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Letter to the Editor

Characterisation of the first extended-spectrum β-lactamase (ESBL)-producing *Shigella sonnei* clinical isolate in Italy

Sir,

Shigella spp. are one of the most common causes of diarrhoea in returned travellers and are a major cause of morbidity and mortality in low-income countries. Nowadays, the emergence of antimicrobial-resistant strains is a matter of concern. In particular, in Asia a significant increase in extended-spectrum β-lactamase (ESBL)-producing strains has recently been recorded [1]. In contrast, in Europe such strains are still very rarely isolated.

In August 2018, *Shigella sonnei* strain LC1477/18 was recovered from a stool sample of an otherwise healthy 10-year-old girl presenting bloody diarrhoea and abdominal pain. The child lived with her parents in Lombardy (Northern Italy, close to Milan) but was returning from a holiday in Albania where she attended a party the day before coming back to Italy by car. Her symptoms appeared during the travel. Her parents decided to refer to a hospital in Croatia, where the patient received only rehydration and was dismissed within a few hours. The day after the girl was hospitalised at the Pediatric Department of Lecco Hospital (Lecco, Italy) where standard stool culture (including *Salmonella*,*Shigella* and *Campylobacter*) was immediately performed. Based on laboratory results showing positivity for *S. sonnei* non-susceptible to extended-spectrum cephalosporins and trimethoprim/sulfamethoxazole (Supplementary Table S1), a single blood culture (paediatric bottle; bioMérieux) was performed and treatment with meropenem was implemented (1 g twice daily for 5 days). The patient was discharged on Day 7 with complete resolution of the infection. Stool culture carried out at hospital discharge as well as the previously performed blood culture resulted negative.

Stools were screened using XLD (xylose–lysine–deoxycholate) selective agar (bioMérieux). Species identification was performed using VITEK®2 (bioMérieux) and was confirmed by latex agglutination (Remel Europe Ltd.). Antimicrobial susceptibility testing was performed using broth microdilution GNX2F and ESB1F Sensititre panels (Thermo Fisher Scientific) and the results were interpreted according to the 2018 European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria (http://www.eucast.org).

Whole-genome sequencing was performed using both HiSeq (Illumina Inc.) and MinION (Oxford Nanopore Technologies) technology. In brief, adapters from Nanopore and Illumina reads were removed using Porechop and Trimmomatic software, respectively. The longest and best-quality Nanopore reads were extracted using Filtlong, were assembled with Canu, and were circularised implementing Circlator software. The assembly was polished with Illumina trimmed reads using Pilon, and annotation was performed with the NCBI Prokaryotic Genome Annotation Pipeline (PGAP). The final genome was analysed using the tools available at the Center for Genomic Epidemiology (http://www.genomicepidemiology.org/). A multilocus sequence typing (MLST) scheme for *S. sonnei* is not available, thus the sequence type (ST) was determined using the *Escherichia coli* MLST scheme as done previously [2].

Shigella sonnei strain LC1477/18 was ST152, a specific hyperepidemic clone previously reported in the USA, Iran and Brazil. It harboured $bla_{CTX\text{-}M\text{-}3}$, *aadA1*, *aph(6)-Id*, *aph(3″)-Ib*, *dfrA1*, *sul2*, *tet*(A) and *mdf(A)* antimicrobial resistance genes (ARGs). The virulence factors *celB* (endonuclease colicin E2), *gad* (glutamate decarboxylase), *ipaH9.8* (invasion plasmid antigen), *lpfA* (long polar fimbriae protein A), *senB* (plasmid-encoded enterotoxin) and *sigA* (*Shigella* IgA-like protease homologue) were also found, but not Shiga toxin genes [2].

Strain LC1477/18 carried three plasmids: pLC1477/18-1 (86 kb, IncI1, and pST57; GenBank accession no. CP035009); pLC1477/18-2 (6.8 kb, not typeable; CP035010); and pLC1477/18-3 (8.4 kb, Col156; CP035011). The $bla_{CTX\text{-}M\text{-}3}$ gene was the only ARG possessed by pLC1477/18-1 and was associated with a truncated IS*Ecp1* insertion sequence with an overall genetic environment structure not previously reported (Fig. 1).

Plasmid pLC1477/18-2 did not harbour ARGs, whilst plasmid pLC1477/18-3 possessed *aph(6)-Id*, *aph(3″)-Ib*, *sul2* and *tet*(A). The remaining ARGs were chromosomally located (GenBank no. CP035008). Finally, conjugation experiments using rifampicin-resistant *E. coli* J53 as recipient performed at 37 °C and selecting on MacConkey plates containing ampicillin and rifampicin (both at 50 μg/mL) were successful (frequency, 1.2×10^{-4}) and confirmed the presence of the IncI1-$bla_{CTX\text{-}M\text{-}3}$ plasmid (Supplementary Table S1).

This is the first report of an ESBL-producing *Shigella* spp. strain in Italy. In Europe, clonally-related CTX-M-3-producing strains, originating from an unknown source, were isolated from paediatric patients only in Turkey more than 10 years ago [3]. More recently, a few ESBL-producing *S. sonnei* strains have been detected in Austria and the UK (CTX-M-27 producers), but only in subjects without travel history (i.e. refugees or men who had sex with men, respectively) [4,5]. On the other hand, in the present clinical case the girl acquired the infection during a trip to Albania and imported the ESBL-producing *S. sonnei* to Italy where it had never been observed.

Taking into account that $bla_{CTX\text{-}M\text{-}3}$ has a relatively high prevalence in the Balkan and Central European regions, it can be hypothesised that a β-lactam-susceptible *S. sonnei* strain belonging to a hyperepidemic clone (ST152) picked up the IncI1-$bla_{CTX\text{-}M\text{-}3}$ plasmid from another enterobacterial species (e.g. *E. coli*) via conjugation. Nevertheless, the complete lack of epidemiological

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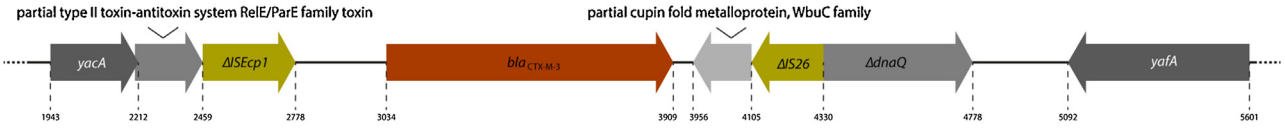

Fig. 1. Genetic environment of the $bla_{CTX\text{-}M\text{-}3}$ gene region in plasmid pLC1477/18-1. The $bla_{CTX\text{-}M\text{-}3}$ gene, together with a partial cupin fold metallo-protein gene, is surrounded by two truncated transposase genes (Δ*ISEcp1* and Δ*IS26*). NCBI Prokaryotic Genome Annotation Pipeline (PGAP) resulting annotation of the $bla_{CTX\text{-}M\text{-}3}$ region was manually curated by blasting the annotated coding sequence (CDS) using BLASTX.

data regarding the spread of such pathogens and/or the specific bla_{ESBL} genes circulating among Enterobacteriaceae in Albania cannot support this speculation. Epidemiological surveys are therefore advised to study the possible spread of ESBL-producing *Shigella* spp. among the local sources of human and non-human origin.

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Competing interests

None declared.

Ethical approval

Not required.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jgar.2019.03.004.

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