ESBL-Producing
Salmonella
enterica Serovar
Typhi in Traveler
Returning from
Guatemala
to Spain

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We report a case of typhoid fever in a traveler returning
to Spain from Guatemala that was caused by Salmonella
enterica serovar Typhi which produced an extended-spe-
ctrum β-lactamase (ESBL). This finding demonstrates the
presence of ESBL-producing S. enterica ser. Typhi strains
in the Americas. Enhanced surveillance is necessary to pre-
vent further spread.

Salmonella enterica serovar Typhi is the causative agent
of typhoid fever, an enteric bacterial infection that re-
results in systemic febrile illness. S. enterica ser. Typhi is
strictly adapted to humans; its transmission occurs through
the fecal-oral route, person-to-person contact, or contami-
nated water or food. An estimated 22 million new cases
type typhoid fever occur each year worldwide, resulting in
200,000 deaths (1). S. enterica ser. Typhi infection is un-
common in industrialized countries, where infections oc-
cur sporadically and mainly in travelers returning from
disease-endemic areas and in newly arrived immigrants.

Chloramphenicol was used successfully as the first-
line agent for the treatment of typhoid fever from the
1950s through the 1970s. Strains resistant to this com-
pound emerged in 1972, associated with self-transferable
IncHI plasmids. Trimethoprim-sulfamethoxazole and am-
picillin were then employed, but strains resistant to all 3
drugs arose rapidly during the 1980s and 1990s in southern
and Southeast Asia, the Middle East, and Africa (2,3). To
overcome these new resistances, fluoroquinolones were
proposed as the drug of choice. However, during the past
decade, ciprofloxacin-resistant strains have been reported
in the Asian subcontinent (2). This situation has resulted in
the use of ceftriaxone or cefotaxime as alternatives for
treatment of enteric fever (3).

Until now, extended-spectrum β-lactamase (ESBL)–
producing S. enterica ser. Typhi strains have been uncom-
mon and have been described only in a few patients of
Asian origin and in travelers returning from that region (4–
7). We report the detection and molecular characterization
of an ESBL-producing S. enterica ser. Typhi strain isolated
in Barcelona, Spain, from a patient with typhoid fever who
had traveled to Guatemala.

The Study

In September 2013, a 41-year-old man residing in Bar-
celona visited the emergency room of the Vall d’Hebron
Hospital, reporting 10 days of fever and pain in the right
upper abdominal quadrant, preceded by 3 days of diarrhea.
At the time of his hospital visit, the patient had been back
in Spain for 4 days after a 5-month stay in rural Guatemala.
Physical examination revealed a temperature of 37.8°C
and unremarkable blood pressure and heart rate results.
Abdominal examination showed tenderness of the right
upper and lower quadrants. General biochemistry values
and blood cell counts were within reference ranges, but C-
reactive protein level and erythrocyte sedimentation rate
were elevated. Results of initial blood and stool culture
testing were negative. Because of the patient’s persistent
abdominal pain and newly documented fever, computed
tomographic scan of the abdomen was performed; results
showed thickening of the terminal ileum, adjacent fat tis-
sue stranding, and regional lymphadenopathy. Antimicro-
bial drug treatment with amoxicillin-clavulanate acid was
begun. New blood cultures, performed because of the pa-

tient’s persistent fever, yielded S. enterica ser. Typhi, and
the strain produced an ESBL. Subsequently, intravenous
ertapenem (1 g/d) was administered for 14 days; the patient
experienced complete clinical recovery, and subsequent
blood and stool culture results were negative.

The isolate was identified by using the VITEK2 sys-
tem (bioMérieux, Marcy l’Etoile, France). Serotyping by
slide agglutination using commercial antisera according
to the Kauffmann-White scheme yielded the antigenic for-
mula 9,12,[Vi]:d:-. Multilocus sequence typing (MLST),
which was done using primers described elsewhere (8) with
allele sequences and allelic profiles verified in the MLST
database (http://mlst.ucc.ie/mlst/dbs/Senterica), showed
that the strain belonged to sequence type (ST) 2. This ST
is one of the most prevalent among S. enterica ser. Typhi;
isolates of this ST have been detected in Asia, Africa, and
South America (9).
Antimicrobial susceptibility to β-lactams was assessed by disk diffusion following Clinical Laboratory Standards Institute recommendations (http://www.clsi.org). Suggestive evidence of ESBL production was observed as synergy between amoxicillin/clavulanate and ≥1 of the following: cefotaxime, ceftazidime, aztreonam, and cefepime. In addition, MICs of β-lactams, quinolones, trimethoprim/sulfamethoxazole, chloramphenicol, and azithromycin were determined by E-test (bioMérieux) (Table). According to Clinical Laboratory Standards Institute interpretative criteria, the isolate was resistant to all β-lactams evaluated except amoxicillin/clavulanate, piperacillin/tazobactam, cefoxitin, and carbenepens and was susceptible to chloramphenicol, trimethoprim/sulfamethoxazole, nalidixic acid, ciprofloxacin, and gentamicin. The MIC of azithromycin was 4 μg/mL; according to the European Committee on Antimicrobial Susceptibility Testing (http://www.eucast.org), isolates with an MIC ≤16 μg/mL of this drug should be considered wild-type organisms that are expected to respond to treatment (10).

To screen for TEM and SHV β-lactamases, CTX-M ESBL genes, and genes encoding resistance to quinolones (qnrA, qnrB, qnrS, qepA, oqxAB, and acc(6′)-Ib-cr), we used PCR as described previously (11,12). Mutations in acc(6′)-Ib that confer quinolone resistance and in the quinolone resistance–determining regions of gyrA, gyrB, parC, and parE, were studied by sequencing (12,13). These experiments showed that the S. enterica ser. Typhi isolate possessed blaTEM-1, blaCTX-M-15, and acc(6′)-Ib but none of the studied quinolone resistance genes or detectable quinolone resistance–determining region mutations.

ISEcp1, IS26, and orf477 are elements that previously have been identified in the genetic environment surrounding blaCTX-M-15. The presence of such elements was investigated by PCR mapping and sequencing in combination with blaCTX-M-15–specific primers, as reported previously (14). These results showed that the ESBL gene was situated between a nontruncated ISEcp1 and orf477, as noted previously in other Enterobacteriaceae.

Identification and characterization of the location of blaCTX-M-15 was carried out by conjugation, using a nalidixic acid–resistant derivative of Escherichia coli HB101 as recipient, PFGE of total DNA of donor and transconjugant digested with S1-nuclease, Southern hybridization with specific probes, and PCR-based replicon typing, as described previously (14). These studies showed that the S. enterica ser. Typhi blaCTX-M-15 was located in an IncI/M self-transferrable plasmid of 65 kb that also carried the blaTEM-1 and acc(6′)-Ib genes. MICs to antimicrobial agents for donor, recipient, and transconjugant are shown in the Table.

### Conclusions

We describe an ESBL-producing S. enterica ser. Typhi strain isolated from a man in Spain who had traveled to Guatemala. It is well documented that ESBL-producing Enterobacteriaceae are spreading worldwide. CTX-M-15 is one of the most commonly identified ESBLs; its high prevalence has been driven mainly by the pandemic spread and expansion of the ST131 E. coli clonal group. Extended-spectrum cephalosporin resistance has increased during the past few years in nontyphoidal S. enterica, principally in developing countries, where such resistance appears to be endemic in some areas (15).

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**Table. Susceptibility profiles of the CTX-M-15–producing Salmonella enterica serovar Typhi strain from a patient in Spain who had traveled to Guatemala in 2013, compared with profiles of the Escherichia coli recipient strain and the transconjugant strain**

<table>
<thead>
<tr>
<th>Antimicrobial agent(s)</th>
<th>S. enterica ser. Typhi 301812 (donor) MIC, μg/mL</th>
<th>E. coli HB101-Nal (recipient) 301812</th>
<th>E. coli HB101-Nal TC-301812 (transconjugant)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>&gt;256</td>
<td>1.5</td>
<td>&gt;256</td>
</tr>
<tr>
<td>Amoxicillin-clavulanate</td>
<td>16</td>
<td>3</td>
<td>16</td>
</tr>
<tr>
<td>Piperacillin-tazobactam</td>
<td>3</td>
<td>1</td>
<td>1.5</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>12</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>&gt;256</td>
<td>0.064</td>
<td>0.016</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>&gt;256</td>
<td>0.016</td>
<td>&gt;256</td>
</tr>
<tr>
<td>Cefotaxime-clavulanate</td>
<td>0.19</td>
<td>0.016</td>
<td>0.016</td>
</tr>
<tr>
<td>Cecefeprin</td>
<td>&gt;256</td>
<td>0.032</td>
<td>24</td>
</tr>
<tr>
<td>Aztreonem</td>
<td>&gt;256</td>
<td>&lt;0.016</td>
<td>32</td>
</tr>
<tr>
<td>Imipenem</td>
<td>0.25</td>
<td>0.38</td>
<td>0.38</td>
</tr>
<tr>
<td>Meropenem</td>
<td>0.064</td>
<td>0.023</td>
<td>0.023</td>
</tr>
<tr>
<td>Ertapenem</td>
<td>0.064</td>
<td>0.002</td>
<td>0.002</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Trimethoprim-sulfamethoxazole</td>
<td>0.047</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>3</td>
<td>&gt;256</td>
<td>&gt;256</td>
</tr>
<tr>
<td>Ciproflaxacin</td>
<td>0.023</td>
<td>0.125</td>
<td>0.125</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>4</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>1.5</td>
<td>0.75</td>
<td>3</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>24</td>
<td>1</td>
<td>&gt;256</td>
</tr>
<tr>
<td>Amikacin</td>
<td>32</td>
<td>2</td>
<td>&gt;256</td>
</tr>
</tbody>
</table>
To our knowledge, 5 cases of S. enterica ser. Typhi resistant to β-lactams by ESBL production have been reported, all with Asian origins (Bangladesh, the Philippines, Iraq, and India) (4–7). For the isolate originating in the Philippines, SHV-12 was the enzyme responsible for ESBL resistance (7); for the other 4 isolates, a CTX-M enzyme was detected, and in 3 of those, the CTX-M-15 variant was identified (4–6). In our case, however, we found a different genetic environment of bla\textsubscript{CTX-M-15}. Specifically, we found a nontruncated IS\text{Ecp}\textsubscript{1} upstream of bla\textsubscript{CTX-M-15}, whereas in the previously reported Iraq-origin strain, IS\text{Ecp}\textsubscript{1} was truncated by IS\text{26} (5). Genetic environments of bla\textsubscript{CTX-M-15}-producing S. enterica ser. Typhi isolates from India are not clear, but according to the methods used by the authors (4), a truncated IS\text{Ecp}\textsubscript{1} or a different structure may have been involved. Our results also confirm that the plasmid harboring bla\textsubscript{CTX-M-15}, which also carries bla\textsubscript{TEM-1} and acc(6)-Ib, carries an IncI/M replicon.

In summary, we report a case of typhoid fever caused by an ESBL-producing S. enterica ser. Typhi isolate from a traveler returning to Spain from Guatemala. This case represents the acquisition of an ESBL-producing S. enterica ser. Typhi strains in the Americas. Because typhoid fever is a serious public health issue, meticulous microbiological and epidemiologic investigation of strains of this sort are necessary to prevent further spread of this disease.

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References

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