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ORIGINAL RESEARCH

An *Enterobacter* plasmid as a new genetic background for the transposon Tn*1331*

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Background: Genus *Enterobacter* includes important opportunistic nosocomial pathogens that could infect complex wounds. The presence of antibiotic resistance genes in these microorganisms represents a challenging clinical problem in the treatment of these wounds. In the authors' screening of antibiotic-resistant bacteria from complex wounds, an *Enterobacter* species was isolated that harbors antibiotic-resistant plasmids conferring resistance to *Escherichia coli*. The aim of this study was to identify the resistance genes carried by one of these plasmids.

Methods: The plasmids from the *Enterobacter* isolate were propagated in *E. coli* and one of the plasmids, designated as pR23, was sequenced by the Sanger method using fluorescent dye-terminator chemistry on a genetic analyzer. The assembled sequence was annotated by search of the GenBank database.

Results: Plasmid pR23 is composed of the transposon Tn*1331* and a backbone plasmid that is identical to the plasmid pPIGDM1 from *Enterobacter agglomerans*. The multidrug-resistance transposon Tn*1331*, which confers resistance to aminoglycoside and beta lactam antibiotics, has been previously isolated only from *Klebsiella*. The *Enterobacter* plasmid pPIGDM1, which carries a ColE1-like origin of replication and has no apparent selective marker, appears to provide a backbone for propagation of Tn*1331* in *Enterobacter*. The recognition sequence of Tn*1331* transposase for insertion into pPIGDM1 is the pentanucleotide TATTA, which occurs only once throughout the length of this plasmid.

Conclusion: Transposition of Tn*1331* into the *Enterobacter* plasmid pPIGDM1 enables this transposon to propagate in this *Enterobacter*. Since Tn*1331* was previously isolated only from *Klebsiella*, this report suggests horizontal transfer of this transposon between the two bacterial genera.

Keywords: transposon Tn*1331, Enterobacter*, wound infection

Introduction

Bacteria from the genus *Enterobacter*, particularly *Enterobacter cloacae, Enterobacter agglomerans*, and *Enterobacter aerogenes*, are important opportunistic nosocomial pathogens responsible for skin, soft tissue, urinary tract, and gastrointestinal tract infections.[1–5] These microorganisms also infect various types of complex wounds and represent a challenging clinical problem in their treatment.[6,7] Consequently, the emergence of multidrug resistant strains of *Enterobacter*, thought to be correlated with the ubiquitous and empirical use of antibiotics,[6,8] is of great concern to clinicians treating wound infections.

Bacteria acquire drug-resistant phenotypes through either spontaneous mutations or acquisition of resistance genes from the environment. Horizontal gene transfer (HGT) is

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one of the most effective mechanisms for a bacterium to gain new functionally relevant genes from other microorganisms in their environment.[9,10] Mobile genetic elements such as transposons carrying antibiotic-resistance genes can be transmitted by HGT and, as a result, play a significant role in the transfer and spread of antibiotic resistance in bacterial populations. To date, several transposons have been identified in pathogenic *E. cloacae* that carry resistance markers to a wide spectrum of antibiotics. These include Tn*2101*;[11] Tn*402* derivatives, often in association with Tn*21*;[12–14] Tn*7*;[15] and Tn*6005*.[16] Some of these transposons, like Tn*402* derivatives, have also been identified in *Pseudomonas*, indicating exchange of antibiotic-resistance genes between *Enterobacter* and *Pseudomonas* species, possibly by HGT.[14]

Previously, the authors have reported isolation of a multiple antibiotic-resistant *Enterobacter* species from a clinical wound sample that was closely related to *E. cloacae* and was designated *Enterobacter* sp W001.[17] This bacterium carries plasmids that could confer kanamycin and ampicillin resistance to an antibiotic-sensitive laboratory strain of *Escherichia coli*. In the present study, the sequence and organization of one these plasmids, which suggests direct or indirect horizontal transfer of a transposon between *Klebsiella* and *Enterobacter*, is reported.

Materials and methods

Bacterial strains and cultivation

The plasmid analyzed in this study originated from a bacterial isolate from a clinical wound sample, as previously described.[17] Briefly, the clinical sample was screened for presence of ampicillin- and kanamycin-resistant bacteria by antibiotic-selective agar method. One of the resistant colonies was subjected to 16S ribosomal DNA sequence analysis and biochemical assays using a Phoenix Automated Microbiology System (BD, Franklin Lakes, NJ). This bacterium was determined to be 99% similar to *E. cloacae* by both 16S rDNA and Phoenix analysis and was designated *Enterobacter* sp W001. *Enterobacter* sp W001 was maintained on Tryptic Soy Agar and grown in Tryptic Soy Broth (both Thermo Fisher Scientific, Rockford, IL) for plasmid extraction using QIAprep Spin MiniPrep Kit (Qiagen, Valencia, CA).

Plasmid extracted from *Enterobacter* sp W001 was transformed into the commercially available competent *E. coli* TOP10 cells (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. The transformed cells were selected on kanamycin- and ampicillin-selective agar plates. A resistant colony from the transformants was selected for plasmid extraction and analysis. Plasmid extracted from this colony was designated pR23.

Cloning, sequencing, and bioinformatics analysis

To clone and sequence the plasmid pR23, it was double digested with restriction enzymes HindIII/PstI or BamHI/PstI (New England Biolabs, Ipswich, MA). The fragments were cloned into a pUC19 plasmid, which was either singly digested with PstI, HindIII, or BamHI, or double digested with PstI/HindIII or PstI/BamHI. The recombinant plasmids were propagated in *E. coli* TOP10 cells and purified using QIAprep Spin MiniPrep Kit. Plasmids were sequenced in the authors' laboratory by the Sanger method using M13 forward and reverse primers and fluorescent dye-terminator chemistry on an ABI 3100-Avant Genetic Analyzer (Life Technologies Corp, Carlsbad, CA). To complete the sequence of large fragments, primers were designed based on the first round of sequencing and used for DNA walking of the larger fragments. In those instances where DNA walking of the large fragments was difficult due to presence of repeats in DNA sequence, the primers were used in polymerase chain reaction amplification of the interior sections, which we cloned the amplicons into TOPO TA Cloning® (Invitrogen) according to the manufacturer's instructions and sequenced. Sequencing reads were assembled into contigs, then assembled into the final complete plasmid sequence using Geneious Pro (v 5.1; Biomatters Ltd, Auckland, New Zealand). The accuracy of the assembly was confirmed by resequencing different parts of the plasmid after amplification by polymerase chain reaction using primers based on the assembled sequence. The plasmid sequence was annotated by a BLAST search of the GenBank®[18] database according to the highest similarity scores. The sequence of the pR23 plasmid has been submitted to the GenBank and the Accession number is JF703130.

Results and discussion

Plasmid pR23 harbors transposon Tn*1331*

The plasmid originating from the clinical isolate *Enterobacter* sp W001 and carrying antibiotic-resistance genes was designated as pR23. As shown in Figure 1, pR23 is composed of a transposon that carries antibiotic-resistance genes and a backbone plasmid. The transposon is 100% identical to the multidrug-resistance transposon Tn*1331* from plasmid pJHCMW1 (Accession number NC_003486), which was isolated from a *Klebsiella pneumoniae* strain and confers resistance to aminoglycoside and beta lactam antibiotics.[19]

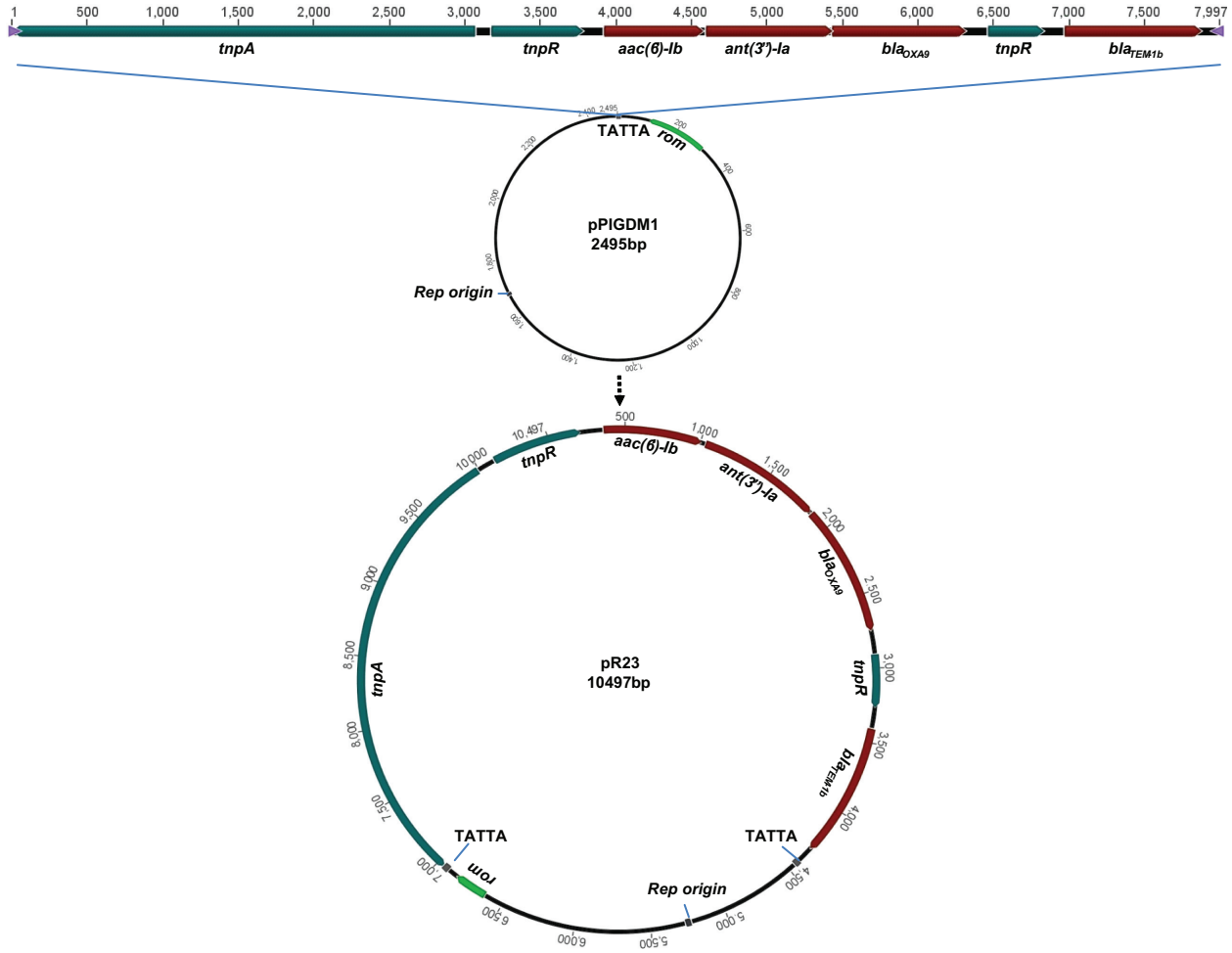

Figure 1 Possible mechanism for generation of plasmid R23. Transposon Tn*1331* insertion into pPIGDM1 at the insertion site TATTA could lead to generation of pR23 plasmid. Two direct repeats of pentanucleotide TATTA flank inverted repeats (not shown) at the ends of Tn*1331* transposon in pR23. *Enterobacter* plasmid pPIGDM1 carries an origin of replication (rep origin) in enterobacteria and the RNA one modulator (*rom*) gene, which helps maintain a low plasmid copy number. Tn*1331* consists of genes coding for transposase (*tnpA*), resolvase (*tnpR*), aminoglycoside 6′-N-acetyltransferase type Ib (*aac(6′)-Ib*), Aminoglycoside (3″) adenylyltransferase (*ant(3″)-Ia*), oxacillinase-carbapenemases beta-lactamase (bla_{OXA-9}), partial coding sequence of resolvase (*tnpR′*), and TEM beta-lactamase (bla_{TEM-1b}). The Accession numbers for the coded proteins are TnpA: NP_608305.1, TnpR: NP_608306.1, AAC(6′)-Ib: NP_608307.1, ANT(3″)-Ia: NP_608308.1, OXA-9: NP_608309.2, and Bla-TEM-1b: NP_608310.1.

Tn*1331* consists of a Tn*3* transposon and two gene cassettes carrying three antibiotic-resistance genes. One of the cassettes carries the aminoglycoside 6′-N-acetyltransferase type Ib (*aac(6′)-Ib*) gene that confers resistance to aminoglycosides including amikacin and kanamycin. The other cassette contains the streptomycin 3′-adenyltransferase (*ant(3″)-Ia*) and oxacillinase-carbapenemases beta-lactamase (bla_{OXA-9}) genes that code for resistance to streptomycin and beta lactams, respectively. The two gene cassettes have integrated between the resolvase (*tnpR*)-resistance and ampicillin-resistance (bla_{TEM-1}) genes of transposon Tn*3* by a mechanism that has resulted in partial duplication of the resolvase gene (*tnpR′*).

Although Tn*1331*-related sequences and partial components have also been detected in *Serratia marcescens*, *Salmonella serovars*, *Pseudomonas putida*, and *Proteus mirabilis*,[20–24] this transposon and its cluster of antibiotic-resistant genes – in its entirety – has been previously isolated only from *K. pneumoniae* clinical isolates[25,26] (also refer to NCBI Accession number NC_009650 and GU553923). To the best of the authors' knowledge, the present study is the first report of Tn*1331* association with an *Enterobacter* species.

Genetic background for Tn*1331* on pR23

The second major feature of the plasmid pR23 is the genetic context into which Tn*1331* has transposed. The sequence of this backbone is 100% identical to the plasmid pPIGDM1 (Accession number AF014880) from an *E. agglomerans* originally isolated from a kitchen sink.[27] This plasmid carries a ColE1-like origin of replication, and the *rom* gene, which is responsible for controlling the copy number of the

Table 1 Transposition sites of Tn*1331* identified in different plasmids

Bacterial isolate	Plasmid	Size (kbps)	Target sequence of Tn*1331*	Number of targets in pPIGDM1	Accession numbers
K. pneumoniae JHCK1	pJHCMW1	11.354	AAAGC	9	AF479774
K. pneumoniae strain FC1	pMET-1	41.723	TTAGA	5	EU383016
K. pneumoniae strain 4003	pRMH712	21.828	TTGTT	3	GU553923
K. pneumoniae (subsp. *pneumoniae* MGH 78578)	pKPN4	107.576	TTGTT	3	NC_009650
Enterobacter sp. W001	pR23	10.497	TATTA	1	JF703130

Notes: Recognition sequences of Tn*1331* for transposition into plasmids isolated from different *Klebsiella* species and *Enterobacter* sp. W001 are A-T rich pentanucleotides. Tn*1331* insertion sites into pPIGDM1 is TATTA, which occurs only once in this plasmid. Insertion sites of Tn*1331* into plasmids isolated from *Klebsiella* are also present at multiple sites on pPIGDM1. The order of isolation or sequencing of Tn*1331*-harboring plasmids is from the earliest at the top to the latest at the bottom of the table Recognition sequences of Tn*1331* for transposition into plasmids isolated from different *Klebsiella* species and *Enterobacter* sp. W001 are A-T rich pentanucleotides. Tn*1331* insertion sites into pPIGDM1 is TATTA, which occurs only once in this plasmid. Insertion sites of Tn*1331* into plasmids isolated from *Klebsiella* are also presents at multiple sites on pPIGDM1. The order of isolation or sequencing of Tn*1331*-harboring plasmids is from the earliest at the top to the latest at the bottom of the table.

plasmid. Another plasmid, pAH2504, which is 99% identical to pPIGDMI, has also been isolated from *Aeromonas hydrophila* (Accession number DQ389104), suggesting that pPIGDM1 may be harbored by bacterial species from different genera. Plasmids pPIGDM1 and pAH2504, which are small in size, have a low copy number, and no apparent selective markers, are examples of cryptic plasmids that may circulate within different bacteria and serve as a natural backbone plasmid for mobile genetic elements.

The recognition sequence of Tn*1331* transposase for insertion into pPIGDM1 was identified as direct repeats of the pentanucleotide TATTA, which flank the inverted repeats at the ends of Tn*1331* (Figure 1). This sequence occurs only once throughout the length of the plasmid. The recognition site for Tn1331 transposition appears to be adenine/thymine rich with a frequency of 60%–100%. Transposition sites of Tn*1331* into plasmids from *K. pneumoniae* isolates are present in multiple locations in those plasmids (Table 1). These recognition sequences are also present many times across the length of pPIGDM1, and could represent potential sites for Tn*1331* transposition.

Transposons appear to have played an important role in the emergence of antibiotic resistance in members of genus *Enterobacter*. The first report of a transposon carrying antibiotic-resistance genes in an *Enterobacter* species was published in 1982.[11] A subsequent study has provided strong evidence for association of transposons with antibiotic resistance genes in *Enterobacter*.[28] The present study adds Tn*1331* transposon to the list of mobile genetic elements that could contribute to spread of antibiotic-resistance genes in *Enterobacter*.

In summary, this report suggests transposition of Tn*1331* into a new genetic background that allows propagation of its associated antibiotic-resistance genes in a genus other than *Klebsiella*. The plasmid pPIGDM1 from *E. agglomerans*, for which no functional relevance had previously been defined, appears to serve as a backbone to facilitate propagation of Tn*1331* in an *Enterobacter* species, perhaps after horizontal transfer of this transposon. Given that clinically important members of *Enterobacter* are already highly resistant to antibiotics, appearance of new combination of antibiotic-resistance genes and their propagation in these microorganisms is alarming and may further complicate management of complex wound infections.

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Disclosure

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