Plasmid-Mediated Resistance to Cephalosporins in Salmonella enterica Serovar Typhi

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This study characterized a cephalosporin-resistant Salmonella enterica serovar Typhi isolate. The organism possessed a plasmid encoding the CTX-M-15 extended-spectrum β-lactamase. This plasmid is the determinant for the phenotype of cephalosporin resistance and is transferrable among Enterobacteriaceae.

Typhoid fever is a systemic infection caused by Salmonella enterica serovar Typhi and has become a predominantly travel-associated disease in developed countries (3, 6). Fluoroquinolones have been the most effective drugs for treatment since the spread of multidrug-resistant (resistant to ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole) S. Typhi strains. Furthermore, in the era of emergence of fluoroquinolone-resistant S. Typhi, it appears that alternative antimicrobial agents are necessary to ensure therapy for typhoid fever (7, 8). Actually, expanded-spectrum cephalosporins and macrolide antibiotics are considered to be viable alternatives (12). However, a few cephalosporin-resistant S. Typhi strains, which produce extended-spectrum β-lactamas (ESBLs), have been reported (1, 9, 11).

Forty-nine clinical isolates of S. Typhi were collected from 48 patients with typhoid fever in 2008. All isolates were identified by regional public health centers and sent to the Department of Bacteriology I, National Institute of Infectious Diseases. Isolates were routinely phage typed by the standard technique with the phage set kindly provided by the Health Protection Agency, London, United Kingdom, and MICs of antimicrobials were determined by the broth microdilution method. The results were interpreted according to the criteria of the Clinical and Laboratory Standards Institute (CLSI) (2, 5). One isolate with the phage type E1 (080049Ty) displayed a high level of resistance to cefazidime, cefotaxime, and ceftriaxone. This isolate was also resistant to nalidixic acid, showed reduced susceptibility to fluoroquinolones, and was susceptible to imipenem, aztreonam, kanamycin, gentamicin, chloramphenicol, tetracycline, and trimethoprim-sulfamethoxazole. The MICs of cefazidime and cefotaxime decreased significantly in the presence of clavulanic acid at a fixed concentration of 4 mg liter⁻¹ (Table 1). A plasmid profile of the resistant S. Typhi isolate showed one specific band, which was absent in the susceptible strain (08004Ty) with the same phage type, E1 (Fig. 1A). The plasmid DNA prepared by alkaline lysis with SDS was dissolved in Milli-Q water at a suitable concentration and was used for electroporation of Escherichia coli W3110. Transformants were selected on LB agar containing ampicillin. The resultant transformant produced a β-lactam resistance pattern similar to that produced by S. Typhi isolate 080049Ty (Table 1). These results suggest that a certain gene(s) located on the plasmid conferred the phenotype of resistance against expanded-spectrum cephalosporins. The presence of the gene conferring the ESBL phenotype was verified by PCR from the plasmid DNA with the primer pair for detection of the CTX-M-type β-lactamase gene (5‘-CA

Table 1. MICs for the strains used in this study

<table>
<thead>
<tr>
<th>Strain</th>
<th>AMP (≥32)</th>
<th>CAZ (≥32)</th>
<th>CAZ-CLA</th>
<th>CTX (≥32)</th>
<th>CTX-CLA</th>
<th>CRO (≥32)</th>
<th>NAL (≥32)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. Typhi 080049Ty (phage type E1)</td>
<td>&gt;64</td>
<td>64</td>
<td>0.25</td>
<td>&gt;128</td>
<td>&lt;0.125</td>
<td>&gt;128</td>
<td>&gt;64</td>
</tr>
<tr>
<td>S. Typhi 080048Ty (phage type E1)</td>
<td>&lt;2</td>
<td>1</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125</td>
<td>0.125</td>
<td>&gt;64</td>
</tr>
<tr>
<td>Transformant (E. coli W3110 derivative)</td>
<td>&gt;64</td>
<td>64</td>
<td>0.25</td>
<td>&gt;128</td>
<td>&lt;0.125</td>
<td>&gt;128</td>
<td>32</td>
</tr>
<tr>
<td>Transconjugant (S. Typhi 080048Ty derivative)</td>
<td>&gt;64</td>
<td>64</td>
<td>0.25</td>
<td>&gt;128</td>
<td>&lt;0.125</td>
<td>&gt;128</td>
<td>&gt;64</td>
</tr>
</tbody>
</table>

AMP, ampicillin; CAZ, cefazidime; CTX, cefotaxime; CRO, ceftriaxone; NAL, nalidixic acid; CLA, clavulanic acid. Numbers in parentheses are CLSI breakpoints for resistance.

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verified (Fig. 1). The doubly resistant colonies appeared in the presence of a plasmid with the same size as that of the donor was supplemented with ampicillin and nalidixic acid, and the presence of a plasmid with the same size as that of the donor was confirmed by ethidium bromide. (B) Southern blot analysis with a digoxigenin-labeled probe specific for CTX-M-15.

AGCGCAGGTGGGCACAGCAGC-3' and 5'-CTTTTGCCGTCTAAGGCGATAAAC-3'; data not shown) and subsequent Southern hybridization of undigested plasmid DNA with a digoxigenin-labeled probe generated with DIG-High Prime (Roche Diagnostics) according to the manufacturer’s instructions (Fig. 1B). In addition, sequence analysis revealed that this bla<sub>CTX-M-15</sub> was 100% identical to bla<sub>CTX-M-15</sub> (data not shown). Most of the bla<sub>CTX-M-15</sub> genes have been identified in Enterobacteriaceae, mainly in E. coli and Klebsiella spp., and CTX-M-15-producing E. coli strains were isolated from healthy people as well (4, 10). Therefore, the cephalosporin-resistant S. Typhi isolate in this study may have acquired the transferable plasmid containing bla<sub>CTX-M-15</sub> from another enteric bacterium in the patient’s intestine. E. coli W3110 harboring the above transformed plasmid with bla<sub>CTX-M-15</sub> was used as the donor of the plasmid in the transconjugation analysis to confirm the transferability. The expanded-spectrum cephalosporin-susceptible S. Typhi isolate 080048Ty was used as the recipient strain; this strain was also resistant to nalidixic acid. A mixture of each bacterial culture was incubated overnight at 37°C. Transconjugants were isolated on LB agar plates supplemented with ampicillin and nalidixic acid, and the presence of a plasmid with the same size as that of the donor was verified (Fig. 1). The doubly resistant colonies appeared with frequencies of 10<sup>-4</sup> to 10<sup>-3</sup> (defined as the number of ampicillin- and nalidixic acid-resistant S. Typhi colonies divided by the number of nalidixic acid-resistant S. Typhi colonies). These results indicate the transfer of the plasmid between enteric bacteria. Another transferable plasmid containing bla<sub>CTX-M-15</sub> was also observed in S. Typhi from an Iraqi patient (9). Obviously, the dissemination of the plasmids as an ESBL phenotype determinant, in addition to the emergence of fluoroquinolone-resistant S. Typhi, would make typhoid fever treatment increasingly difficult. Therefore, continued and careful observations and investigations regarding the characterization of S. Typhi isolates are necessary, especially in the identification of drug resistance profiles, and the development of novel therapeutic options should also be reconsidered.

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REFERENCES