BRIEF REPORT

Emergence of lineage III of *Shigella sonnei* ST152 belonging to a high-risk clone harboring the $\text{bla}_{\text{CTX-M-15}}$ gene in Peru

Arturo Gonzales Rodríguez\textsuperscript{a,*}, Edgar Gonzales Escalante\textsuperscript{a}, Lizet Lezameta Abarca\textsuperscript{b,c,d}, Jordana Saavedra Gutierrez\textsuperscript{a}

\textsuperscript{a} Facultad de Medicina Humana, Universidad de Piura, Peru
\textsuperscript{b} Facultad de Medicina, Universidad Peruana Cayetano Heredia, Peru
\textsuperscript{c} Laboratorio de Resistencia Antibiótica e Inmunopatología, Universidad Peruana Cayetano Heredia, Peru
\textsuperscript{d} Clínica Centenario Peruano Japonesa, Lima, Peru

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**Abstract** Multidrug-resistant *Shigella sonnei* ST152, global lineage III, is a high-risk clone, whose dissemination has limited therapeutic options for shigellosis. This study aimed to characterize two isolates of *S. sonnei*, which were recovered in Lima, Peru, during November 2019, exhibiting resistance to extended-spectrum cephalosporins and quinolones, and concurrently harboring $\text{bla}_{\text{CTX-M-15}}$ and $\text{qnrS1}$ genes, in addition to mutations in gyra-S83L. These isolates were resistant to ceftriaxone, ciprofloxacin and trimethoprim/sulfamethoxazole. The molecular analysis showed that both isolates belonged to lineage III, sublineages IIIa and IIIb. The $\text{bla}_{\text{CTX-M-15}}$ gene was located in the same genetic platform as $\text{qnrS1}$, flanked upstream by ISKpn19, on a conjugative plasmid belonging to the IncI-\textgamma group. To the best of our knowledge, this would be the first report on *S. sonnei* isolates carrying the $\text{bla}_{\text{CTX-M-15}}$ gene in Peru. The global dissemination of *S. sonnei* ST152, co-resistant to β-lactams and quinolones, could lead to a worrisome scenario in the event of potential acquisition of genetic resistance mechanisms to azithromycin.

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**Palabras clave**

*Shigella sonnei*; Beta-lactámicos; Multi-resistencia; Perú

**Resumen** La bacteria multidrogorresistente *Shigella sonnei* ST152, del linaje global III, es un clon de alto riesgo, cuya diseminación ha limitado las opciones terapéuticas contra la shigellosis. En este estudio se caracterizaron dos aislamientos de *S. sonnei* resistentes a cefalosporinas

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Corresponding author.

E-mail address: arturo.gonzalez@udep.edu.pe (A. Gonzales Rodríguez).

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Shigellosis is a bacillary dysenteric disease that is responsible for approximately 1.1 million deaths annually, mainly involving children under 5 years old. Shigella comprises four related species (S. flexneri, S. boydii, S. dysenteriae and S. sonnei), exhibiting a heterogenous geographic distribution. Shigella flexneri traditionally exhibits a predominant prevalence in low and middle income countries. However, in the last decade, an abnormal increase in S. sonnei incidence has been observed globally, including in Peru. Despite shigellosis being a self-limiting disease, antimicrobial therapy is recommended in patients with severe or invasive infections and in cases associated with malnutrition and prolonged dysenteries. Furthermore, the World Health Organization (WHO) has proposed antimicrobial use in asymptomatic carriers as an approach for limiting its dissemination.

However, the increase in Shigella antimicrobial resistance has modified the therapeutic alternatives for first- and second-line drugs. Since 2005, given the high levels of resistance against trimethoprim-sulfamethoxazole, tetracycline, chloramphenicol, ampicillin, and nalidixic acid, the WHO has suggested the use of ciprofloxacin and ceftriaxone, as first-line agents, and azithromycin as a second option. Nevertheless, the global dissemination of S. sonnei sequence type (ST) 152, known for its antimicrobial resistance profile to extended-spectrum cephalosporins (ESC), macrolides and quinolones, drastically limits the therapeutic options and has raised a global alarm.

At the beginning of 2022, the United Kingdom reported a three-fold increase in S. sonnei isolates displaying resistance to ESC, quinolones, tetracycline, azithromycin and sulfamethoxazole. A similar scenario has been reported in several countries of the European Union and Southeast Asia. In Latin America, the Pan American Health Organization (PAHO) recently issued an alert, highlighting the need to strengthen the epidemiological surveillance of S. sonnei. The aim of this study was to report the genetic features of two S. sonnei clinical isolates coharboring blaCTX-M-15 and qnrS1 that belong to an internationally successful clone.

Two isolates of Shigella sonnei resistant to ESC (SS1 and SS2) were isolated from stool samples of a 2-year-old male and a 37-year-old female in November 2019, at the “Clínica Centenario Peruano – Japonesa” in Lima, Peru, respectively. Susceptibility tests to ampicillin/subactam (AMS), ceftazidime (CAZ), ceftriaxone (CRO), cefepime (FEP), ertapenem (ETP), meropenem (MER), amikacin (AMI), gentamicin (GEN), ciprofloxacin (CIP) and trimethoprim/sulfamethoxazole (TMS) were performed using automated systems (VITEK® 2 COMPACT, Biomerieux). Azitromycin (AZT) resistance and extended-spectrum β-lactamase (ESBL) production were determined by the disk diffusion method. Interpretation was performed by following the recommendations outlined in CLSI M100-ED33 2023 (https://clsi.org/all-free-resources/).

Bacterial DNA was extracted using the GeneJetGenomic DNA Purification kit (ThermoScientific), following the manufacturer’s recommendations. The presence of the blaCTX-M gene was performed by polymerase chain reaction (PCR) amplification. In addition, the identification of blaCTX-M-1, blaCTX-M-2, blaCTX-M-9 groups was conducted by PCR using specific primers: Fw-blaCTX-M-1 (5′-ATGTTTTAAAAATCCTGCTG-3′), Rv-blaCTX-M-1 (5′-GTTGAGATTTGACCCG-3′); Fw-blaCTX-M-2 (5′-CGTTAACCAGCGAGATGAC-3′), Rv-blaCTX-M-2 (5′-CGATATCGTTGTGTGCGCAT-3′); Fw-blaCTX-M-9 (5′-GATTGACCTATTGCTT-3′), Rv-blaCTX-M-9 (5′-CGCGCTGGTAAATAGGTCA-3′). Plasmid conjugation assays were performed by a mating-out assay using Escherichia coli J53 sodium azide resistant (A2) as the recipient strain (ECJ53) and SS1 and SS2 as donor isolates. Strains were grown overnight in 5.0 ml of Luria Bertani (LB) broth and incubated until an optical density of 0.6 was reached. Subsequently, in each case, equal parts (0.5 ml) of the cultures were mixed, centrifuged, and the pellet was resuspended in 100 μl of LB broth. Transconjugants were initially selected on LB agar containing ampicillin (50 μg/ml) and sodium azide (150 μg/ml). Grown colonies were subcultured in LB agar supplemented with cefotaxime (2 μg/ml) and sodium azide (150 μg/ml). Transconjugants (TCSS1 and TCSS2) were evaluated by analyzing the blaCTX-M group and the antibiotic susceptibility profile as mentioned.

Next generation sequencing (NGS) analysis was performed on both strains. Genomic libraries were prepared using the MiSeq chemistry, Illumina, with paired-end 2 × 250 bp. Poor quality readings (Phred scores below 30) were filtered out using the Trimmomatic v0.39 program.
De novo genome assembly was conducted using Unicycler v0.4.8. Assembled final sequence genomes were annotated using Prokka v1.14.6 and manually curated. Sequence type (ST) was identified with MLSTfinder v2.0, resistance genes with ResFinder v1.1 and plasmid incompatibility groups with PlasmidFinder v2.1 software. Genetic relationship based on single-nucleotide polymorphisms (SNPs) was assessed using genomic sequences of \textit{S. sonnei} ST152 available on the GenBank public database. SNPs were identified and extracted using SNP-sites v2.5.1 and maximum-likelihood clustering was inferred by IQ-TREE Phylogenomic v1.5.5.3 using the best-fit model found and 1000 bootstrap. The complete, raw sequences were deposited in NCBI under BioProject ID: PRJNA968049.

Both \textit{S. sonnei} bacterial isolates, designated as SS1 and SS2, respectively, exhibited a multidrug-resistant (MDR) profile against AMS, CRO, TMS, and CIP, remaining susceptible to CAZ, FEP, ETP, IMP, GEN, AMI, and AZT (Table 1).

<table>
<thead>
<tr>
<th>Isolates</th>
<th>AMS</th>
<th>CAZ</th>
<th>CRO</th>
<th>FEP</th>
<th>ETP</th>
<th>MER</th>
<th>AMI</th>
<th>GEN</th>
<th>CIP</th>
<th>TMS</th>
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<tbody>
<tr>
<td>SS1</td>
<td>8\textsuperscript{c}</td>
<td>2\textsuperscript{c}</td>
<td>≥64\textsuperscript{R}</td>
<td>0.5\textsuperscript{c}</td>
<td>&lt;0.12\textsuperscript{c}</td>
<td>&lt;0.25\textsuperscript{c}</td>
<td>≤1\textsuperscript{c}</td>
<td>≤1\textsuperscript{c}</td>
<td>&lt;4\textsuperscript{R}</td>
<td>≥320\textsuperscript{R}</td>
</tr>
<tr>
<td>TCSS1\textsuperscript{a}</td>
<td>8\textsuperscript{c}</td>
<td>2\textsuperscript{c}</td>
<td>32\textsuperscript{R}</td>
<td>0.5\textsuperscript{c}</td>
<td>&lt;0.12\textsuperscript{c}</td>
<td>&lt;0.25\textsuperscript{c}</td>
<td>≤1\textsuperscript{c}</td>
<td>&lt;1\textsuperscript{c}</td>
<td>&lt;4\textsuperscript{R}</td>
<td>≥320\textsuperscript{R}</td>
</tr>
<tr>
<td>SS2</td>
<td>8\textsuperscript{c}</td>
<td>2\textsuperscript{c}</td>
<td>≥64\textsuperscript{R}</td>
<td>0.5\textsuperscript{c}</td>
<td>&lt;0.12\textsuperscript{c}</td>
<td>&lt;0.25\textsuperscript{c}</td>
<td>≤1\textsuperscript{c}</td>
<td>≤1\textsuperscript{c}</td>
<td>&lt;4\textsuperscript{R}</td>
<td>≥320\textsuperscript{R}</td>
</tr>
<tr>
<td>TCSS2\textsuperscript{a}</td>
<td>8\textsuperscript{c}</td>
<td>2\textsuperscript{c}</td>
<td>32\textsuperscript{R}</td>
<td>0.5\textsuperscript{c}</td>
<td>&lt;0.12\textsuperscript{c}</td>
<td>&lt;0.25\textsuperscript{c}</td>
<td>≤1\textsuperscript{c}</td>
<td>&lt;1\textsuperscript{c}</td>
<td>&lt;4\textsuperscript{R}</td>
<td>≥320\textsuperscript{R}</td>
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<tr>
<td>EJ53</td>
<td>≤2</td>
<td>≤0.12</td>
<td>≤0.25</td>
<td>≤0.12</td>
<td>&lt;0.25</td>
<td>≤1</td>
<td>≤1</td>
<td>≤0.06</td>
<td>≤20</td>
<td></td>
</tr>
</tbody>
</table>

AMS: ampicillin/sulbactam; CAZ: ceftazidime; CRO: ceftriaxone; FEP: cefepime; ETP: ertapenem; MER: meropenem; AMI: amikacin; GEN: gentamicin; CIP: ciprofloxacin; TMS: trimethoprim/sulfamethoxazole; R: resistant; S: susceptible; I: intermediate.

\textsuperscript{a} \textit{E. coli} J53 transconjugant with the \textit{bla}_{CTX-M-group-1}\textsuperscript{-}harboring plasmid, obtained from strain SS1.

\textsuperscript{b} \textit{E. coli} J53 transconjugant with the \textit{bla}_{CTX-M-group-1}\textsuperscript{-}harboring plasmid, obtained from strain SS2.

\textsuperscript{c} R: resistant; S: susceptible.

*Table 1* Antibiotic susceptibility profiles of \textit{Shigella sonnei} strains and their transconjugants.

Incompatibility group in both genomes. When analyzing the \textit{bla}_{CTX-M-15} genetic context in SS1 and SS2, both genomes exhibited the same genetic platform, accompanied by the sequences: \textit{bla}_{CTX-M-15} -cupin \textit{Wbuc-Tn3}Δ-\textit{IS3}Δ-\textit{qnrS1}-\textit{hin-ISKpn19}.

Phylogenetic analysis using a representative group composed of 230 genomes of \textit{S. sonnei} from PulseNet Latin America and Caribbean surveillance network\textsuperscript{1}, indicated that SS1 was closely related to ERR200468, isolated in Argentina in 2005, with 374 SNPs differences, while SS2 was related with SRR7693709, isolated in Ecuador in 2014, with 261 SNPs differences (Fig. 1). Additionally, a further analysis indicates that SS1 and SS2 correspond to IIib and IIia sublineages, respectively.

The high level of antimicrobial resistance in \textit{S. sonnei}, attributed to the dissemination of ST152, global III lineage, has narrowed the antimicrobial options against shigellosis\textsuperscript{13} and has alerted about the need to strengthen epidemiological surveillance systems. Nowadays, there are several reports of \textit{S. sonnei} ESBL producers, primarily related to CTX-M-15 and CTX-M-3, mainly\textsuperscript{10}. To the best of our knowledge, although ESBL-producing \textit{S. flexneri} isolates have been previously described in Peru by Gonzales et al.,\textsuperscript{3} this study would be the first documentation of \textit{S. sonnei} isolates harboring the \textit{bla}_{CTX-M-15} gene in Peru. The ESBL gene was detected on a conjugative plasmid, conveying antimicrobial resistance capacity to \textbeta-lactams, TMS and reduced susceptibility to CIP.

As mentioned above in SS1, \textit{bla}_{CTX-M-15} was identified in the \textit{IncI-\gamma} group-contig (contig 3: 84778 bp). In \textit{S. sonnei}, \textit{bla}_{CTX-M-15} has been associated with several incompatibility groups (IncFII, IncCZ), with \textit{IncI} being relevant for its successful dissemination since 2006\textsuperscript{6}. Contig 3 in SS1 exhibited an identity of 99.2% with the plasmid p202102843-3 (GenBank: OP038292.1), recently reported in France in 2022\textsuperscript{2}. The genetic environment of \textit{bla}_{CTX-M-15} is similar to that described in an IncFII plasmid from \textit{S. sonnei} in 2020 (GenBank: CP045525\textsuperscript{5}) and in an IncFII plasmid from \textit{K. pneumoniae} isolated in China in 2014 (GenBank: CP02615B)\textsuperscript{11}. The genetic context of \textit{bla}_{CTX-M-15} described in the present study suggests the risk of dissemination of this genetic platform in different types of plasmids.

The global dissemination of ciprofloxacin-resistant \textit{S. sonnei}, evolved from the sequential accumulation of
mutations in gyrA-S83L, parC-S80I and gyrA-D87G, from Central Asia\textsuperscript{14}. The former isolates reported only the gyrA-S83L mutation, accompanied by the qnrS1 gene, harbored in the same genetic environment as \textit{blac}_{\text{CTX-M-15}}. A Latin American surveillance study with 22,273 \textit{S. sonnei} collected over 15 years revealed that Peru was one of the countries in the region with the highest annual increase in resistance to ciprofloxacin (1–5\%)\textsuperscript{12}. Fortunately, the regional levels of resistance are still as low as 2.7\% (9/329)\textsuperscript{1}. Nevertheless, in several European and Asian countries, such as France or India, ciprofloxacin resistance has reached levels of up to 38.7\% and 61.5\%, respectively\textsuperscript{6}.

The isolates SS1 and SS2, belonging to the global III lineage, were recognized for their role in the global dissemination of multidrug-resistant \textit{S. sonnei} clones. We highlight their belonging to sublineage IIIb, described for the very first time in Peru\textsuperscript{1}. These results support the evidence of clonal expansion of sublineages IIIa and IIIb in Latin America, as postulated by Baker et al.\textsuperscript{1}

However, the low number of isolates and the lack of clinical information limit the scope of this study. Nevertheless, this research contributes to the characterization of the circulating strains of \textit{S. sonnei} in our environment. Both isolates presented \textit{blac}_{\text{CTX-M-15}} associated with qnrS1, presumably on a conjugative plasmid of the IncI-\gamma group; in both cases azithromycin susceptibility was observed. The global spread of strains with a profile of extreme resistance to antimicrobials makes it necessary to strengthen epidemiological surveillance systems.

Conflict of interest

The authors declare that they have no conflicts of interest.

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