Mercury Resistance Determinants Related to Tn21, Tn1696, and Tn5053 in Enterobacteria from the Preantibiotic Era

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Three mer transposons from the Murray collection of preantibiotic era enterobacteria show >99% sequence identity to current isolates. Tn5073 is most closely related to Tn5036 and Tn1696, and Tn5074 is most closely related to Tn5053. Tn5075 is most closely related to Tn21 but lacks integron In2 and is flanked by insertion elements.

Tn21 encodes mercuric ion resistance (Hg₁) and contains the class I integron In2, encoding resistance to sulfonamides (sul) and streptomycin-spectinomycin (aadA) (9, 18). It is carried by the conjugative plasmid NR1 (R100), which was isolated in Japan in the 1950s (21). More recently, Tn21 (18) and other Tn21-like transposons carrying integron-associated antibiotic resistance (7) have been detected in Escherichia coli from agricultural (1, 28) and nonclinical sources (19), as well as from mercury amalgam-exposed, gram-negative gut bacteria (16, 17, 33), clinical bacterial isolates (13, 31, 36), and intercontinental plasmids carried by clinical isolates (10, 22).

It is now thought that Tn21 evolved by the insertion of an In2 ancestor (lacking IS353) into the urf2M gene of a hypothetical mercury resistance transposon, Tn21Δ (18) (also called TnX [25]), probably catalyzed by transposition proteins encoded in trans (2, 18). A similar event led to the formation of Tn1696 from plasmid R1033, where In4 inserted at the res site of a Tn5036-like mer transposon (25).

Hughes and Datta identified three Hg₁ bacterial strains, M426, M567, and M634, from a total of 433 strains from the Murray collection of preantibiotic era enterobacteria (11). In this study, we sequenced the mer operons from M426, M567, and M643 in order to investigate the relationships between Hg₁ sequences from clinical bacteria that had been isolated before antibiotics came into widespread use and present-day Hg₁ sequences.

The plasmids, bacterial strains, antimicrobial resistance of these strains, and the 16S ribosomal DNA sequence identifications of the three Hg₁ strains from this study (determined as previously described [32]) are shown in Table 1. All bacteria were grown at 37°C in Luria broth (LB) or on LB agar (27). Hg₁ plasmids from M426, M567, and M634 were mated with E. coli TG2 (20), and Hg₁ transconjugants were grown overnight on LB agar plates containing tetracycline (15 μg/ml) and HgCl₂ (20 μg/ml).

The E. coli TG2 Hg₁ transconjugants from each of the three Murray strains contained an ~60-kb plasmid that conferred Hg₁. Plasmid DNA was isolated by standard methods (27), and PCR was performed with part of the mer operon from each plasmid as described elsewhere (3). PCR products purified by using a QIAquick PCR purification kit (Qiagen, Ltd., Crawley, United Kingdom) were sequenced with the Big Dye terminator cycle sequencing kit (PE Applied Biosystems, Warrington, United Kingdom) and an Applied Biosystems 3700 sequencer, according to the manufacturer’s protocols. Further sequence analysis was performed by using primers designed from the sequences so obtained and from merA gene primers (5) (the primers used are described at http://www.biosciences.bham.ac.uk/labs/brown/mer_primers.htm). The transposon terminal inverted repeat DNA and flanking sequences were amplified by inverse PCR (26). Genetic maps of the sequenced mer operons are shown in Fig. 1A. DNA alignments and analysis were performed with the University of Wisconsin Genetics Computer Group version 9.0 suite of programs at the University of Birmingham.

The three mer operons that we sequenced represent different lineages and are not closely related to each other, but they are closely related to mer transposons isolated since the 1950s. Table 2 shows the percent identities between the sequences of the genes from Tn5073 (Klebsiella pneumoniae M426), Tn5074 (Morganella morganii M567), Tn5075 (E. coli M634) and published DNA sequences.

The sequenced merRTPCAD genes (3,788 bp) of the Tn5073 mer operon (Fig. 1A) had the highest identity at the DNA level to those from Tn5036 (35), a Tn5036-like mer transposon from Salmonella enterica serovar Typhi CT18 plasmid pHCM1 (24), and to the sequenced merAD genes of Tn1696, which carries In4 (25) (Table 2). In comparison to the sequence encoded by Tn5036, there were two amino acid differences in the sequence encoded by Tn5036: MerR A17→V and MerA V250→A. The Tn5073 merT gene carries five GTCTGACCCACAAAA duplications at the 5’ end (Fig. 1A). Multiple repeats of this sequence have also been observed in enterobacterial mercury resistance determinants from primates (16) and in Tn5036, Tn5059, and pKLI272 (35).

The sequenced merRTPFADE genes (3,647 bp) from the Tn5074 mer operon (Fig. 1A) had the highest identity at the DNA level to those from Tn5053 (14) and pMER327/419 (8) (Table 2). In comparison to the sequence encoded by Tn5053, there were four amino acid differences in the sequence encoded by Tn5074: MerR A119→S and K121→Q and MerA

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TABLE 1. Bacterial strains and plasmids, antibiotic resistance phenotypes, 16S rRNA sequences, and sources of the strains used in this study

<table>
<thead>
<tr>
<th>Strain or plasmid</th>
<th>Antibiotic resistance</th>
<th>Original tube date (yr)</th>
<th>Genotype</th>
<th>16S rRNA identification</th>
<th>Source or reference</th>
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<tr>
<td>Murray collection</td>
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<tr>
<td><em>Klebsiella</em> sp. strain M426</td>
<td>Hg&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1940</td>
<td><em>Klebsiella pneumoniae</em> with 99% identity to sequence of accession no. AF144323.1</td>
<td>Urine NCTC</td>
<td></td>
</tr>
<tr>
<td><em>Proteus</em> morganii M567</td>
<td>Hg&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1935</td>
<td><em>Morganella morganii</em> with 98% identity to sequence of accession no. AD010681</td>
<td>Stool sample, child with dysentery NCTC</td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli</em> M634</td>
<td>Hg&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1931</td>
<td><em>Escherichia coli</em> K-12 with 100% identity to sequence of accession no. NC000913</td>
<td>Cerebrospinal fluid NCTC</td>
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<table>
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<tr>
<th>Laboratory strains</th>
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<tr>
<td><em>Escherichia coli</em> TG2</td>
<td>Te&lt;sup&gt;b&lt;/sup&gt;</td>
<td>K-12 lac-proΔ&lt;sup&gt;Δ&lt;/sup&gt;recA 306::Tn10 supE thi hsdD5 [F&lt;sup&gt;r&lt;/sup&gt; truD36 proA&lt;sup&gt;+&lt;/sup&gt; B&lt;sup&gt;+&lt;/sup&gt; lac&lt;sup&gt;+&lt;/sup&gt; lacZ&lt;sup&gt;Δ&lt;/sup&gt;M15]</td>
<td>Laboratory stock (4)</td>
<td></td>
<td></td>
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<tr>
<td><em>Escherichia coli</em> KH802</td>
<td>Rif&lt;sup&gt;c&lt;/sup&gt;</td>
<td>F&lt;sup&gt;−&lt;/sup&gt; gox&lt;sup&gt;4&lt;/sup&gt; proB merB hsdS&lt;sub&gt;λ&lt;/sub&gt; (r&lt;sup&gt;+&lt;/sup&gt; m&lt;sup&gt;+&lt;/sup&gt;)</td>
<td>P. Strike (34)</td>
<td></td>
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</table>

Plasmid RK2 | Ap<sup>a</sup> Te<sup>a</sup> Km<sup>a</sup> | C. Thomas |

<sup>a</sup> Te<sup>a</sup>, tetracycline resistance; Rif<sup>c</sup>, rifampicin resistance; Ap<sup>a</sup>, ampicillin resistance; Km<sup>a</sup>, kanamycin resistance.

<sup>b</sup> NCTC, National Collection of Type Cultures, Colindale, London, United Kingdom.

V232→A and S289→G. The Tn5053 type mer operon was first isolated in environmental bacteria (8, 14) and has also been found in the feral flora of primates and humans exposed to dental amalgam (16, 20).

In total, 11,298 bp of Tn5075 were sequenced. The 3,962-bp mer operon from Tn5075 carrying merRTPCADE has the highest identity at the DNA level to the equivalent regions of Tn21 (18) and the Tn21-like mer transposon from *S. enterica* serovar Typhi CT18 plasmid pCHM1 (24) (Fig. 1A) (Table 2). The Tn5075 res site and the transposition genes (3,529 bp) tnpR and tnpA again had the highest identity to those of Tn21. In comparison to the sequence encoded by Tn21, there were three amino acid differences in the sequence encoded by Tn5075: MerA, Q558→H; TnpR, T165→A; and TnpA R455→A. Most importantly, Tn5075 did not carry the integron In2, which in Tn21 is located between urf2 and the putative transposition gene tnpM (12) (Fig. 1A). Instead, in Tn5075, there is a single 945-bp open reading frame (urf2M) of unknown function rather than the predicted 987-bp hypothetical urf2M from Tn21A proposed by Liebert et al. (18). Tn5075 urf2M is 97.1% identical to the DNA level to the hypothetical urf2M that Liebert et al. (18) postulated to exist in the ancestor of Tn21. The reason for this lower percent identity is a 23-bp deletion in the Tn5075 urf2M sequence compared to the tnpM sequence in Tn21, resulting in a frameshift and a 314-amino-acid protein, rather than the 328-amino-acid protein predicted previously (18).

The insertion (IS) elements IS5075L and IS5075R (which are 1,351 bp and >99.5% identical to each other) flank Tn5075, forming a composite transposon (Fig. 1A). IS5075L and IS5075R belong to the IS110 family and are >99.6% identical to the IS elements flanking the Tn21-like mer transposon from plasmid pCHM1 (Fig. 1A). IS5075L and IS5075R are between 91 and 93% identical to IS element sequences flanking other mercury resistance-encoding genes from gram-negative bacteria (Table 2).

Transposition of the Hg<sup>a</sup> phenotype was determined by a mate-out assay (15) with *E. coli* TG2 carrying plasmid RK2, conjugated with Hg<sup>a</sup> plasmids from M426, M567, or M634, into *E. coli* KH802. Transconjugants were selected on LB agar plates containing HgCl<sub>2</sub> (20 μg/ml) and carbenicillin (200 μg/ml), and the donor strain was counterselected with rifampin (50 μg/ml). Purified plasmid RK2 DNA from the transconjugants was analyzed for transposon insertion by *Pst*I digestion and gel electrophoresis (27). Tn5073 transposed into plasmid RK2 at a frequency of 6.3 × 10<sup>−5</sup> per donor cell. Tn5073 transposed into RK2 at a frequency of 2.9 × 10<sup>−4</sup> per donor cell.
**A**

![Diagram showing the structure of Tn5074 and Tn5073.]

**B**

![Diagram illustrating the genetic changes leading to Tn21 and pHCM1 from Tn5075.]

- (a) Hypothetical ancestral Mer transposon
- (b) Acquisition of IS5075L and R
- (c) Insertion of In2
- (d) Tn21 variants
- (e) Acquisition of IS-elements
- (f) Composite Hg' transposon pHCM1

Insertion of In2

Insertion of IS-elements carrying antibiotic resistance genes

Deletion of res, tmpA and partial deletion of ln2, and tmpA. Insertion of IS-elements carrying antibiotic resistance genes.
we found no transposition of Tn5075 in our assays, which could detect frequencies of $>10^{-7}$.

In conclusion, the internal genetic structure of Tn5075 is consistent with the recently proposed structure of Tn21 (18) and Tn5073 is closely related to Tn5075 and Tn1696 (25). The sequence data from Tn5075 support the hypothesis that Tn21 evolved from an Hg$^+$ transposon similar to Tn5075, rather than from Tn2613 (29). Tn1696 and Tn21 represent independent lineages of mer transposons that have acquired integrons (25). The dates of isolation of Tn5073 (1940) and Tn5075 (1931) and the close relationships of these transposons to other lineages (Tn5073 merAD genes are $>99.9\%$ identical to those of Tn1696; Tn5075 is $>99.6\%$ identical to Tn21 except where In2 is not present) are consistent with the idea that integrons transposed into preexisting clinical Hg$^+$ transposons. The Tn5074 mer operon, isolated from a clinical source, has the greatest DNA identity to the Tn5053 and pMER327/419 mer operons, which have been isolated from both environmental and nonclinical (16, 20) sources.

The DNA sequence data suggest that Tn5075, the Tn21-like mer transposon in pHCM1, and Tn21 had a common ancestor and may have evolved as shown in Fig. 1B; i.e., an ancestral mer transposon acquired IS5075L and IS5075R, leading to the formation of Tn5075. Alternatively, an integrase related to In2 inserted into the ancestral mer transposon, leading to the formation of Tn21. Gene insertions and deletions within the integron in Tn21 could lead to the formation of Tn21 variants (7). Acquisition of IS5075-like elements by Tn21 could have led to the formation of a hypothetical mer transposon. Deletion of res and tnpR and partial deletion of In2 and tnpA from this transposon followed by insertion of antibiotic resistance-carrying IS elements, could have led to the formation of the Tn21-like transposon in pHCM1 (24). Although the Tn21-like transposon in pHCM1 is flanked by IS elements which are 99.6\% identical to IS5075L and IS5075R from Tn5075, it is more closely related to Tn21 than to Tn5075 because it contains a vestige of the In2 sequence, which Tn5075 does not and is 100\% identical to Tn21 across the mer genes and tnpA.

**Nucleotide sequence accession numbers.** The GenBank accession numbers of the mer sequences determined in this study are as follows: Tn5073 (strain M426), AF461013; Tn5074 (strain M567), AF461012; and Tn5075 (strain M634), AF457211. The 16S rRNA gene sequence accession number for strain M567 is AF461011.

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