First Description of Shigella sonnei Harboring bla_{CTX-M-55} Outside Asia

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Shigella sonnei is one of the most common causes of infectious diarrhea in developed countries and is a frequent agent in traveller’s diarrhea. However, over the last decade, infections caused by S. sonnei have increased in transitional middle-income countries [1]. In Ecuador, reports of shigellosis cases have become more frequent in recent years, increasing from 399 in 2013 to 563 in 2015 [12]. Additionally, many cases go unreported.

Although most S. sonnei isolates remain sensitive to third-generation cephalosporins, this species has a great capacity to acquire ESBL genes from Escherichia coli from the human and animal gut [16]. Therefore, the evolution of S. sonnei producing ESBL is a global concern that may compromise the empirical treatment of infectious diarrhea [6].

There have been no reports of S. sonnei producing ESBL in Ecuador and there is only one report in South America [15]. However, reports of the isolation of S. sonnei ESBL is a concerning issue in several parts of the world [19]. Previous reports describe bla\(_{CTX-M-15}\) as the most prevalent S. sonnei variant; bla\(_{CTX-M-55}\) has been observed in this species only in Asia [11, 14] (Fig. 1). The bla\(_{CTX-M-15}\) and bla\(_{CTX-M-55}\) variants belong to bla\(_{CTX-M}\) group 1 and show similar activity against cefotaxime and ceftazidime, owing to the D240G amino acid mutation. These two variants differ in a single amino acid substitution (A77V) [8]. bla\(_{CTX-M-15}\) appears to be ubiquitous and is recognized as the dominant variant of the CTX-M family in Enterobacteriaceae [7]. In contrast, bla\(_{CTX-M-55}\) appears to be mainly restricted to Asia [18].

The dissemination of bacterial resistance worldwide is facilitated by travellers and migrants, particularly those from high-risk regions with poor hygiene and weak antimicrobial policies [10]. Furthermore, Valverde et al. [17] demonstrated that international travellers play an important role in the transfer of different bla\(_{CTX-M}\) variants between regions.

Here, we present the first report of S. sonnei harboring bla\(_{CTX-M-55}\) isolated from Ecuador and outside Asia.

A 7-year-old girl who had not travelled abroad came to the hospital with watery diarrhea, abdominal pain, and fever (39.3°C). The child lives in Santo Domingo, an Ecuadorian city in a rainy subtropical zone. Stool samples were inoculated in MacConkey, Salmonella-Shigella, and Hektoen enteric agars, as well as in Selenite broth. The plates and broth were incubated at 35°C for 20 h. Negative lactose colonies were identified as S. sonnei using the Vitek2 system (bioMérieux, France), and positive agglutination was observed with antiserum Poly Shigella Group D serotypes I & II. The susceptibility profile was evaluated using the Sensititre broth microdilution (MIC) method (Trek Diagnostics, USA) (Table 1) and phenotypic ESBL
First Report of \textit{S. sonnei} \textit{bla}_{CTX-M-55} in Ecuador  

production was confirmed using the double disk method. Results were interpreted according to the CLSI-2016 guidelines. The patient was treated with azithromycin on an outpatient basis and is progressing well.

Plasmid DNA of an \textit{S. sonnei} strain and a laboratory susceptible \textit{E. coli} strain were purified using the Qiagen Plasmid Midi Kit (Qiagen, Germany), digested with S1 nuclease (Promega, USA), and resolved by pulsed-field gel electrophoresis (PFGE). The assay showed a unique plasmid of \(-130\) kb only in the \textit{S. sonnei} strain. A mating assay produced \textit{E. coli} transconjugant lac\(^+\) colonies in McConkey agar plates supplemented with 5 mg/l cefotaxime. The species identification and susceptibility profile of the transconjugant (TcSs-55) was conducted as described above (Table 1). Plasmid DNA of \textit{S. sonnei} and the TcSs-55 strain were digested with the EcoR1 restriction enzyme and resolved in 2% agarose gels. The restriction profiles of the two strains were identical.

**Table 1.** MIC profiles of the \textit{S. sonnei} strain harboring the \textit{bla}_{CTX-M-55} variant, the laboratory susceptible \textit{E. coli} strain, and \textit{E. coli} transconjugant strain with the plasmid (TcSs55).

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>\textit{Shigella sonnei}</th>
<th>\textit{E. coli} strain</th>
<th>TcSs-55</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amikacin</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Trimethoprim/sulfamethoxazole</td>
<td>4/76</td>
<td>0.5/9.3</td>
<td>0.5/9.5</td>
</tr>
<tr>
<td>Cefazidine</td>
<td>16</td>
<td>1</td>
<td>16</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>32</td>
<td>1</td>
<td>32</td>
</tr>
<tr>
<td>Cefepime</td>
<td>16</td>
<td>2</td>
<td>16</td>
</tr>
<tr>
<td>Ertapenem</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Meropenem</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Imipenem</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>4</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>4</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>16</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Minocycline</td>
<td>8</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Colistin</td>
<td>0.5</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>0.5</td>
<td>0.25</td>
<td>0.25</td>
</tr>
</tbody>
</table>

MICs were performed using the Sensititre broth microdilution (MIC) method.

Fig. 1. CTX-M variants reported in \textit{Shigella sonnei} isolates. The map was drawn based on the review of Zhao and Hu [19] and updated to 2016 with a PubMed and GenBank review. *Sequences of CTX-M-55 and CTX-M-57 are identical.*

nuclease (Promega, USA), and resolved by pulsed-field gel electrophoresis (PFGE). The assay showed a unique plasmid of \(-130\) kb only in the \textit{S. sonnei} strain. A mating assay produced \textit{E. coli} transconjugant lac\(^+\) colonies in McConkey agar plates supplemented with 5 mg/l cefotaxime. The species identification and susceptibility profile of the transconjugant (TcSs-55) was conducted as described above (Table 1). Plasmid DNA of \textit{S. sonnei} and the TcSs-55 strain were digested with the EcoR1 restriction enzyme and resolved in 2% agarose gels. The restriction profiles of the two strains were identical.

PCR screening for \textit{bla}_{CTX-M} genes was carried out in the \textit{S. sonnei} strain and TcSs-55. PCR products were sequenced and the \textit{bla}_{CTX-M-55} variant was identified in both strains (Fig. 2C). The association of the \textit{ISEcp-1} mobile element with \textit{bla}_{CTX-M-55} was determined by PCR using a forward primer complementary to a region in the \textit{ISEcp-1} and reverse primer located in \textit{orf477}. The PCR products were sequenced to establish their identities (GenBank Accession No. KX196197). Plasmid PCR typing was conducted as described by Carattoli et al. [3] and a plasmid belonging to the II incompatibility group was identified.

Recently, Qu et al. [14] showed the potential transfer of \textit{bla}_{CTX-M-55} from fecal \textit{E. coli} to \textit{S. sonnei} using a whole plasmid sequencing approach. This transference appears to be more probable in regions with high prevalence of \textit{E. coli} harboring \textit{bla}_{CTX-M-55}, such as in Asia [9] and particularly China, where \textit{bla}_{CTX-M-55} is displacing \textit{bla}_{CTX-M-14} and \textit{bla}_{CTX-M-15} as the most prevalent variant [18, 19]. To date, reports of \textit{S. sonnei} harboring \textit{bla}_{CTX-M-55} have been confined to Asian countries [11, 14]. In South America, the most prevalent
The presence of \textit{bla}_{CTX-M-15} has been reported recently in low occurrence in \textit{E. coli} causing urinary tract infection in Bolivia \cite{2}, in healthy travellers returning from Peru \cite{17}, and in Ecuador in clinical isolates \cite{4,13}.

Two explanations may describe the appearance of this strain in our location. The first is that \textit{bla}_{CTX-M-15} may convert into \textit{bla}_{CTX-M-55}. The second explanation, which is more plausible, is that travellers and products originating from Asia may have introduced this variant into the Andean Region in recent years \cite{5}.

Recently, commercial relationships between China and Ecuador have increased, leading to an increase in the mobilization of people among both countries \cite{5}; 80,000 Chinese citizens immigrated to Ecuador between 2011 and 2015 \cite{https://issuu.com/elcomerciocom/docs/informacion_oficial_de_entrellas_s-s}. Human mobilization and trade relationships may explain, at least in part, the recent reports of Asiatic resistance determinants in our location: \textit{E. coli} harboring \textit{mcr-1} and \textit{bla}_{CTX-M-55} in a clinical sample in Quito-Ecuador \cite{13}, \textit{bla}_{CTX-M-55}, \textit{bla}_{CTX-M-14}, and \textit{bla}_{CTX-M-15} in gram-negative bacteria in Southern Ecuador \cite{4}, \textit{bla}_{CTX-M-14} in \textit{E. coli} isolated from bacteremia in hospitals in Quito-Ecuador (Zurita J, et al. 2016. Abstr. 26th ECCMID. Amsterdam, Netherlands P0497), and \textit{E. coli} harboring \textit{bla}_{CTX-M-55} and \textit{bla}_{CTX-M-14} in an urban river in Quito-Ecuador (Ortega-Paredes D, et al. 2016. Abstr. ASM Microbe. Boston, USA P080-Sunday).

This report and recent data suggest that Asiatic bacteria resistance determinants, including \textit{bla}_{CTX-M-55}, are present in our location and appear to be increasing. However, additional studies of the genetic environment of \textit{bla}_{CTX-M-55} are needed to establish the epidemiology dynamics of this variant, entryways and dispersion, and possible health implications in our region.

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