Inhibitor-Resistant TEM-33 β-Lactamase in a Shigella sonnei Isolate

It has been shown that *Shigella* species produce a low-level chromosomally mediated β-lactamase which does not appreciably affect the level of resistance to β-lactams (2, 10). High rates of resistance to ampicillin among *Shigella* isolates are due to the production of β-lactamasess similar to TEM-1 or OXA-1. The OXA-type β-lactamase is more prevalent than TEM-1 in ampicillin-resistant *Shigella flexneri* (2, 6, 7). In *Shigella sonnei* the most commonly produced β-lactamase is the TEM-1 enzyme (2, 6, 7).

We describe here an *S. sonnei* isolate which was identified by the API ID 32E system (Bio-Merieux) and by antiserum agglutination and which was obtained in 1998 from stool samples of an 8-year-old child hospitalized at Orléans Hospital (Orléans France) for diarrheal disease that appeared after a 2-month stay in Turkey. The strain was resistant to amoxicillin and ticarcillin, either as single drugs or in combination with clavulanate and piperacillin. It was also resistant to trimethoprim, to nalidixic acid, and to fluoroquinolones.

The purpose of the present study was to find the molecular basis of the resistance to amoxicillin-clavulanate in the *S. sonnei* isolate (UCK strain). A TEM-1-hyperproducing *S. sonnei* strain (CFS01) and an OXA-1-producing *S. flexneri* strain (CFS02) were studied as comparators for MIC determinations.

The MICs of amoxicillin, amoxicillin-clavulanate, ticarcillin, and ticarcillin-clavulanate (clavulanic acid at a fixed concentration of 2 μg/ml) and combined with clavulanate (2 μg/ml), while MICs for the OXA-1-producing *S. flexneri* CFS02 were much lower (amoxicillin, 256 μg/ml; amoxicillin-clavulanate, 64 μg/ml; ticarcillin, 256 μg/ml; ticarcillin-clavulanate, 32 μg/ml). Against *S. sonnei* CFS01 (TEM-1 hyperproducing), clavulanate partially restored the activities of amoxicillin and ticarcillin (MICs, 64 μg/ml for both combinations).

No transconjugant was obtained by mating of *S. sonnei* UCK with *Escherichia coli* HB101 as recipient strain with ticarcillin (32 μg/ml) as selective agent. Plasmid DNA content was determined by comparison with plasmids Rsa (39 kb), TP114 (61 kb), pCFF04 (85 kb), and pCFF14 (180 kb). Strain UCK contained one large plasmid of ca. 90 kb which hybridized with the TEM probe (data not shown).

The nucleotide sequence analysis obtained by direct sequencing of the PCR product revealed that the sequence of the *bla* gene differed from that of *blaTEM-1*, at two positions: the G-162→T transversion (at position 1 of the −10 consensus sequence) in the promoter region and the change A-407→C, leading to the amino acid substitution Met-69→Leu in the coding region. The latter change has already been observed in the inhibitor-resistant TEM β-lactamase IRT-5/TEM-33 (4).

Table 1 shows the sequences of the structural genes and of the promoter regions specifying this IRT enzyme. Three different *blaTEM-33* genes which had the mutation A-407→C in common were reported by Goussard and Courvalin (3): *blaTEM-33a*, derived from *blaTEM-1b*, with the change T-226→C, which is under the control of the weak P3 promoter (9); *blaTEM-33b*, derived from *blaTEM-1b*, which is under the control of the strong promoters Pa and Pb; and *blaTEM-33c*, derived from *blaTEM-2* with the change A-317→C (Lys-39→Gln), with the promoter region corresponding to the strong P4 promoter with the T-32→C and G-162→T mutations. The structural gene of IRT-5/TEM-33 produced by *S. sonnei* CFS01 (TEM-1 hyperproducing), clavulanate partially restored the activities of amoxicillin and ticarcillin (MICs, 64 μg/ml for both combinations).

### Table 1

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<th>Position</th>
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<th>blatem1</th>
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<th>blatem2</th>
<th>blatem33a</th>
<th>blatem33b</th>
<th>blatem33c</th>
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<tr>
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<td>A (Lys)</td>
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<td>G</td>
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</tr>
</tbody>
</table>

* Numbering according to Sutcliffe (10).
* Numbering according to Ambler et al. (1).
* Only silent mutations occur.
* From reference 9.
* From reference 3.
* This study.
* P3 in TEM-1a, -1b, and -33a; Pa and Pb in TEM-2 and -33b; and P4 in TEM-33c and -33b-like.
SONNEI UCK is identical to blaTEM-33b. The promoter region had the change G-162→T (P4), which has been shown to be responsible for the hyperproduction of TEM-1 and which is commonly found upstream from the genes for inhibitor-resistant β-lactamases (3). This fourth gene coding for TEM-33 was designated blaTEM-33b-like.

The high-level resistance to amoxicillin-clavulanate (MIC₉₀ = 5 µg/ml) associated with susceptibility to cephalothin (8 µg/ml) observed in S. sonnei (UCK strain) suggested the presence of a hitherto unreported TEM-33 variant in the species. The sequence diversity observed for the IRT-3/TEM-33 genes lends weight to the idea that there is a great variety of TEM genes in nature, as previously suggested by Goussard and Courvalin (3) and as recently reported by Leflon-Guibout et al. (5). In this study, the patient had received no antibiotics before the resistant strain was isolated.

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REFERENCES

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