Letter to the Editor

First Canadian Salmonella enterica Serovar Typhi Isolate Harboring an Integron

Two recent reports in this journal have described multidrug-resistant Salmonella enterica serovar Typhi strains which harbor integrons (7, 9). The emergence of multidrug-resistant S. enterica serovar Typhi poses a serious public health concern, resulting in treatment failures and limiting therapeutic options (11). Multidrug-resistant strains have been isolated in Canada from individuals returning from Asia, primarily from India and Pakistan (2). Recently, an S. enterica Typhi strain was isolated from an asymptomatic sibling of the patient about 30 years before and had a history of typhoid fever. There was no record of recent travel, however; the patient’s family had emigrated from Sri Lanka approximately 5 years before and had a history of typhoid fever. A strain with an indistinguishable DNA fingerprint generated using pulsed-field gel electrophoresis was also obtained from a stool specimen from an asymptomatic sibling of the patient (13). The clinical isolate, labeled N02-542, was identified using standard biochemical and serological procedures for enteric bacteriology (4). Antimicrobial susceptibility testing was performed initially using agar dilution and was repeated using broth microdilution for additional antimicrobials (4). The strain was found to be resistant to ampicillin, chloramphenicol, trimethoprim-sulfamethoxazole, streptomycin, tetracycline, and nalidixic acid (5) (Table 1). A second isolate from the same blood culture specimen was also identified as S. enterica serovar Typhi. It was fully susceptible as determined by agar dilution, and the fingerprint was indistinguishable from that of the resistant isolate, suggesting that the strain had lost the multidrug-resistant plasmid (see below). Plasmid DNA was isolated from S. enterica serovar Typhi N02-542 with a commercial plasmid isolation kit (Qiagen) and used to transform electrocompetent Escherichia coli DH10B (Invitrogen). The transformant, E. coli FJ542, was resistant to all the same antimicrobials as the parent strain with the exception of nalidixic acid (Table 1). Plasmid profiling showed that E. coli FJ542 contained the same plasmid as S. enterica serovar Typhi N02-0542, and PCR analysis identified the presence of the tetracycline resistance gene tet(A)B, the chloramphenicol resistance gene catA1, and blaTEM-1. PCR using primers 5’-CS and 3’-CS to detect cassettes of class 1 integrons (3) produced an ampli- con of approximately 750 bp in size. Sequence analysis of the ampli con revealed a 617-bp gene cassette (10) which had 100% identity to the cassette containing the dihydrofolate reductase gene dfrA47 from Shigella flexneri (GenBank accession number AF139109) (unpublished data). The dfrA47 gene cassette is also found in integrons in E. coli plasmid pDOGO100 (1) and E. coli plasmid R751 (6). The S. enterica serovar Typhi plasmid pHCM1 (218 kb) also carries blaTEM-1, tet(A)B, and catA1; however, it carries a dfrA45 gene and an incomplete class 1 integron (8). The related S. enterica serovar Typhi plasmid R27 (180 kb) contains only the tet(A)B gene and no class 1 integron (12). PCR analysis of the fully susceptible strain of S. enterica serovar Typhi isolated from the patient yielded no amplicons for any of the resistance genes.

The plasmid DNA from strain FJ542(pFJ-1) was restricted with HpaI, EcoRI, and BglII, and the sizes of the fragments were used to estimate the size of pFJ-1 to be 180 kb. Southern hybridization analysis with tet(A)B, blaTEM-1, catA1, and dfrA7 probes confirmed the presence of these genes on pFJ-1 (data not shown). Most plasmids associated with S. enterica serovar Typhi have been shown to belong to the H1 incompatibility group, and we have used PCR to confirm that plasmid pFJ-1 also belongs to this group (data not shown) (9).

In addition to the resistance gene content, the plasmid size and the compatibility group of pFJ-1 are identical to those recently described for isolates from India and Vietnam (9). This observation provides evidence to suggest that the integron-harboring plasmid has disseminated to North America. Recently, a 50-kb plasmid harboring a class 1 integron containing six drug resistance genes has been described in an S. enterica serovar Typhi isolate from Korea (7). These two findings stress the need for molecular laboratories to include an integron detection PCR for S. enterica serovar Typhi strains displaying resistance to sulfonamides, as class 1 integrons typically carry this resistance determinant (3).

We thank Romeo Hizon for his contribution related to antimicrobial susceptibility testing and Shaun Tyler and the staff of the DNA Core Facility at the National Microbiology Laboratory for generating the sequence information and synthesizing oligonucleotides.

REFERENCES


TABLE 1. MICs of various antimicrobials for study strains as determined by broth microdilution

<table>
<thead>
<tr>
<th>Antimicrobial(s)</th>
<th>E. coli DH10B</th>
<th>S. enterica serovar Typhi N02-542</th>
<th>E. coli FJ542a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amikacin</td>
<td>&lt;4</td>
<td>&lt;4</td>
<td>&lt;4</td>
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<tr>
<td>Ampicillin</td>
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<td>&gt;32</td>
<td>&gt;32</td>
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<tr>
<td>Apramycin</td>
<td>&lt;2</td>
<td>&lt;2</td>
<td>&lt;2</td>
</tr>
<tr>
<td>Amoxicillin and clavulanate</td>
<td>4 and 2</td>
<td>8 and 4</td>
<td>8 and 4</td>
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<tr>
<td>Cephalothin</td>
<td>0.25</td>
<td>16</td>
<td>18</td>
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<tr>
<td>Ceftriazone</td>
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<td>&lt;0.25</td>
<td>&lt;0.25</td>
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<tr>
<td>Chloramphenicol</td>
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<td>&lt;32</td>
<td>&lt;32</td>
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<tr>
<td>Trimethoprim-sulfamethoxazole</td>
<td>&lt;0.12-0.23</td>
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<td>Gentamicin</td>
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<td>&lt;0.25</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>32</td>
<td>16</td>
<td>32</td>
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<td>Nalidixic acid</td>
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<td>&gt;256</td>
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<td>&lt;0.015</td>
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<td>Streptomycin</td>
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<td>&gt;32</td>
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<tr>
<td>Cefoxitinb</td>
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<td>6</td>
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<tr>
<td>Ceftotaxin</td>
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<td>1</td>
</tr>
</tbody>
</table>

a Susceptibility testing conducted using Etest.

b Strain E. coli FJ542 is a transformant of S. enterica serovar Typhi N02–542.


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