Persistent isolation of \textit{Salmonella} Concord harbouring CTX-M-15, SHV-12 and QnrA1 in an asymptomatic adopted Ethiopian child in Spain also colonized with CTX-M-14- and QnrB-producing Enterobacteriaceae

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Sir,

Endemicity of extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae in orphanages has been reported in different developing countries where children and caregivers are colonized with these type of isolates.\textsuperscript{1} ESBLs in salmonellae are increasing in prevalence, with the propensity to carry more than one ESBL with or without other transmissible resistance mechanisms. We report the persistent recovery of CTX-M-15- and SHV-12-carrying \textit{Salmonella} enterica serotype Concord isolates also harbouring QnrA1 from the stool cultures of an Ethiopian child in Madrid. The 1-year-old boy, transiently adopted in December 2008 through a non-governmental organization, came from an orphanage in Addis Ababa, Ethiopia, and was immediately admitted to the Paediatric Intensive Care Unit of the Hospital Universitario Ramón y Cajal in Madrid (Spain). Prior to his arrival and due to a febrile syndrome, he had been exclusively treated with standard doses of ceftriaxone, piperacillin/tazobactam and amoxicillin/clavulanate; the latter was suspended due to persistent diarrhoea. Once in Spain, although it was not microbiologically documented, he was clinically diagnosed with urinary sepsis and acute obstructive renal failure. The patient stayed in an intensive care unit for 7 days until surgery to correct an obstructive uropathy. He received meropenem and fluconazole for 19 days. After hospital discharge, oral amoxicillin/clavulanate was administered.

A routine stool culture submitted at admission rendered the isolation of an ESBL-producing \textit{Salmonella} Concord isolate (S1). Due to the resistance pattern and the infrequent serotype in our country, subsequent stool samples were requested. In addition to standard stool culture plating, the chromogenic agar medium chromID ESBL (bioMérieux, Marcy l’Étoile, France) was used. Intrafamilial faecal carriage of ESBL-producing Enterobacteriaceae was also screened during January–March 2009. The CTX-M-15-producing \textit{Salmonella} Concord strain 3728 and its \textit{Escherichia coli} (JS3 Azí\textsuperscript{30}) transconjugant were used as controls.\textsuperscript{2,3} Molecular methods were performed as previously described.\textsuperscript{4–7}

Three additional ESBL-producing \textit{Salmonella} Concord isolates (S2–S4) as well as three ESBL-producing \textit{Escherichia coli} (E1–E3) and three ESBL-producing \textit{Klebsiella pneumoniae} (K1–K3) isolates were recovered monthly (January–March 2009) from the patient. We cannot rule out the potential acquisition of the ESBL-producing \textit{E. coli} and \textit{K. pneumoniae} isolates after the child’s arrival in Spain as the search for these isolates was not performed for the first faecal culture. The four \textit{Salmonella} Concord isolates were resistant to all β-lactams except cefoxitin and carbapenems. Cefotaxime, ceftazi-dime and cefepime MICs (standard microdilution) were $\geq$256 mg/L, while those of the combinations cefotaxime/clavulanate and ceftazo-dime/clavulanate (fixed clavulanate concentration of 4 mg/L) were 1 and 2 mg/L, respectively. These isolates simultaneously produced a CTX-M-15 and an SHV-12 ESBL. Moreover, they were resistant to nalidixic acid (MIC $\geq$32 mg/L) with a ciprofloxacin MIC of 0.25 mg/L. All isolates were resistant to gentamicin and tobramycin and susceptible to kanamycin, amikacin and netilmicin. They were also resistant to trimethoprim, sulphamethoxazole, tetracycline and chloramphenicol, but susceptible to tigecycline (Table 1).

The first recovered \textit{Salmonella} isolate (S1) harboured three plas-mids of $\sim$50, 100 and 340 kb. Both bla\textsubscript{CTX-M-15} and bla\textsubscript{SHV-12} genes were demonstrated to be located in the latter non-conjugative plasmid of incompatibility group InhI2 by hybridization studies. The other three \textit{Salmonella} isolates contained at least three plasmids ranging from $\sim$50 to 250 kb, but did not harbour the 340 kb plasmid, as did S1 [Figure S1, available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/)]. The location of both bla\textsubscript{CTX-M-15} and bla\textsubscript{SHV-12} was also demonstrated by hybridization and genes were found on the InhI2 non-conjugative plasmid of 250 kb. It is of note that the four \textit{Salmonella} isolates belonged to the same clone as the PFGE patterns were indistinguishable (Table 1) and presented high similarity (two bands of difference) to an isolate previously recovered in France from the stools of an adopted child who had come from Ethiopia.\textsuperscript{2} Serotype Concord is very unusual in Spain and, during the last 5 years (2004–08), only three \textit{Salmonella} Concord isolates (0.01%) were identified in the Spanish Reference Salmonella Laboratory (National Reference Centre, Majadahonda, Spain) and none of them was an ESBL producer (A. Echeita, National Reference Centre, personal communication).

Two out of the three \textit{E. coli} isolates were genetically related (E1 and E3). The three isolates produced a CTX-M-14 enzyme and one of them (E2) was also positive for qnrB4. In all cases, bla\textsubscript{CTX-M-14} was detected on an $\sim$120 kb conjugative plasmid. Two of these plasmids belonged to the IncA/C group, and the other one was non-typeable (Table 1). The three ESBL-producing \textit{K. pneumoniae} isolates presented highly related PFGE patterns.
Table 1. Characteristics of all isolates recovered during the period of study

<table>
<thead>
<tr>
<th>Isolatesa</th>
<th>PFGE type</th>
<th>Month/year</th>
<th>Non-β-lactam resistance phenotypeb</th>
<th>bla genes</th>
<th>Plasmid size (approximate kb)c</th>
<th>Plasmid Inc groupd</th>
<th>Qnr type</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>SCA</td>
<td>12/08</td>
<td>TMP CHL TET SUL STR GEN TOB NAL</td>
<td>CTX-M-15; SHV-12</td>
<td>340, 100, 50</td>
<td>IncHI2</td>
<td>A1</td>
</tr>
<tr>
<td>S2</td>
<td>SCA</td>
<td>01/09</td>
<td>TMP CHL TET SUL STR GEN TOB NAL</td>
<td>CTX-M-15; SHV-12</td>
<td>250, 100, 50</td>
<td>IncHI2</td>
<td>A1</td>
</tr>
<tr>
<td>S3</td>
<td>SCA</td>
<td>02/09</td>
<td>TMP CHL TET SUL STR GEN TOB NAL</td>
<td>CTX-M-15; SHV-12</td>
<td>250, 100, 50</td>
<td>IncHI2</td>
<td>A1</td>
</tr>
<tr>
<td>S4</td>
<td>SCA</td>
<td>03/09</td>
<td>TMP CHL TET SUL STR GEN TOB NAL</td>
<td>CTX-M-15; SHV-12</td>
<td>250, 100, 50</td>
<td>IncHI2</td>
<td>A1</td>
</tr>
<tr>
<td>E1</td>
<td>ECA</td>
<td>01/09</td>
<td>SUL STR</td>
<td>CTX-M-14</td>
<td>260, 170, 120, 50, 30</td>
<td>IncA/C</td>
<td>none</td>
</tr>
<tr>
<td>E2</td>
<td>ECB</td>
<td>02/09</td>
<td>TMP CHL TET SUL STR NET KAN GEN TOB AMK</td>
<td>CTX-M-14</td>
<td>170, 120, 40</td>
<td>NA</td>
<td>B4</td>
</tr>
<tr>
<td>E3</td>
<td>ECA</td>
<td>03/09</td>
<td>SUL STR</td>
<td>CTX-M-14</td>
<td>260, 170, 120, 50, 30</td>
<td>IncA/C</td>
<td>none</td>
</tr>
<tr>
<td>K1</td>
<td>KPA1</td>
<td>01/09</td>
<td>SUL STR FOF</td>
<td>CTX-M-14</td>
<td>120, 50</td>
<td>IncA/C</td>
<td>none</td>
</tr>
<tr>
<td>K2</td>
<td>KPA2</td>
<td>02/09</td>
<td>TMP CHL TET SUL STR SPT NET KAN GEN TOB AMK</td>
<td>CTX-M-14</td>
<td>130, 50</td>
<td>IncA/C</td>
<td>none</td>
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<tr>
<td>K3</td>
<td>KPA3</td>
<td>03/09</td>
<td>SUL STR FOF</td>
<td>CTX-M-14</td>
<td>120, 50</td>
<td>IncA/C</td>
<td>none</td>
</tr>
</tbody>
</table>

aS, Salmonella Concord; E, E. coli; K, K. pneumoniae.
bTMP, trimethoprim; CHL, chloramphenicol; TET, tetracycline; SUL, sulphonamides; SPT, spectinomycin; STR, streptomycin; GEN, gentamicin; TOB, tobramycin; AMK, amikacin; NET, netilmicin; KAN, kanamycin; NAL, nalidixic acid; FOF, fosfomycin.
cPlasmid sizes harbouring bla genes (approximate kb) are underlined.
dPlasmid Inc group 1, IncHI2, IncA/C, none.

Supplementary data

Figure S1 is available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

Transparency declarations

None to declare.

No Ethics Committee approval was required for this study. Patient and adoptive-family privacy was strictly maintained during the study.

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References