl (2%) for 2 h at 60°C. After two phenol–chloroform extractions and one chloroform extraction, DNA was precipitated by the addition of two volumes of ethanol (at −20°C) and washed in 70% ethanol.

#### PCR and sequencing

PCR amplifications were performed as follows. Initial denaturation was performed at 96°C for 5 min followed by 30 cycles of denaturation at 94°C for 45 s, annealing for 45 s, at the appropriate temperature for each primer set, and extension at 72°C for 90 s. Ten picomoles of each primer was used. Streptococcal isolates were identified using *sodiA* gene sequence analysis. Isolates belonging to other genera were identified by 16S rRNA gene sequence analysis as described previously. 16S rRNA genes were amplified using primers 27F 5'-AGAGTTTGTATCMTGGCTCAG-3' and 1492R 5'-TACGTYACCTTGTTACGACTT-3' and partially sequenced with 519R 5'-GWATTACCGCGGCKGTG-3'. The *tet(M)* gene was amplified using primers *tet(M)2F* 5'-GAACTGAAAGGCTCGG-3' and *tet(M)3R* 5'-ATG GAA GCC CAG AAA GGA T-3'.

#### Typing methods

Bacterial isolates were typed using repetitive extragenic palindromic (REP) gene amplification. PCR amplification was performed using primers REP1R-Dt (5'-NCG NCC NCA TCN GCC-3') and REP2-Dt (5'-NGC NCT TAT CNG GCC TAC-3'). Initial denaturation was performed at 95°C for 7 min, followed by 32 cycles of denaturation at 94°C for 30 s, annealing at 40°C for 1 min and extension at 65°C for 8 min, with final extension at 65°C for 16 min. Amplification products were visualized by electrophoretic separation of 10 µL of the PCR product on a 1.5% agarose gel stained with ethidium bromide and photographed under UV illumination.

#### Determination of transposon insertion point by inverse PCR

Chromosomal DNA was digested with *DraI* restriction endonuclease (New England Biolabs, UK) for 2 h at 37°C. After incubation at 60°C for 20 min to denature the restriction enzyme, the DNA fragments were diluted 1:10, 1:50, 1:100, 1:500 and 1:1000 and ligated with T4 DNA ligase (Promega, UK). Inverse-PCR was performed using two sets of specifically designed oligonucleotides for each end of the *Tn*. The PW01 primer set [PW01(3)-F 5'-AGGAAGAAAAAATCGTATAGG-3' and PW01-R 5'-CTGTTAGGAAGATCCTTTCAG-3'] and the PW03 primer set [PW03-F 5'-GAATGACTTTGTTACGACG-3' and PW03(2)-R 5'-TTTACGAGGCCTTTTCAAGCG-3'] were designed to amplify an internal fragment of the ends of the *Tn*, allowing them to be used to test for the presence of the desired fragments. Primer sets PW02 [PW02 IPCR-F 5'-CGTGAAGTATCTTCCTACAG-3' and PW02(3) IPCR-R 5'-GGCTAACAGTTTTTCTTCCT-3'] and PW04 [PW04(2) IPCR-F 5'-CCGATGAAAACAGGTCGTGA-3' and PW04 IPCR-R 5'-CGTCGTATCACCAGGCTT-3'] were the reverse complement of the internal primer sets PW01 and
PW03, respectively, and were designed to extend outwards in to the flanking regions. PW01 and PW02 amplified the left-hand side of the CTn, whereas PW03 and PW04 were used to amplify the right-hand side.

Conjugal-mating experiments

Conjugal-mating experiments were performed using a standard membrane method. The conjugation frequency of the CTn was expressed as colony forming units (cfu) of transconjugants per cfu of donor.

Determination of number of copies of transposon

Southern blot hybridization was performed on Hybond-H+ membranes (Amersham, UK) using a digoxigenin (DIG)-labelled fragment of the int-Tn and xis-Tn genes generated by PCR using the primers intxis1 and intxis2 described above as the probe, and detected by an anti-DIG-conjugated alkaline phosphatase/BCIP colorimetric system (Roche). Chromosomal DNA was digested with HindIII prior to hybridization.

The GenBank accession number for the nucleotide sequence of CTn6002 is AY898750.

Results

Streptococcal populations before and after treatment with doxycycline

Total anaerobic bacterial counts in the plaque samples were reduced after doxycycline treatment: $7.8 \times 10^6 - 4.2 \times 10^6$ in Subject A and $9.6 \times 10^6 - 2.6 \times 10^6$ in Subject B, while the proportion of anaerobic organisms resistant to doxycycline rose for Subject A from 2.4% to 51.9% but fell in Subject B from 18.8% to 2.9%. Table 1 shows the identity of the streptococcal isolates before and after treatment. In Subject A, 8 of the 122 streptococcal isolates were found to be resistant to doxycycline and were identified as Streptococcus sanguinis (6) and Streptococcus oralis (2). The susceptible and resistant S. sanguinis strains constituted distinct phylogenetic subgroups by sodA gene sequence analysis (Figure 1). Ninety-eight of the 122 resistant isolates after treatment were identified as S. sanguinis. REP-PCR genotyping showed that these isolates belonged to the same clonal type as the resistant S. sanguinis strains seen pre-treatment (Figure 2).

In Subject B before treatment, representatives of five streptococcal species were detected among the isolates cultured on TYC medium. All of the isolates grown on TYC were sensitive to doxycycline, although, as stated above, resistant organisms were detected on the BAD and FAAD media, and included streptococci. After treatment, five species resistant to doxycycline were detected on TYC but only one of these, S. cristatus, was also found before treatment, although it was then doxycycline-sensitive. REP-PCR genotyping (Figure 3) showed that the pre-treatment, sensitive strains belonged to the same clonal type as the post-treatment, resistant strains. PCR for tet(M) and int-Tn and xis-Tn demonstrated that the resistant strains had acquired the TetM resistance determinant and integration and excision genes typically found in the Tn916 family.

Table 1. Identification of bacterial isolates cultured on TYC medium and resistance to doxycycline before and after treatment

<table>
<thead>
<tr>
<th>Species</th>
<th>No. of strains (no. doxycycline-resistant)</th>
<th>Subject A</th>
<th>Subject B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pre</td>
<td>post</td>
<td>pre</td>
</tr>
<tr>
<td>Streptococcus australis</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Streptococcus constellatus</td>
<td>49</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Streptococcus cristatus</td>
<td>6</td>
<td>0</td>
<td>18</td>
</tr>
<tr>
<td>Streptococcus gordonii</td>
<td>10</td>
<td>0</td>
<td>13</td>
</tr>
<tr>
<td>Streptococcus infantis</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Streptococcus intermedius</td>
<td>47</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Streptococcus oralis</td>
<td>2</td>
<td>4 (4)</td>
<td>0</td>
</tr>
<tr>
<td>Streptococcus parasanguinis</td>
<td>0</td>
<td>20 (20)</td>
<td>0</td>
</tr>
<tr>
<td>Streptococcus sanguinis</td>
<td>8 (6)</td>
<td>98 (98)</td>
<td>0</td>
</tr>
<tr>
<td>Total streptococci</td>
<td>122</td>
<td>122</td>
<td>45</td>
</tr>
<tr>
<td>Non-streptococcal isolates</td>
<td>94</td>
<td>15</td>
<td>169</td>
</tr>
<tr>
<td>Total</td>
<td>216</td>
<td>137</td>
<td>214</td>
</tr>
</tbody>
</table>

*Numbers of doxycycline-susceptible streptococci were not determined post-treatment.

Figure 1. Phylogenetic tree based on the sodA gene sequence analysis compared over 341 aligned bases, showing resistant streptococcal isolates from the pre- (S1A) and post-treatment (S1B) samples. Subject A. Representative isolates from each resistant species are shown in bold.
of conjugative transposons. Antimicrobial susceptibility testing showed that the post-treatment strains were also resistant to erythromycin. Pre-treatment *Streptococcus cristatus* strains displayed doxycycline and erythromycin MICs of 0.5 and 0.25 mg/L respectively, whereas post-treatment strains of *S. cristatus* had MICs of 32 and >512 mg/L. The post-treatment strains were demonstrated by PCR to harbour *erm*(B).

### Analysis of the novel conjugative transposon

Sequencing of the transposon showed that the novel CTn was 20.88 kb in length, with 27 putative open reading frames (ORFs) (Table 2) and an overall G + C content of 38.2%. It shared significant structural and sequence homology with Tn916, with the exception of the *tet*(M) gene which had higher sequence identity to the same gene in Tn3872 rather than that of the original *tet*(M) gene in *Enterococcus faecalis*, but included an additional 2847 bp element which included *erm*(B), inserted within *orf*20 of Tn916 (Figure 4). This insertion created an extended ORF, designated *orf*20F, consisting of two hypothetical proteins from unrelated sources, part of *orf*20 from Tn916 and *orf*14 from *Lactobacillus* plasmid pLME300 (designated *orf*P0). In addition to *orf*P0, four other complete ORFs were identified on the novel element containing *erm*(B). Three of these showed high homology with genes from other erythromycin resistance plasmids, while *orf*P4, interestingly, although it did not match any sequence in the nucleotide database, did show 45% amino acid homology with a transposase located on insertion sequence IS1165 from *Leuconostoc mesenteroides*, suggesting that a novel transposase was identified in the CTn sequenced here. The non-Tn916 element was found to have a G + C content of 34.5%, lower than that of the remainder of the transposon at 38.8%. In view of its structure and function, the novel *S. cristatus* S2B-T25 CTn was named CTn6002 and its details logged on the Transposon Registry web site (http://www.ucl.ac.uk/eastman/tn/). The prefix ‘C’ was also given to indicate that the element was capable of conjugal transfer. Southern blot analysis of *S. cristatus* S2B-T25 using a fragment of the *int*-Tn and *xis*-Tn genes as probe showed that a single chromosomal HindIII fragment was recognized by the probe in the post-treatment *S. cristatus* isolate S2A-T220 while no band was seen in the pre-treatment susceptible *S. cristatus* isolate (Figure 5). It is therefore likely that only a single copy of the transposon was present in the isolates exhibiting a band.

### Insertion point of the transposon

Analysis of the regions flanking CTn6002 by means of inverse-PCR revealed genes homologous to hypothetical genes described in the *S. pneumoniae* R6 genome. A gene with high sequence identity to spr1199 was identified to the left of CTn6002, whereas a gene closely related to spr1206 was identified to the right (Figure 4).
<table>
<thead>
<tr>
<th>Open reading frame</th>
<th>Position in CTn6002</th>
<th>Start codon</th>
<th>Stop codon</th>
<th>Putative ribosome binding site</th>
<th>Closest homologue</th>
<th>Nucleotide identity (%)</th>
<th>Accession number</th>
</tr>
</thead>
<tbody>
<tr>
<td>orf24</td>
<td>194–313</td>
<td>ATG</td>
<td>TAA</td>
<td>AGACACTT</td>
<td>orf24 in Tn916 from E. faecalis</td>
<td>100</td>
<td>U09422</td>
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<tr>
<td>orf23</td>
<td>336–650</td>
<td>ATG</td>
<td>TAA</td>
<td>AGGTAAA</td>
<td>orf23 in Tn916 from E. faecalis</td>
<td>100</td>
<td>U09422</td>
</tr>
<tr>
<td>orf22</td>
<td>666–1052</td>
<td>ATG</td>
<td>TAG</td>
<td>GAGAGGA</td>
<td>orf22 in Tn916 from E. faecalis</td>
<td>99</td>
<td>U09422</td>
</tr>
<tr>
<td>orf21</td>
<td>1081–2466</td>
<td>ATG</td>
<td>TGA</td>
<td>AAAGGAGA</td>
<td>orf21 in Tn916 from E. faecalis</td>
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<tr>
<td>orf20F</td>
<td>2860–4062</td>
<td>ATG</td>
<td>TGA</td>
<td>GAGCTCC</td>
<td>orf20 in Tn916 from E. faecalis</td>
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<tr>
<td>orfP0</td>
<td>3895–4062</td>
<td>ATG</td>
<td>TGA</td>
<td>AAAAAGA</td>
<td>orf14 in pLME300 from L. fermentum</td>
<td>100</td>
<td>AJ488494</td>
</tr>
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<td>orfP1</td>
<td>4111–4194</td>
<td>ATG</td>
<td>TAA</td>
<td>AAGGAGG</td>
<td>rRNA methylase regulatory protein from L. fermentum</td>
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<tr>
<td>orfP2</td>
<td>4319–5056</td>
<td>ATG</td>
<td>TAA</td>
<td>AGGAGAGA</td>
<td>erm(B) from L. johnsonii</td>
<td>100</td>
<td>DQ518904</td>
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<tr>
<td>orfP3</td>
<td>5001–5192</td>
<td>ATG</td>
<td>TAA</td>
<td>AAGTACCA</td>
<td>orf11 from pLME300 from L. fermentum</td>
<td>100(^a)</td>
<td>AJ488494</td>
</tr>
<tr>
<td>orfP4</td>
<td>6560–5313</td>
<td>ATG</td>
<td>TAA</td>
<td>AAAGAAGG</td>
<td>no match</td>
<td></td>
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<tr>
<td>orf19</td>
<td>6740–6961</td>
<td>ATG</td>
<td>TAA</td>
<td>GAGGAGG</td>
<td>orf19 in Tn916 from E. faecalis</td>
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<tr>
<td>orf18</td>
<td>7078–7575</td>
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<td>TAA</td>
<td>AGAAAGG</td>
<td>orf18 in Tn916 from E. faecalis</td>
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<td>orf17</td>
<td>7550–8056</td>
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<td>TAA</td>
<td>TCAACCA</td>
<td>orf17 in Tn916 from E. faecalis</td>
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<td>orf16</td>
<td>8040–10 487</td>
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<td>TAA</td>
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<tr>
<td>orf15</td>
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<td>TGA</td>
<td>AAAGAAGG</td>
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<tr>
<td>orf14</td>
<td>12 664–13 449</td>
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<td>TAA</td>
<td>GTAAGAA</td>
<td>orf14 in Tn916 from E. faecalis</td>
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<tr>
<td>orf13</td>
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<td>TAA</td>
<td>GAATCAA</td>
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<td>orf12</td>
<td>14 869–14 955</td>
<td>ATG</td>
<td>TGA</td>
<td>GGAATAA</td>
<td>tet(M) leader protein from S. oralis</td>
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<tr>
<td>tet(M)</td>
<td>14 971–16 890</td>
<td>ATG</td>
<td>TAG</td>
<td>GAGAAAAGA</td>
<td>tet(M) from S. mitis</td>
<td>99</td>
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<tr>
<td>orf6</td>
<td>16 988–17 176</td>
<td>ATT</td>
<td>TAA</td>
<td>GAGGAGG</td>
<td>orf6 in Tn916 from E. faecalis</td>
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<td>orf9</td>
<td>17 589–17 236</td>
<td>ATT</td>
<td>TAA</td>
<td>AGAGGTGG</td>
<td>orf9 in Tn2009 from A. junii</td>
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<td>17 794–17 865</td>
<td>ATT</td>
<td>TAA</td>
<td>GAGGTCAA</td>
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<td>TGA</td>
<td>AGATGGA</td>
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<td>orf8</td>
<td>18 513–18 743</td>
<td>ATA</td>
<td>TGA</td>
<td>TGGAGGAA</td>
<td>orf8 in Tn916 from E. faecalis</td>
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<td>orf5</td>
<td>19 220–18 969</td>
<td>ATG</td>
<td>TAA</td>
<td>AATAGGA</td>
<td>orf5 in Tn916 from E. faecalis</td>
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<tr>
<td>xis-Tn</td>
<td>19 204–19 407</td>
<td>ATG</td>
<td>TAG</td>
<td>AAGGAGG</td>
<td>xis-Tn in Tn916 from E. faecalis</td>
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<tr>
<td>int-Tn</td>
<td>19 489–20 706</td>
<td>ATG</td>
<td>TAG</td>
<td>GAAAGGAG</td>
<td>int-Tn in Tn1545 from S. pneumoniae</td>
<td>100</td>
<td>X61025</td>
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</table>

\(^a\)orf20F shows 100% homology to orf20 from Tn916, however, orf20F is extended.

\(^b\)orfP3 shows 100% homology to orf11 from pLME300, however, orfP3 is extended.
Identification of a possible donor organism

Although no doxycycline-resistant streptococci were found on the TYC + doxycycline plates post-treatment in Subject B, resistant isolates were subcultured from the doxycycline-containing BA and FAA plates and these were screened for their potential to be the donor organism for the novel transposon. Six isolates were found to harbour both the tet(M) and int-Tn and xis-Tn genes by PCR and, of these, two had tet(M) genes with 100% sequence identity with the tet(M) of S. cristatus S2B-T25. sodA gene sequence analysis identified these strains as S. oralis. Sequence analysis of the transposon termini, erm(B), tet(M), orfP4 and orf20F in S. oralis strain S2A-B25 found them to be identical to the corresponding regions of CTn6002. In filter-mating experiments, using S. oralis S2A-B25 as a donor and a rifampicin-resistant mutant derived from the pre-treatment doxycycline-resistant strain S. cristatus S2A-T220 as the recipient, transconjugants were observed at a frequency of 3.0 \times 10^{-7} per donor cell.

Discussion

In this study, investigation of the doxycycline-resistant populations of streptococci before and after doxycycline treatment has revealed both clonal expansion of an already resistant bacterial population and conjugal transfer of doxycycline resistance mediated by a novel transposon. Sequence analysis of the transposon revealed it to be a complex transposon made up of Tn916 and a fragment of DNA encoding five genes of unknown source. We believe this is to be the first direct demonstration, within the oral cavity, of conjugal transfer of doxycycline resistance in vivo. The ability of the novel transposon to mediate conjugal transfer was demonstrated in vitro. A possible donor strain of S. oralis was found, which harboured a transposon apparently identical to CTn6002, in the pre-treatment sample of Subject B. Interestingly, this strain was not found on the TYC + doxycycline medium but was found on the BAD medium. We would have expected S. oralis to grow on the TYC medium but this particular strain may not have been able to grow on TYC on primary isolation or might have been present in the sample in small numbers and was therefore not present in the inoculum for the TYC plates by chance. Although it is most likely that this S. oralis strain was the donor organism, it is also possible that another organism carrying the same transposon was the donor and that this organism may have colonized the periodontal pocket during treatment, facilitated by the selective pressure of the doxycycline in the local environment.

A number of previously described transposons related to Tn916 encode resistance to both tetracyclines and erythromycin. They are typically composite transposons, with an element carrying the gene for erythromycin resistance inserted within a Tn916-like element. Structural similarities between CTn6002 and that of Tn3872 and Tn2009 suggest CTn6002 also belongs in this group. However, unlike all the other Tn916-based composite transposons which have been shown to...
be non-conjugative, CTn6002 was shown to be capable of in vivo conjugation. It is suspected that this difference is due to the location of the smaller element within Tn916. In Tn3872, for example, the Tn917-like element is reported to have inserted at orf6, downstream of the tet(M) gene. The same is true for Tn2009, which has the MEGA element located within orf9. The insertion of these elements downstream of the tet(M) gene is thought to prevent the formation of the long transcripts responsible for the regulation of transfer, and thus impairs the host element’s ability to transpose. However, in CTn6002, the insertion of the novel element is within orf20, upstream of the tet(M) gene, and may therefore explain why this composite transposon is able to conjugatively transpose.

The structural homology between CTn6002 and Tn916 is strong evidence that CTn6002 is a previously uncharacterized member of the Tn916/Tn1545 family, and suggests that both CTn6002 and Tn916 share an evolutionary ancestor. Of the 27 putative ORFs in CTn6002, 19 show high homology to those described in Tn916. However, it is suspected that this element has, at some stage, undergone homologous recombination, as both the tet(M) gene and the gene encoding the Tet(M) leader protein showed greater homology to those found in Tn5251 than those of Tn916. The additional 2.847 kb fragment inserted into Tn916 was the source of the erythromycin resistance gene, erm(B), and accounted for the differences in size between CTn6002 and Tn916. The novel element consisted of five putative ORFs, one of which was found to be part of orf20 as a result of the insertion and subsequent displacement of the stop codon in orf20. The fusion of orf20 from Tn916 and the orf14 homologue from pLME300 has created an extended version of orf20, designated orf20E. It is unknown whether this alteration has created a structurally modified protein, a potential two-part protein, or rendered the putative gene non-functional. In addition, orfP1–orfP3 also showed homology to genes found on plasmids carried by Lactobacillus species, suggesting an origin for the additional element. In contrast, however, orfP4 was found to be novel in that it displayed no significant shared homology with any nucleotide sequence in GenBank, sharing only 45% amino acid homology with a transposase from IS1165, suggesting the discovery of a previously uncharacterized and potentially novel transposase.

Although more than 14 different members of the Tn916–Tn1545 family have been identified, relatively few transposons within this group have been fully sequenced. Transposons have often been identified as belonging to the Tn916 family solely on the basis of the presence of tet(M) and/or Int-Tn genes. Although these two genes are present on the majority of the transposons belonging to the Tn916 family, the group also contain transposons that do not carry the tet(M) gene, such as Tn916S and EfcTn1. In addition, although all carry tetracycline resistance genes, a few have been shown to carry additional antimicrobial resistance genes, with those conferring resistance to erythromycin being the most common. Furthermore, associations between tetracycline and erythromycin resistance, particularly between the tet(M) and erm(B) genes have been demonstrated. Recently, elements similar to CTn6002, containing both tet(M) and erm(B), have been described, suggesting that these elements may be common. When members of the Tn916 family are detected in future studies, a thorough characterization should be performed, in addition to PCR detection of tet(M) and int-Tn genes, to determine the relative prevalence of individual members of the family and, also, the extent of carriage of genes for resistance to antimicrobials other than tetracycline.

This study has demonstrated the transfer of antimicrobial resistance from one oral streptococcal species to another during antimicrobial treatment of periodontitis. This confirms that the administration of antimicrobial agents for common chronic bacterial infections, such as periodontitis, can contribute to increased levels of bacterial resistance to antimicrobials. Further work is required to determine the importance of CTn6002 and related transposons in disseminating antimicrobial resistance among the oral microbiota and related human pathogens.

Acknowledgements

Dr Adam Roberts is thanked for his helpful discussions.

Funding

This study was funded by the Medical Research Council via a studentship awarded to P. J. W.

Transparency declarations

None to declare.

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