J. Microbiol. Biotechnol. (2016), 26(12), 2224–2227
http://dx.doi.org/10.4014/jmb.1605.05069

jmb

First Description of *Shigella sonnei* Harboring $bla_{CTX\text{-}M\text{-}55}$ Outside Asia

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Received: May 23, 2016
Revised: August 10, 2016
Accepted: August 17, 2016

First published online
August 24, 2016

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pISSN 1017-7825, eISSN 1738-8872

Shigella sonnei harboring $bla_{CTX\text{-}M\text{-}55}$ was isolated outside of Asia for the first time. The $bla_{CTX\text{-}M\text{-}55}$ gene was found to be downstream of *ISEcp-1* and located in a ~130 kb conjugative plasmid belonging to the *I1* incompatibility group. The strain was recovered from a 7-year-old Ecuadorian girl with watery diarrhea who had not travelled abroad. Recent local data describe the emergence of $bla_{CTX\text{-}M\text{-}55}$ and other variants typically found in Asia in the Andean Region, suggesting that increased travel of humans and trade relationships with Asian countries are influencing the current Ecuadorian bacterial resistance situation.

Keywords: *Shigella sonnei*, $bla_{CTX\text{-}M\text{-}55}$, Ecuador, *ISEcp-1*, Asia

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 $bla_{CTX\text{-}M\text{-}15}$ as the most prevalent *S. sonnei* variant; $bla_{CTX\text{-}M\text{-}55}$ has been observed in this species only in Asia [11, 14] (Fig. 1). The $bla_{CTX\text{-}M\text{-}15}$ and $bla_{CTX\text{-}M\text{-}55}$ variants belong to $bla_{CTX\text{-}M}$ 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]. $bla_{CTX\text{-}M\text{-}15}$ appears to be ubiquitous and is recognized as the dominant variant of the CTX-M family in Enterobacteriaceae [7]. In contrast, $bla_{CTX\text{-}M\text{-}55}$ 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 $bla_{CTX\text{-}M}$ variants between regions.

Here, we present the first report of *S. sonnei* harboring $bla_{CTX\text{-}M\text{-}55}$ 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

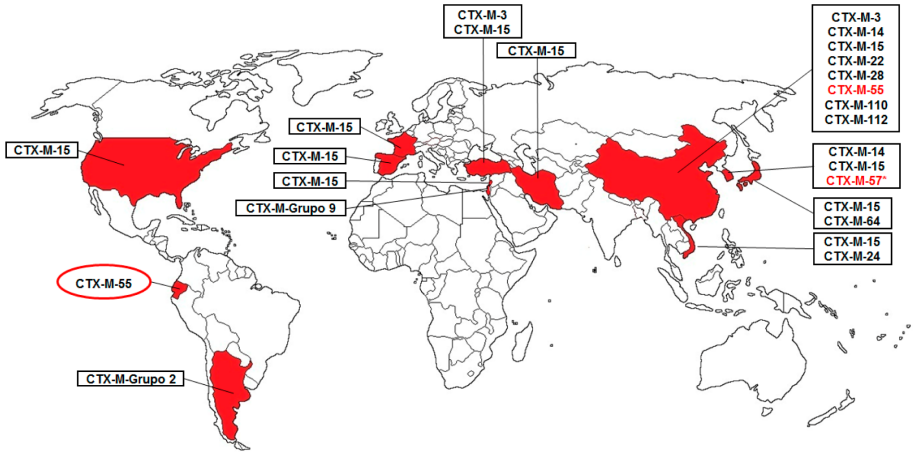

Fig. 1. CTX-M variants reported in *Shigella sonnei* isolates.
The map was drawn based on the review of Zhao and Hu [19] and updated to 2016 with a PubMed and GenBank review. *Sequences of CTX-M-55 and CTX-M-57 are identical.

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 *S. sonnei* strain and a laboratory susceptible *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 *S. sonnei* strain. A mating assay produced *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 *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 *S. sonnei* strain harboring the $bla_{CTX\text{-}M\text{-}55}$ variant, the laboratory susceptible *E. coli* strain, and *E. coli* transconjugant strain with the plasmid (TcSs55).

Antibiotic	*Shigella sonnei*	*E. coli* strain	TcSs-55
Amikacin	4	4	4
Trimethoprim/ sulfamethoxazole	4/76	0.5/9.5	0.5/9.5
Ceftazidime	16	1	16
Cefotaxime	32	1	32
Cefepime	16	2	16
Ertapenem	0.25	0.25	0.25
Meropenem	1	1	1
Imipenem	1	1	1
Gentamicin	4	1	1
Tobramycin	4	1	1
Ciprofloxacin	0.25	0.25	0.25
Levofloxacin	1	1	1
Doxycycline	16	2	2
Minocycline	8	2	2
Colistin	0.5	1	1
Tigecycline	0.5	0.25	0.25

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

PCR screening for $bla_{CTX\text{-}M}$ genes was carried out in the *S. sonnei* strain and TcSs-55. PCR products were sequenced and the $bla_{CTX\text{-}M\text{-}55}$ variant was identified in both strains (Fig. 2C). The association of the *ISEcp-1* mobile element with $bla_{CTX\text{-}M\text{-}55}$ was determined by PCR using a forward primer complementary to a region in the *ISEcp-1* and reverse primer located in *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 *I1* incompatibility group was identified.

Recently, Qu *et al.* [14] showed the potential transfer of $bla_{CTX\text{-}M\text{-}55}$ from fecal *E. coli* to *S. sonnei* using a whole plasmid sequencing approach. This transference appears to be more probable in regions with high prevalence of *E. coli* harboring $bla_{CTX\text{-}M\text{-}55}$, such as in Asia [9] and particularly China, where $bla_{CTX\text{-}M\text{-}55}$ is displacing $bla_{CTX\text{-}M\text{-}14}$ and $bla_{CTX\text{-}M\text{-}15}$ as the most prevalent variant [18, 19]. To date, reports of *S. sonnei* harboring $bla_{CTX\text{-}M\text{-}55}$ have been confined to Asian countries [11, 14]. In South America, the most prevalent

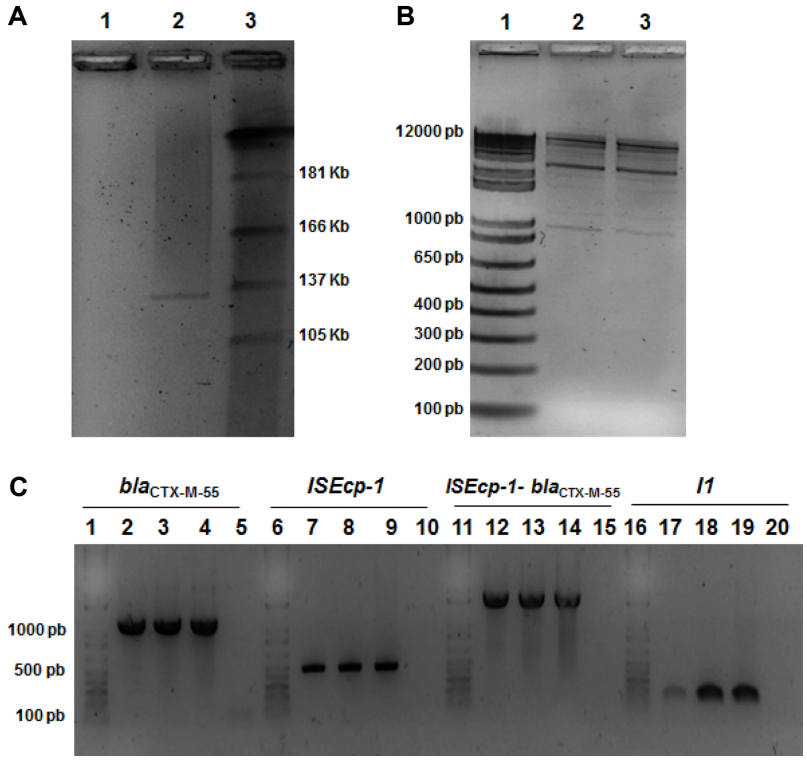

Fig. 2. Isolation and characterization of the plasmid from the *Shigella sonnei* strain harboring the $bla_{CTX\text{-}M\text{-}55}$ variant.
(**A**) Plasmids digested with endonuclease S1 resolved by PFGE; lane 1, laboratory susceptible *E. coli* strain; lane 2, *S. sonnei* strain; lane 3, PFGE marker. (**B**) Plasmids digested with EcoR1; lane 1, 1 kb plus DNA ladder; lane 2, *S. sonnei* strain; lane 3, *E. coli* transconjugant (TcSs-55). (**C**) Association of *ISEcp-1* mobile element with $bla_{CTX\text{-}M\text{-}55}$ and plasmid incompatibility group typing; lanes 1, 6, 11, and 16, 1 kb plus DNA; lanes 2, 7, 12, and 17, positive controls; lanes 3, 8, 13, and 18, *S. sonnei* strain; lanes 4, 9, 14, and 19, *E. coli* transconjugant (TcSs-55); lanes 5, 10, 15, and 20, negative controls.

variant is $bla_{CTX\text{-}M\text{-}15}$ [7]. The presence of $bla_{CTX\text{-}M\text{-}55}$ has been reported recently in low occurrence in *E. coli* causing urinary tract infection in Bolivia [2], in healthy travellers returning from Peru [17], and in Ecuador in clinical isolates [4, 13].

Two explanations may describe the appearance of this strain in our location. The first is that $bla_{CTX\text{-}M\text{-}15}$ may convert into $bla_{CTX\text{-}M\text{-}55}$. 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 [5].

Recently, commercial relationships between China and Ecuador have increased, leading to an increase in the mobilization of people among both countries [5]; 80,000 Chinese citizens immigrated to Ecuador between 2011 and 2015 (https://issuu.com/elcomerciocom/docs/informacion_oficial_de_entradas_-_s). Human mobilization and trade relationships may explain, at least in part, the recent reports of Asiatic resistance determinants in our location: *E. coli* harboring *mcr-1* and $bla_{CTX\text{-}M\text{-}55}$ in a clinical sample in Quito-Ecuador [13], $bla_{CTX\text{-}M\text{-}55}$, $bla_{CTX\text{-}M\text{-}65}$, and $bla_{CTX\text{-}M\text{-}14}$ in gram-negative bacteria in Southern Ecuador [4], $bla_{CTX\text{-}M\text{-}14}$ in *E. coli* isolated from bacteremia in hospitals in Quito-Ecuador (Zurita J, *et al*. 2016. Abstr. 26th ECCMID. Amsterdam, Netherlands P0497), and *E. coli* harboring $bla_{CTX\text{-}M\text{-}65}$ and $bla_{CTX\text{-}M\text{-}14}$ 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 $bla_{CTX\text{-}M\text{-}55}$, are present in our location and appear to be increasing. However, additional studies of the genetic environment of $bla_{CTX\text{-}M\text{-}55}$ are needed to establish the epidemiology dynamics of this variant, entryways and dispersion, and possible health implications in our region.

Acknowledgments

This study was supported by Zurita & Zurita Laboratorios by grant MIC-05, and Pontificia Universidad Católica del

Ecuador by grant L13313. We want to thank Ana Cecilia Vargas and Elena Sierra for their valuable support during diagnosis in the Hospital Vozandes, Quito-Ecuador, and Iliana Alcocer for the laboratory facilities at the Pontificia Universidad Católica del Ecuador.

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