New integron-associated gene cassette encoding a trimethoprim-resistant DfrB-type dihydrofolate reductase

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New Integron-Associated Gene Cassette Encoding a Trimethoprim-Resistant DfrB-Type Dihydrofolate Reductase

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A sixth gene cassette containing a dfrB-type gene, dfrB6, was found in a dfrB6-aadA1 cassette array in class 1 integrons. This array was isolated from several multiply antibiotic-resistant Salmonella enterica serovar Infantis strains that appear to be clonally related. The DfrB6 dihydrofolate reductase conferred resistance to trimethoprim.

Resistance to trimethoprim, which inhibits the production of the essential cofactor tetrahydrofolate, is generally achieved by a bypass mechanism. Acquired genes that confer resistance to trimethoprim encode dihydrofolate reductases that are inhibitor resistant. These enzymes fall into two quite distinct groups (5), designated DfrA and DfrB, that are encoded by dfrA1 and dfrB genes (12). Members of the DfrA group are about 160 amino acids (aa) long and related to the chromosomally encoded dihydrofolate reductases of bacteria. Several of the known dfrA genes are found in gene cassettes (3). Members of the second, smaller group, DfrB (encoded by dfrB genes), are proteins of 78 aa that form a tetramer that binds both the substrate, dihydrofolate, and the cofactor, NADP, in equivalent positions, thus allowing reduction of the dihydrofolate to occur (1, 5). The five known dfrB genes (Table 1), which are all found in gene cassettes, confer resistance to substantially lower levels of trimethoprim than the dfrA genes (1).

Here we report the identification of a sixth dfrB gene cassette, dfrB6, found in class 1 integrons in multiply drug-resistant Salmonella enterica serovar Infantis strains, most of which were not recorded as resistant to trimethoprim.

Isolates. Eight multiply antibiotic-resistant S. enterica serovar Infantis strains isolated from chickens or chicken meat (six isolates) or infected animals (one isolate from a cat and one from a dog) were identified in a larger collection of 136 S. enterica strains of various serovars because they all carried an unusual array of gene cassettes (see below). The strains were serotyped using procedures standard to the Kauffman and White scheme (10), and the resistance profiles were determined as described previously (7). These strains were mostly resistant to streptomycin, spectinomycin, sulfathiazole, and tetracycline but susceptible to ampicillin, gentamicin, chloramphenicol, kanamycin, nalidixic acid, and ciprofloxacin (Table 2). Only one strain was recorded as resistant to trimethoprim.

Gene cassettes in class 1 integrons. Whole-cell DNA isolated from the S. enterica serovar Infantis strains by using

<table>
<thead>
<tr>
<th>Gene namea</th>
<th>Old or other name(s)</th>
<th>Cassette length (bp)</th>
<th>59-base length (bp)</th>
<th>GenBank accession no.</th>
<th>Reference or source</th>
</tr>
</thead>
<tbody>
<tr>
<td>dfrB1</td>
<td>dfrB, dfr2a</td>
<td>411b</td>
<td>57</td>
<td>AF141719</td>
<td>16</td>
</tr>
<tr>
<td>dfrB2</td>
<td>dfrB, dfr2b</td>
<td>384</td>
<td>57</td>
<td>AJ009557</td>
<td>15</td>
</tr>
<tr>
<td>dfrB3</td>
<td>dfrB, dfr2c</td>
<td>408</td>
<td>57</td>
<td>X72858</td>
<td>11</td>
</tr>
<tr>
<td>dfrB4</td>
<td>dfr2d</td>
<td>408</td>
<td>57</td>
<td>AJ429132</td>
<td>4</td>
</tr>
<tr>
<td>dfrB5</td>
<td></td>
<td>411</td>
<td>57</td>
<td>U189871</td>
<td>8</td>
</tr>
<tr>
<td>dfrB6</td>
<td></td>
<td>410</td>
<td>57</td>
<td>DQ274503</td>
<td>This study</td>
</tr>
</tbody>
</table>

a Cassettes are named after the gene.
b An earlier sequence for this cassette is 485 bp long and contains a duplication of 72 of 73 bp (GenBank accession no. U36276).

<table>
<thead>
<tr>
<th>Strain</th>
<th>Source</th>
<th>Yr isolated</th>
<th>Antibiotic resistance phenotypea</th>
<th>Cassette array</th>
<th>tet(B)b</th>
</tr>
</thead>
<tbody>
<tr>
<td>SRC70</td>
<td>Feline</td>
<td>2001</td>
<td>Sm Sm Sp Su Tc</td>
<td>dfrB6-aadA1</td>
<td>+</td>
</tr>
<tr>
<td>SRC71</td>
<td>Chicken</td>
<td>2001</td>
<td>Sm Sm Su Tc</td>
<td>dfrB6-aadA1</td>
<td>+</td>
</tr>
<tr>
<td>SRC72</td>
<td>Chicken</td>
<td>2001</td>
<td>Sm Sm Su Tc</td>
<td>dfrB6-aadA1</td>
<td>+</td>
</tr>
<tr>
<td>SRC92</td>
<td>Canine</td>
<td>2000</td>
<td>Sm Sp Su Tc Tp</td>
<td>dfrB6-aadA1</td>
<td>+</td>
</tr>
<tr>
<td>SRC93</td>
<td>Chicken</td>
<td>2000</td>
<td>Sp Su Tc</td>
<td>dfrB6-aadA1</td>
<td>+</td>
</tr>
<tr>
<td>SRC94</td>
<td>Chicken</td>
<td>2000</td>
<td>Sm Sp Su Tc</td>
<td>dfrB6-aadA1</td>
<td>+</td>
</tr>
<tr>
<td>SRC95</td>
<td>Chicken</td>
<td>2000</td>
<td>Sm Sp Su Tc</td>
<td>dfrB6-aadA1</td>
<td>+</td>
</tr>
<tr>
<td>SRC96</td>
<td>Chickencarcass</td>
<td>2000</td>
<td>Cm Sm Sm Sp Su Tc</td>
<td>dfrB6-aadA1</td>
<td>+</td>
</tr>
</tbody>
</table>

a Cm, chloramphenicol; Sm, streptomycin; Sp, spectinomycin; Su, sulfanamides; Tc, tetracycline; Tp, trimethoprim.
b, +, gene present.
standard methods (13) was screened for the presence of class 1 integrons by using primers within the intI1 gene (L2 and L3) and in the 3′-conserved segment (3′-CS) (QS-1 and QS-2) and primers L1 and R1 to amplify the cassette array (see reference 7 for primer details). PCR amplification was carried out in PCR buffer (Roche Molecular Biochemicals, Mannheim, Germany) containing 160 μM deoxynucleoside triphosphates, 20 pmol of each primer, approximately 10 to 50 ng template, and 1 U of Taq DNA polymerase (Roche). Reaction conditions were 94 to 96°C for 5 min, followed by 30 cycles of 96°C for 30 s, 54°C, 60°C, or 57°C, respectively, for 30 to 60 s, and 72°C for 90 s, and a final incubation at 72°C for 15 min. The gene and cassette were named, using the next available number, as dfrB6, and the protein was named DfrB6. The second cassette in the integron is identical to the aadA1 cassette in GenBank accession no. AF313471 (9).

The sequence of the amplicon from a single strain (SRC70) revealed two gene cassettes (GenBank accession no. DQ274503). The first contains an open reading frame with an ATG start codon at positions 71 to 73 relative to the start of the cassette that is preceded by a potential ribosome binding site AGG at positions 61 to 63. Translation from this ATG predicts a protein of 78 aa that is quite closely related to the known DfrB proteins (77 to 92% identical), and alignment of the sequences (Fig. 1) revealed only 7 and 10 amino acid differences from the closest relatives DfrB1 and DfrB5, respectively. The gene and cassette were named, using the next available number, as dfrB6, and the protein was named DfrB6. The second cassette in the integron is identical to the aadA1 cassette in GenBank accession no. AF313471 (9).

The dfrB6 cassette is 410 bp long, with 70 bp preceding the initiation codon and 53 bp between the termination codon and the 59-be (59-base element). The closest relatives of this cassette are the dfrB1 cassette and the dfrB5 cassette, both of which are 90% identical over the full length of the cassette. The 59-be is made up of two simple sites and a central region, which are 90% identical over the full length of the cassette. The 59-be is identical to that in the dfrB1 cassette and very closely related to those of other dfrB cassettes, which form a distinct group that are the shortest known, at 57 bp (12).

The dfrB6 gene confers resistance to trimethoprim. As most of the original S. enterica serovar Infantis strains were not recorded as resistant to trimethoprim, the promoter in the integron 5′-CS was also amplified from SRC70 and sequenced. The dfrB6 cassette is preceded by the strong promoter of class 1 integrons (2). The dfrB6-aadA1 cassette array from SRC70 was amplified by PCR and cloned into pPCR-Script as described previously (7). The cloned fragment was recovered by transformation with selection on LB agar plates containing ampicillin (50 μg/ml) and trimethoprim (25 μg/ml). Susceptibility to trimethoprim for the Escherichia coli DH5α strain containing either pPCR-Script or pPCR-Script with the cassette array was determined using the gradient plate method. The cloned fragment conferred resistance to 550 μg/ml of trimethoprim (control was <1 μg/ml). The plasmid also conferred resistance to streptomycin and spectinomycin, as expected from the presence of the aadA1 cassette.

The S. enterica serovar Infantis strains are clonally related. The strains were also screened by PCR for the presence of several additional antibiotic resistance genes by use of primer pairs internal to the genes (7). The tetracycline resistance determinant was identified as tet(B) [not tet(A) or tet(G)], and the strAB spectinomycin resistance determinant and sul2 sulfonamide resistance gene were not present. IS200 profiles, determined as described by Weill et al. (17), were identical for all of the eight strains, indicating that the strains are clonally related. This raises the possibility that the presence of rare gene cassettes may be an indicator for closely related strains. The infections of companion animals, a cat and a dog, may have arisen from the consumption of chicken meat.

**REFERENCES**


FIG. 1. Alignment of DfrB proteins. Amino acids completely conserved in all sequences are shown as white on black. The sequences were obtained from GenBank accession numbers listed in Table 1.

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**TABLE 1**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Accession No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>intI1</td>
<td>AF313471</td>
</tr>
<tr>
<td>dfrB6</td>
<td>DQ274503</td>
</tr>
</tbody>
</table>

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