

1 **Ciprofloxacin facilitates the transfer of XDR plasmids from commensal *E. coli* into**  
2 **epidemic fluoroquinolone-resistant *Shigella sonnei***

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18 **Running title**

19 XDR *Shigella sonnei* in Vietnam

20

21 **Key words**

22 *Shigella*; fluoroquinolones; ESBL; multi-drug resistance; diarrheal disease; antimicrobial  
23 resistance

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27

28 **Abstract**

29 The global dissemination of a ciprofloxacin-resistant (cipR) *S. sonnei* clone outlines the mobility  
30 of this important agent of diarrheal disease, and threatens the utility of ciprofloxacin as a first-line  
31 antimicrobial for shigellosis. Here, we aimed to track the emergence of cipR *S. sonnei* in Vietnam  
32 to understand how novel antimicrobial resistant (AMR) *Shigella* clones become established in  
33 new locations. From 2014 to 2016, we isolated and genome sequenced 79 *S. sonnei* from children  
34 hospitalized with dysenteric diarrhea in southern Vietnam. The novel cipR *S. sonnei* clone  
35 displaced the resident ciprofloxacin-susceptible lineage while acquiring resistance against third-  
36 generation cephalosporins, macrolides, and aminoglycosides. This process was not the result of a  
37 single clonal expansion, as we identified at least thirteen independent acquisitions of ESBL-  
38 encoding plasmids. The frequency and diversity of the variable AMR repertoire in an expanding  
39 clonal background of *S. sonnei* is unprecedented and we speculated that it was facilitated by  
40 horizontal gene transfer from commensal organisms in the human gut. Consequently, we  
41 characterized non-*Shigella* Enterobacteriaceae from *Shigella*-infected and healthy children by  
42 shotgun metagenomics. We identified a wide array of AMR genes and plasmids in the  
43 commensal Enterobacteriaceae, including an *E. coli* isolated from a *Shigella*-infected child with  
44 an identical ESBL plasmid to that characterized in the infecting *S. sonnei*. We confirmed that  
45 these AMR plasmids could be exchanged between commensal *E. coli* and *S. sonnei* and found  
46 that supplementation of ciprofloxacin into the conjugation media significantly increased the  
47 conjugation frequency of IncI/*bla*<sub>CTX-M-15</sub>, IncB/O/*bla*<sub>CTX-M-27</sub> and IncF/*bla*<sub>CTX-M-27</sub> plasmids. In a  
48 setting with high antimicrobial use and a high prevalence of AMR commensals, cipR *S. sonnei*  
49 may be propelled towards pan-resistance by adherence to outdated international treatment  
50 guidelines. Our work highlights the role of the gut microbiota in transferring resistance plasmids  
51 into enteric pathogens and provides essential data to restrict the use of ciprofloxacin globally.

52

## 53 **Introduction**

54 *Shigella* is one of the leading bacterial agents of diarrhea globally; responsible for >165 million  
55 diarrheal episodes annually, shigellosis principally affects children in low- to middle-income  
56 countries (LMICs) <sup>1</sup>. Correspondingly, it has been estimated that shigellosis is responsible for  
57 28,000-48,000 deaths in children aged <5 years annually <sup>2</sup>. In 2014, the Global Enteric  
58 Multicenter Study (GEMS) identified *Shigella* as one of the top four pathogens associated with  
59 moderate-to-severe diarrheal disease in young children in sub-Saharan Africa and South Asia <sup>3</sup>.

60

61 The genus *Shigella* comprises of four species: *S. flexneri*, *S. sonnei*, *S. dysenteriae*, and *S. boydii*,  
62 but the current international *Shigella* landscape is dominated by *S. flexneri* and *S. sonnei*. Of these  
63 two species, *S. sonnei* is increasingly being isolated, replacing *S. flexneri* as the predominant  
64 *Shigella* species in many LMICs in Asia, Latin America, and the Middle East <sup>4</sup>. Unlike other  
65 *Shigella*, *S. sonnei* exists as a single serotype and has a population structure encompassing five  
66 lineages, of which lineage III successfully disseminated globally from the 1970s onwards. A key  
67 event facilitating the success of this lineage was the acquisition of a multi-drug resistance (MDR)  
68 phenotype, which distinguishes this population <sup>5,6</sup>. Antimicrobials are important for *Shigella*  
69 treatment and disease control, and the World Health Organization (WHO) currently recommends  
70 ciprofloxacin (fluoroquinolone) as a first-line treatment, followed by pivmecillinam (beta-  
71 lactam), ceftriaxone (cephalosporin), and azithromycin (macrolide) as alternatives <sup>7</sup>.

72

73 Fluoroquinolones are well-tolerated and were highly effective for shigellosis until resistance  
74 began to emerge in the early 2000s with sporadic cases of ciprofloxacin-resistant (cipR) *S.*  
75 *dysenteriae*, *S. flexneri*, and *S. boydii* being detected in India, Nepal, Pakistan, China, and  
76 Vietnam <sup>8-11</sup>. Concurrently, *S. sonnei* with reduced susceptibility to fluoroquinolones became  
77 common across Asia <sup>6</sup>, and fully cipR *S. sonnei* were characterized in India and Nepal soon after  
78 <sup>9,12</sup>. These organisms carried the classical chromosomal point mutations in the quinolone

79 resistance determining regions (QRDRs) at codon 83 (serine to leucine) and codon 87 (aspartic  
80 acid to glycine/asparagine) in *gyrA*, and at codon 80 (serine to isoleucine) in *parC*<sup>11,12</sup>. From  
81 2010 onwards, cipR *S. sonnei* emerged as a major global health concern, becoming widely  
82 distributed through international travel and ensuing domestic transmission<sup>13-15</sup>. To date, cipR *S.*  
83 *sonnei* has been reported in children across Asia<sup>16,17</sup>, as well as homeless individuals and men-  
84 who-have-sex-with-men (MSM) in Canada, the US, Taiwan, and the UK<sup>14,18-20</sup>. Of further  
85 concern is the observation that cipR *S. sonnei* have the ability to acquire resistance to second-line  
86 alternative drugs such as ceftriaxone<sup>21</sup>, which further limits alternative treatment options.

87

88 Our previous work demonstrated that all cipR *S. sonnei* globally were clonal and emerged once  
89 from a single lineage that likely arose in South Asia<sup>22</sup>. Here, we describe the expansion of a  
90 single lineage III clade of cipR *S. sonnei* by providing phylo-temporal insights into its extant  
91 clonal dynamics using clinical samples obtained from children admitted to three paediatric  
92 facilities in Ho Chi Minh City (HCMC) between 2014 and 2016. We observe replacement of the  
93 resident ciprofloxacin susceptible (cipS) clone by the novel cipR lineage, as well as the  
94 concurrent and independent acquisition of a diverse range of resistance plasmids, which lead to  
95 MDR and XDR phenotypes. Through a detailed analysis of the plasmid content from commensal  
96 *E. coli* and *S. sonnei* isolated from a single patient and a series of conjugation experiments, we  
97 provide compelling evidence that ciprofloxacin exposure influences the *de novo* acquisition of  
98 MDR and XDR plasmids. Our data suggest that following the current international guidelines for  
99 *Shigella* therapy may lead to these cipR variants becoming resistant to alternative antimicrobials.

100

## 101 **Methods**

### 102 *Study Design*

103 The *S. sonnei* used in this study were isolated during a 2-year observational study conducted at  
104 three tertiary hospitals (Children's Hospital 1, Children's Hospital 2, and the Hospital for

105 Tropical Diseases) in HCMC, Vietnam, between May 2014 and April 2016, as previously  
106 described (Supplementary Table 1)<sup>23</sup>. In brief, children aged <16 years admitted to one of the  
107 three study hospitals with diarrhea (defined as  $\geq 3$  passages of loose stools within 24 hours) and  
108 >1 loose stool containing blood and/or mucus were recruited. A fecal sample was collected and  
109 processed within 24 hours after enrolment. All hospitalized patients received standard of care  
110 treatment at each of the study sites. Treatment and clinical outcomes (e.g. patient recovery status  
111 (three days after enrolment) and duration of hospital stay) were recorded by clinical staff at the  
112 study sites. For the phylogenetic analyses, we additionally included two *Shigella sonnei* isolated  
113 from children attending the Hospital for Tropical Diseases in HCMC in October 2013.

114

115 Ethical approval was provided by the ethics committees of all three participating hospitals in  
116 HCMC and the University of Oxford Tropical Research Ethics Committee (OxTREC No.1045-  
117 13). Written consent from parents or legal guardians of all participants was obtained prior to  
118 enrolment.

119

#### 120 *Microbiological methods*

121 Fecal samples were inoculated onto MacConkey agar (MC agar; Oxoid) and xylose-lysine-  
122 deoxycholate agar (XLD agar, Oxoid) and incubated at 37°C. Non-lactose fermenting colonies  
123 grown on MC agar and/or XLD agar were sub-cultured on nutrient agar and identified  
124 biochemically (API20E, Biomerieux). Serological identification was performed by slide  
125 agglutination with somatic (O) antigen grouping sera following the manufacturer's  
126 recommendations (Denka Seiken). Additionally, colony sweeps from MC agar were collected and  
127 suspended in 20% glycerol and stored at -80°C for further characterization.

128

129 Antimicrobial susceptibility testing was initially performed by the Kirby-Bauer disc diffusion  
130 method against ampicillin, chloramphenicol, trimethoprim-sulfamethoxazole, tetracycline,

131 nalidixic acid, ciprofloxacin, azithromycin, gentamicin, amikacin, imipenem, and ceftriaxone.  
132 Subsequently, minimal inhibitory concentrations (MICs) against ciprofloxacin, azithromycin,  
133 gentamicin, and ceftriaxone were measured using the E-test (AB Biodisk), according to the  
134 manufacturer's instructions. All antimicrobial testing was performed on Mueller-Hinton agar and  
135 susceptibility criteria were interpreted following the CLSI 2016 guidelines<sup>15</sup>. MDR was defined  
136 as acquired non-susceptibility to at least one agent in three or more antimicrobial categories;  
137 XDR was defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial  
138 categories (i.e. bacterial isolates remain susceptible to only one or two categories)<sup>24</sup>. Detection of  
139 Extended Spectrum Beta Lactamase (ESBL) activity was performed for all isolates that were  
140 resistant to ceftriaxone using the combination disc method (cefotaxime, 30µg; ceftazidime, 30µg;  
141 with and without clavulanic acid, 10µg). ESBL-producing organisms were defined as those  
142 exhibiting a >5mm increase in the size of the zone of inhibition for the beta-lactamase inhibitor  
143 combinations in comparison to a third-generation cephalosporin without the beta-lactamase  
144 inhibitor.

145

#### 146 *Isolation of commensal bacteria*

147 For the purposes of this study we defined commensal organisms as organisms isolated from the  
148 stool samples of children thought not to be associated with the observed episode of diarrheal  
149 disease. In children with and without *Shigella* infections (i.e. symptomatic and asymptomatic  
150 children), non-*Shigella* organisms grown on MC plates were considered to be commensals.  
151 Colony sweeps from MC agar from *Shigella*-infected children were serially diluted and plated  
152 onto the MC agar without antimicrobial selection. All single colonies with a different color and  
153 morphology from that of *S. sonnei* were harvested, identified and homogenized in 20% glycerol.  
154 Subsequently, DNA was extracted from these commensal bacteria by boiling and was then  
155 subjected to qualitative real-time PCR with primers and probes specific for *Shigella* to detect if  
156 these samples were contaminated with *Shigella*<sup>25</sup>. To determine the AMR gene and plasmid

157 diversity in human commensal bacteria, we also included a subset of commensal bacteria  
158 recovered from the rectal swabs of 18 healthy children enrolled in a longitudinal cohort study  
159 with active surveillance for diarrheal disease in HCMC between 2014 and 2016 <sup>26</sup>.  
160  
161 *Whole genome sequencing (WGS)*  
162 Genomic DNA from *S. sonnei* isolates and commensal bacteria was extracted using the Wizard  
163 Genomic DNA Extraction Kit (Promega, Wisconsin, USA) following the manufacturer's  
164 recommendations. 50ng of genomic DNA from each sample was subjected to library construction  
165 using a Nextera kit, followed by whole genome sequencing on an Illumina MiSeq platform  
166 (Illumina, CA, USA) to generate 150 bp paired-end reads. Raw sequence data are available in the  
167 European Nucleotide Archive (project: PRJEB30967).  
168  
169 SNP calling for *S. sonnei* was performed as previously described <sup>6</sup>. In brief, raw Illumina reads  
170 were mapped against *S. sonnei* reference genome Ss046 chromosome (accession number  
171 NC\_007382) and virulence plasmid pSs046 (NC\_007385.1) using SMALT version 0.7.4  
172 (<http://www.sanger.ac.uk/resources/software/smalt/>). SNPs were called against the reference  
173 sequence and filtered using SAMtools <sup>27</sup>. The allele at each locus in each isolate was determined  
174 by reference to the consensus base in that genome using SAMtools *mpileup* and removal of low  
175 confidence alleles with consensus base quality  $\leq 20$ , read depth  $\leq 5$  or a heterozygous base call.  
176 SNPs occurring in non-conserved regions including prophages or repetitive sequences were  
177 removed. Subsequently, Gubbins <sup>28</sup> was used to identify recombinant regions from the whole  
178 genome alignment produced by SNP-calling isolates, and SNPs detected within these regions  
179 were also removed, resulting in a final set of 1,219 chromosomal SNPs.

180

181

182

183 *Phylogenetic analyses*

184 The best-fit evolutionary model for the SNP alignment of all *S. sonnei* isolates was determined  
185 based on the Bayesian Information Criterion in jModelTest implemented in IQ-TREE software <sup>29</sup>.  
186 A maximum likelihood phylogeny was subsequently reconstructed using IQ-TREE under the  
187 best-fit model (TVM). Support for the maximum likelihood tree was assessed via 1,000 pseudo-  
188 replicates. To explore the temporal signal in the data, the relationship between genetic divergence  
189 and date of sampling was estimated by using TempEst <sup>30</sup> to perform a linear regression analysis  
190 of root-to-tip distances, taken from the maximum likelihood tree, against the year of isolation  
191 (Supplemental Figure 1). Temporal phylogenetic inference was then performed using Bayesian  
192 Markov Chain Monte Carlo (MCMC) implemented in BEAST software (version 1.8.4) <sup>31</sup>. For  
193 BEAST analysis, we first identified best-fit model combinations by performing multiple BEAST  
194 runs using the TVM nucleotide substitution model with constant, exponential growth or Bayesian  
195 skyline <sup>32</sup> demographic models, in combination with either a strict or a relaxed molecular clock  
196 (uncorrelated lognormal distribution) <sup>33</sup>. For each BEAST run, path sampling and stepping-stone  
197 sampling approaches <sup>34,35</sup> were used to obtain the marginal likelihood estimates for model  
198 comparison. Bayes factor (the ratio of marginal likelihoods of two models) comparisons indicated  
199 that the TVM substitution model with a relaxed lognormal molecular clock and Bayesian skyline  
200 demographic model was the best fit to the data (Bayes factor >200). The standard deviation (SD)  
201 of inferred substitution rates across branches was 0.3 (95% highest posterior probability (HPD) =  
202 0.14-0.47), providing additional support for a relaxed molecular clock. For the final analyses, we  
203 performed three independent runs with the best-fit model using a continuous 150 million  
204 generation MCMC chain with samples taken every 15,000 generations, and parameter  
205 convergence (indicated by effective sample size values >500) was assessed in Tracer (version  
206 1.7). LogCombiner (version 1.8) was used to combine triplicate runs, with removal of 10%  
207 burn-in.

208



209 *Resistome analysis of S. sonnei and commensal enterobacteria*

210 From raw Illumina reads of *S. sonnei* and commensal enterobacteria Short Read Sequence Typer-  
211 SRST2<sup>36</sup> was used to identify the acquired resistance genes and their precise alleles using the  
212 ARG-Annot database<sup>37</sup>, as well as the plasmid replicons using the PlasmidFinder database<sup>38</sup>.  
213 Multilocus sequence typing (MLST) of IncI plasmids<sup>39</sup> was also determined using SRST2. Raw  
214 Illumina reads were *de novo* assembled using Velvet, with the parameters optimized by Velvet  
215 Optimizer<sup>40</sup>. Contigs <300 bp in size were discarded from further analyses and assembled  
216 contigs were annotated with Prokka<sup>41</sup>. For the assembled sequences of *S. sonnei*, ABACAS<sup>42</sup>  
217 was used to map all the contigs against a concatenated reference sequence containing *S. sonnei*  
218 Ss046 chromosome (NC\_007382), virulence plasmid pSs046 (NC\_007385.1) and three small  
219 plasmids commonly found in *S. sonnei* belonging to global lineage III: spA (NC\_009345.1), spB  
220 (NC\_009346.1), spC (NC\_009347.1). The unmapped assembled sequences presumably  
221 containing the *bla*CTX-M/*mphA* plasmid were subjected to manual investigation using BLASTN  
222 searching with the plasmid sequences available in GenBank, and comparative analysis was  
223 performed and visualized using ACT<sup>43</sup>. For the assembled sequences of commensal bacteria  
224 carrying IncI and IncB/O plasmids, ABACAS was used to map contigs against full-length  
225 sequences of IncI and IncB/O plasmids identified in *S. sonnei* and sequence comparisons  
226 were visualized using ACT. Nucleotide sequence homology between the mapped contigs and  
227 reference plasmid was subsequently identified using BLASTN. Taxonomic labels of each pooled  
228 sample of commensal bacteria were assigned using Kraken, a k-mer based classification tool<sup>44</sup>.

229

230 *Plasmid profiling*

231 Crude plasmid extractions from all *S. sonnei* isolates was performed using a modified Kado and  
232 Liu method<sup>45</sup>. The resulting plasmid DNA was subjected to electrophoresis in 0.7 % agarose gel  
233 at 90 V for 3 hours, stained with ethidium bromide and photographed. *E. coli* strain 39R861

234 containing four plasmids with known sizes (7 kb, 36 kb, 63 kb, and 147 kb) was used as a marker.

235 Plasmid profiles were compared using Bionumerics v5.1 software (Applied Maths, Austin, TX).

236

### 237 *ESBL plasmid digestion and sequencing*

238 *E. coli* transconjugants resulting from conjugation between an ESBL-positive *S. sonnei* isolate

239 and *E. coli* J53 (sodium azide resistance) were subjected to plasmid extraction using a plasmid

240 Midi kit (Qiagen). For plasmid digestion, 500ng of each extracted plasmid DNA was digested

241 with EcoRI enzyme (10 U/ $\mu$ l) (Fermentas), followed by electrophoresis on 0.8% agarose gel at

242 100V for 4 hours with 1 kb plus DNA ladder (Invitrogen). Plasmid restriction patterns were

243 compared, and cluster analysis was performed using the UPGMA method and Jukes-Cantor

244 correction using Bionumerics v5.1 software. For plasmid sequencing, 50ng of each plasmid DNA

245 was subjected to library construction with a Nextera kit and sequenced using the MiSeq Illumina

246 platform to generate 2x250 bp paired-end reads. *De novo* assembly was subsequently performed

247 using SPADES v3.11<sup>46</sup> and assembled contigs were annotated using Prokka v1.11<sup>41</sup>.

248

### 249 *Nanopore sequencing*

250 Plasmid DNA extracted from the commensal *E. coli* carrying the IncI/blaCTX-M-15 plasmid was

251 initially sequenced using Illumina MiSeq to generate 2x250bp paired-end reads (accession

252 number: ERS3050916). However, the *de novo* assembly failed to produce a complete plasmid

253 sequence. To improve the plasmid assembly, we then performed a single run on a MinION to

254 generate longer reads. For MinION library preparation and sequencing, we used the rapid 1D

255 sequencing kit SQK-RAD001 (Oxford Nanopore Technologies, Oxford, UK), following the

256 manufacturer's recommendations. We used the MinION Mk1 sequencer, FLO-MIN106 flow cell

257 and MinKNOW software v1.1.20 for sequencing, and protocol script

258 NC\_48Hr\_Sequencing\_Run\_FLO-MIN106\_SQK-RAD001\_plus\_Basecaller.py for local base-

259 calling. MinION reads were converted from fast5 to fastq format using the script fast52fastq.py.

260 SPADES version 3.11 was subsequently used to produce a hybrid assembly of MinION data and  
261 Illumina data. Raw MinION reads were deposited in ENA (accession number: ERS3050922).  
262  
263 *Bacterial conjugation*  
264 Bacterial conjugation was first performed between each of the 40 *bla*<sub>CTX-M</sub>/*mphA*-carrying *S.*  
265 *sonnei* isolates associated with all the plasmid acquisitions and *E. coli* J53 (sodium azide  
266 resistant) by combining equal volumes (5mL) of overnight Luria-Bertani (LB) cultures. Bacteria  
267 were conjugated for 12 hours in LB broth at 37°C and *E. coli* transconjugants were selected on  
268 medium containing sodium azide (100 mg/l) plus ceftriaxone (6 mg/l) or sodium azide (100 mg/l)  
269 plus azithromycin (24 mg/l). To measure plasmid transfer from commensal *E. coli* to *cipR S.*  
270 *sonnei* and investigate the effect of ciprofloxacin on the conjugation efficiency, we first screened  
271 commensal *E. coli* isolates for ESBL activity and ciprofloxacin susceptibility from the pooled  
272 colony sweeps on MC agar. Subsequently, bacterial conjugation was performed between each of  
273 the 13 *cipS* ESBL-positive commensal *E. coli* isolates (donor) and the *cipR* ESBL-negative *S.*  
274 *sonnei* 03-0520 (recipient) in LB broth with and without supplementation of ciprofloxacin (0.25,  
275 0.5, 0.75 x MIC of the donor organism). Successful transconjugants were selected on MC agar  
276 containing ciprofloxacin (4 mg/l) and ceftriaxone (6 mg/l). For all conjugation experiments, the  
277 conjugation frequency was calculated as the number of transconjugants per recipient cell.

278

## 279 **Results**

### 280 *The development of an XDR phenotype in ciprofloxacin-resistant Shigella sonnei*

281 Between January 2014 and July 2016, we isolated 79 *S. sonnei* from children hospitalized with  
282 dysenteric diarrhea in our study sites; 75.9% (60/79) of these were *cipR*. A time-scaled  
283 phylogenetic reconstruction demonstrated that all except one *cipR S. sonnei* comprised a distinct  
284 clade, which was distantly related to the ciprofloxacin-susceptible (*cipS*) isolates (Figure 1). The  
285 most recent common ancestor (MRCA) of the *cipR* clade in Vietnam was estimated to date back

286 to late 2008 (95% HPD; 2007.1 – 2010.3) – several years prior to the first known cases of cipR *S.*  
287 *sonnei* in Vietnam, which was detected in HCMC in October 2013. The phylogeny depicted a  
288 clonal expansion from a single cipR organism that we have previously shown to have originated  
289 in South Asia and disseminated internationally<sup>22</sup>. All organisms within the cipR clade were  
290 classical triple mutants (*gyrA*-S83L, *gyrA*-D87G, and *parC*-S80I) conferring high-level  
291 ciprofloxacin resistance (MIC  $\geq$ 8  $\mu$ g/ml). Conversely, isolates belonging to the resident cipS *S.*  
292 *sonnei* clade harbored only a single mutation in *gyrA* (either S83L (16 isolates) or D87Y (4  
293 isolates)).

294

295 Using BEAST, we estimated the median substitution rate of the *S. sonnei* population to be  
296  $8.2 \times 10^{-7}$  substitutions base<sup>-1</sup> year<sup>-1</sup> (95% highest posterior density (HPD);  $5.9 \times 10^{-7}$  to  $10.8 \times 10^{-7}$ ),  
297 which is comparable to previous estimates of the mutation rate within the resident cipS *S. sonnei*  
298 population in Vietnam<sup>47</sup>. Additionally, the cipR isolates exhibited a substantially lower median  
299 pairwise SNP distance (15 SNPs, IQR: 10-20 SNPs) than the resident cipS *S. sonnei* isolates (96  
300 SNPs, IQR: 69-111 SNPs), providing strong evidence of a more recent importation or expansion.  
301 These data suggest that cipR *S. sonnei* underwent a rapid clonal expansion and successfully  
302 persisted, displacing the resident cipS *S. sonnei* as the dominant *S. sonnei* lineage circulating in  
303 the human population of southern Vietnam.

304

305 During the sampling period, the proportion of cipR *S. sonnei* increased significantly from 60.7%  
306 (17/28) in 2014 to 93.8% (15/16) in 2016 ( $p=0.01$ ; Chi-squared test). Almost all isolates carried  
307 AMR genes on a chromosomally integrated class II integron (*dfrA1*, *sat-2A*) and a small spA  
308 plasmid (*strAB*, *sulIII*, *tetAR*) encoding resistance to tetracycline, streptomycin, and co-  
309 trimoxazole. Notably, during their circulation in southern Vietnam, the cipR *S. sonnei* acquired  
310 resistance to further important antimicrobial classes, including third-generation cephalosporins,  
311 macrolides, and aminoglycosides, consequently creating XDR variants. In 2014, the proportion of

312 co-resistance against ceftriaxone, ceftriaxone-azithromycin, and ceftriaxone-azithromycin-  
313 gentamicin was 59% (10/17), 11.8% (2/17), and 5.9% (1/17), respectively. These respective  
314 proportions increased to 87% (13/15), 47% (7/15), and 40% (6/15) in 2016.  
315  
316 Our analyses show that co-resistance in cipR *S. sonnei* was generated by sustained and  
317 independent acquisitions of ESBL-encoding plasmids (plasmid acquisition events, herein referred  
318 to as PAs, Figure 1). These plasmids carried differing variants of the *bla*<sub>CTX-M</sub> gene and/or *mphA*.  
319 Notably, this phenomenon was not characterized by selection of the same plasmid/clone  
320 combination; we identified at least thirteen independent acquisitions of ESBL-encoding plasmids  
321 across the phylogenetic tree (Figure 1). Plasmids of incompatibility groups IncB/O and IncI were  
322 the most common vehicles associated with *bla*<sub>CTX-M</sub>.  
323  
324 More specifically, we found that IncB/O plasmids were independently acquired on at least seven  
325 occasions; these plasmids carried an array of ESBL genes, including *bla*<sub>CTX-M-55</sub> (PA3, 5, 16),  
326 *bla*<sub>CTX-M-14</sub> (PA10, 12), *bla*<sub>CTX-M-15</sub> (PA1), and *bla*<sub>CTX-M-24</sub> (PA13). Critically, in 6/7 IncB/O PAs,  
327 the *bla*<sub>CTX-M</sub> gene was associated with *mphA* and *acc6-IIa* genes, leading to an XDR phenotype  
328 additionally encompassing resistance to third-generation cephalosporins, macrolides, and  
329 aminoglycosides. Similarly, IncI plasmids were acquired on four independent occasions and  
330 carried only a *bla*<sub>CTX-M</sub> gene (*bla*<sub>CTX-M-15</sub> (PA9) and *bla*<sub>CTX-M-55</sub> (PA6, 11, 15)). Furthermore,  
331 *bla*<sub>CTX-M</sub> genes were acquired on IncFI (*bla*<sub>CTX-M14</sub> (PA7)) and IncAnco3 (*bla*<sub>CTX-M-15</sub> (PA14))  
332 plasmid backbones. Three cipR non-ESBL isolates also acquired an *mphA* gene associated with  
333 IncFII (PA4, 8) and IncK (PA2) plasmids.  
334  
335 The acquisition of a resistance plasmid was sporadically followed by continued circulation and  
336 geographical expansion of the resistant clone, as observed for the IncB/O/*bla*<sub>CTX-M-55</sub> (PA3),  
337 IncB/O/*bla*<sub>CTX-M-55</sub>-*mphA*-*aac6-IIa* (PA16), IncI/*bla*<sub>CTX-M-55</sub> (PA6), and IncI/*bla*<sub>CTX-M-15</sub> plasmids

338 (PA9) (Figure 1). The inferred time from the most recent common ancestor to the youngest  
339 isolate in each resistant clone was estimated to be at least three years. We also detected the loss of  
340 IncB/O plasmids on two occasions, suggesting a potential lack of IncB/O plasmid stability in  
341 comparison to the IncI plasmids.

342

### 343 *The structure of XDR plasmids in ciprofloxacin-resistant Shigella sonnei*

344 We assessed the plasmid content of all *S. sonnei* isolates by comparing the banding patterns of  
345 crude undigested plasmid extracts. Our data showed that all cipR isolates exhibited a distinct  
346 plasmid profile from that of the resident Vietnamese *S. sonnei* isolates (Supplementary Figure 2).  
347 Additionally, all cipR *S. sonnei* isolates carrying *bla*<sub>CTX-M</sub> and/or *mphA* consistently harbored a  
348 large (90 kb to 110 kb) plasmid. An analysis of the EcoRI digestion profiles of these ESBL-  
349 encoding plasmids showed two major independent clusters, consistent with IncI and IncB/O  
350 plasmid backbones (Figure 2A). Notably, the genetic structure within each plasmid group  
351 appeared to be highly conserved, with the IncI and IncB/O plasmids sharing ~70% and ~60%  
352 similarity in their respective restriction patterns.

353

354 Additional plasmid sequencing and comparative analyses found that the IncI plasmids, acquired  
355 on four occasions (PA6, 9, 11, 15), shared a conserved backbone of ~84 kb (coverage: 80-100%,  
356 nucleotide identity: 99-100%). This conserved region contained typical structures associated with  
357 self-transmissible IncI plasmids, including a type IV *pil* operon (*pilI-PilV*), *traABC* regulatory  
358 genes, the *tra/trb* type IV secretion system genes, the origin of transfer (*oriT* including *nikA* and  
359 *nikB*), and conjugal leading region (*ssb*, *psiA-psiB*, *parB* homolog, *ardA*). The IncI/*bla*<sub>CTX-M-15</sub>  
360 plasmid belonged to sequence type 16 (ST16) and was nearly identical to the previously  
361 described *S. sonnei* IncI plasmid pKHSB1 (accession number: NC\_020991), which has been  
362 maintained in the resident Vietnamese *S. sonnei* population since 2006<sup>47</sup>. Alternatively, the  
363 IncI/*bla*<sub>CTX-M-55</sub> plasmids belonged to ST167 (one allele different from ST16) and did not harbor

364 the Tn3 transposon-mediated *ISecp1-blaCTX-M-15*, but had an insertion of *ISecp1-blaCTX-M-*  
365 *55* between *yagA* and *yafB* (Figure 2B).

366

367 The IncB/O plasmids, acquired on seven occasions (PA1, 3, 5, 10, 12, 13, 16), also shared a  
368 conserved genetic structure of ~90 kb (coverage: 75-100%, nucleotide identity 99-100%) (Figure  
369 2D). In comparison to plasmid sequences in GenBank, our IncB/O plasmid backbone shared the  
370 highest similarity with IncB/O plasmids from an *E. coli* (pECAZ161, accession number:  
371 CP19011), a *S. flexneri* (pSF150802, accession number: CP030917.1) and an *S. sonnei* (p866,  
372 accession number: CO022673.1); the overall synteny ranged from 72% to 94%, with 99%  
373 sequence identity. The IncB/O plasmid backbone contained comparable conjugative IncI plasmid  
374 modules; however, the *pil* operon (~12 kb) exhibited extremely low sequence identity to that of  
375 the IncI plasmid (coverage 1%, identity 78%) (Figure 2C). The size of IncB/O plasmids varied  
376 from 95 kb to 110kb, depending on the complement of resistance gene cassettes. These plasmids  
377 carried a wide range of *bla*<sub>CTX-M</sub> mobile elements, including *ISecp1-IS5-bla*<sub>CTX-M-55</sub>, *IS66-bla*<sub>CTX-</sub>  
378 *M-55-orf-tnpA*, *ISecp1-bla*<sub>CTX-M-15-orf-tnpA</sub>, *IS5-bla*<sub>CTX-M-14-ISecp1</sub>, and *ISecp1b-bla*<sub>CTX-M-24</sub>.  
379 Additionally, these IncB/O plasmids also contained other transposable elements associated with  
380 *mphA* (*IS6-mphA-mrx-mphr*) and *aac6-IIa* (*IS4-aac6-IIa-tmrB*) adjacent to *bla*<sub>CTX-M</sub>-carrying  
381 elements. One IncB/O plasmid additionally carried the *ermB* gene associated with the ISCR3  
382 family (*ISCR3-groEL-ermB-ermC*).

383

384 Aside from the two main IncI and IncB/O plasmid groups, one cipR *S. sonnei* isolate had gained a  
385 *bla*<sub>CTX-M-14</sub>-carrying IncFI plasmid, which was identical to a previously described plasmid  
386 (pEG356) from a Vietnamese *S. sonnei* isolate (accession number: FN594520); a further isolate  
387 acquired a phage-like IncAnco3 plasmid carrying *bla*<sub>CTX-M-15</sub>; three other isolates gained IncK and  
388 IncFII plasmids carrying the *mphA* gene cassette. The IncAnco3, IncK, and IncFII plasmids were  
389 most similar to described *E. coli* plasmids in GenBank, including pAnco1 (accession number:



390 KY515224.1, coverage 91%, identity 98%), pEC1107 (accession number: MG601057.1,  
391 coverage 78%, identity 94%), and pEC105 (accession number: AY458016.1, coverage 59%,  
392 identity 100%), respectively.

393

394 *Commensal E. coli as a source of ESBL-encoding plasmids for ciprofloxacin-resistant Shigella*  
395 *sonnei in the human gut*

396 Given the diversity of the AMR plasmids observed in cipR *S. sonnei*, their similarity to *E. coli*  
397 plasmids, and the fact that humans are the only natural reservoir for *S. sonnei*, we speculated that  
398 these plasmids had been transferred from commensal *E. coli* into *S. sonnei* during infection.

399 Consequently, we performed additional characterization of AMR genes and plasmid diversity in  
400 commensal Enterobacteriaceae isolated from the same fecal samples that contained *S. sonnei* and  
401 from rectal swabs taken from healthy children. Metagenomic sequencing of these mixed bacterial  
402 populations (lacking *Shigella*) from MC plates indicated that *E. coli* was the most commonly  
403 isolated commensal Enterobacteriaceae (47/48 pooled colonies), followed by *Klebsiella*  
404 *pneumoniae* (7/48 pooled colonies) and *Enterobacter cloacae* (1/48 pooled colonies).

405

406 The resulting sequence data identified a substantial quantity of AMR genes and plasmid  
407 backbones in the commensal Enterobacteriaceae (Supplementary Figure 3 and Figure 3A). We  
408 observed a particularly high prevalence of CipR commensal Enterobacteriaceae; this has been  
409 observed previously and is considered to be associated with sustained antimicrobial exposure and  
410 competition in the gastrointestinal tract<sup>48</sup>. Furthermore, a number of different AMR determinants  
411 were found to be present in both the commensal bacteria and the cipR *S. sonnei*. For example,  
412 *bla<sub>CTX-M</sub>*, *mphA*, *aac3-IIa*, and *ermB* were found to be present in cipR *S. sonnei* and 92% (44/48),  
413 75% (36/48), 52% (25/48), and 38% (18/48) of pooled commensal Enterobacteriaceae,  
414 respectively (Figure 3A). IncF (IncFII, IncFIA, IncFIB, and IncFIC) plasmids were found to be  
415 the most prevalent replicon types in the commensal Enterobacteriaceae. However, we additionally



416 identified IncI and IncB/O plasmids in commensal *E. coli* from the fecal samples of three children  
417 infected with *S. sonnei* and three healthy children, respectively.

418

419 We next aimed to identify comparable plasmid structures between *E. coli* and *S. sonnei*. The  
420 IncB/O plasmids found in commensal *E. coli* from three healthy children (subjects 22889, 22959,  
421 and 22274) exhibited high levels of sequence similarity to the IncB/O plasmid backbone acquired  
422 by cipR *S. sonnei* (coverage/identity: 76/99%, 94/99%, and 99/97%; respectively). Similarly,  
423 among the three commensal *E. coli* samples carrying IncI plasmids, we identified an IncI plasmid  
424 from a commensal *E. coli* without a Tn3 transposon-mediated *bla*<sub>CTX-M-15</sub>, which displayed high  
425 sequence similarity (coverage 81%, identity 98%) to an IncI plasmid from cipR *S. sonnei*. More  
426 significantly, a commensal *E. coli* originating from a patient infected with a cipR *S. sonnei* (01-  
427 0123) carried an analogous IncI/*bla*<sub>CTX-M-15</sub> plasmid. We isolated a single ESBL-producing  
428 commensal *E. coli* from this MC plate and subjected the plasmid to long read Nanopore  
429 sequencing. The sequencing resulted in a 90,786 bp circularized plasmid sequence, harboring  
430 *bla*<sub>CTX-M-15</sub> and *bla*<sub>TEM1</sub> on a Tn3 transposon. The raw IncI plasmid sequence from the  
431 corresponding cipR *S. sonnei* 01-0123 was mapped against the commensal *E. coli* plasmid  
432 sequence and produced a plasmid with 100% coverage (mean mapping coverage: 15, standard  
433 deviation: 7). The assembled plasmid contigs from cipR *S. sonnei* 01-0123 shared 100% sequence  
434 identity (Figure 3B). These data and the location of this organism on the phylogenetic tree  
435 suggest that this resistance plasmid was potentially transferred *in vivo* between commensal *E. coli*  
436 and cipR *S. sonnei* 01-0123 in the gut of the child.

437

438 *Ciprofloxacin increases the conjugation frequency of ESBL plasmids between commensal*  
439 *Escherichia coli and ciprofloxacin-resistant Shigella sonnei*

440 Our data illustrates that commensal *E. coli* are an important reservoir of AMR genes and may be  
441 transferred to *S. sonnei in vivo*. Furthermore, the high diversity of AMR plasmids observed here

442 in a single *S. sonnei* lineage is atypical and has not been previously observed in a geographically  
443 restricted clonal expansion. The reason for this observation is unclear but we suspect is associated  
444 with the combination of a permissive circulating clone, exposure to fluoroquinolones, and a wide  
445 variety of AMR plasmids in the resident commensal population. We also observed that the  
446 majority of *S. sonnei* infected children (85%, 67/79) were treated with ciprofloxacin, an  
447 antimicrobial agent that can trigger the SOS response and promote horizontal gene transfer in  
448 bacteria<sup>49-54</sup>. Consequently, we hypothesized that this array of resistance plasmids was associated  
449 with a *cipR* phenotype and that ciprofloxacin treatment may facilitate plasmid transfer *in vivo*.  
450  
451 To test this hypothesis, we first identified *cipS*/ESBL+ commensal *E. coli* donors and attempted  
452 to mobilize these plasmids into a *cipR*/ESBL- *S. sonnei* recipient. Screening identified that the  
453 majority of commensal *E. coli* (35/48) recovered from the MC plate sweeps were both  
454 *cipR*/ESBL+. The remaining 13 commensal *E. coli* isolates were *cipS* (MIC  $\leq$  1 mg/L)/ESBL+;  
455 nine were derived from children infected with *S. sonnei* and four from healthy children. ESBL  
456 plasmids from 9/13 of the commensal *E. coli* could be conjugated into the *cipR*/ESBL- *S. sonnei*.  
457 The conjugation frequencies were high, ranging from  $4 \times 10^{-7}$  to  $1.6 \times 10^{-3}$ /recipient cells. The  
458 supplementation of 0.25x MIC ciprofloxacin into the conjugation media did not have a significant  
459 effect on the frequency of plasmid transfer. However, when the conjugation media was  
460 supplemented with 0.5x MIC ciprofloxacin (of the *cipS* *E. coli* donor), 4/9 commensal *E. coli*  
461 (22784, 01-0123, 02-1936, and 22959) demonstrated respective increases in conjugation  
462 frequencies of ESBL plasmids to *cipR* *S. sonnei* of 3, 6, 11, and 36-fold, in comparison to media  
463 without ciprofloxacin (Figure 4). These respective conjugation frequencies increased to 4, 10, 25  
464 and 42-fold when the concentration of ciprofloxacin was increased to 0.75x MIC. Additionally, a  
465 single commensal *E. coli* isolate (22978) exhibited a 7-fold increase in conjugation frequency in

466 medium supplemented with 0.75x MIC ciprofloxacin, despite this effect not being observed in  
467 media containing 0.5x MIC ciprofloxacin.  
468  
469 Plasmid sequencing demonstrated that *E. coli* 01-0123 (ciprofloxacin MIC: 0.25 mg/L) carried an  
470 IncI/*bla*<sub>CTX-M-15</sub> plasmid (as described above). *E. coli* 02-1936 (ciprofloxacin MIC: 0.016 mg/L)  
471 and 22784 (ciprofloxacin MIC: 0.38 mg/L) carried IncF/*bla*<sub>CTX-M-27</sub> plasmids that shared high  
472 similarity to the IncF plasmid pC15 in Genbank (accession number: AY458016, ~ 92 kb)  
473 (coverage: 75% and 85%, identity: 99% and 98%, respectively). *E. coli* 22959 (ciprofloxacin  
474 MIC: 0.38 mg/L) harbored an IncB/O/ *bla*<sub>CTX-M-27</sub> plasmid exhibiting high genetic similarity to the  
475 IncB/O plasmid acquired by cipR *S. sonnei* as described above (coverage 94%, identity 99%). *E.*  
476 *coli* 22978 (ciprofloxacin MIC: 0.5 mg/L) carried an IncF/*bla*<sub>CTX-M-27</sub> similar to the IncF plasmid  
477 pDA33135 in Genbank (accession number: CP029577.1, ~ 139 kb) (coverage 94%, identity  
478 99%).

479

## 480 **Discussion**

481 Since its introduction in the 1980s, ciprofloxacin has become one of the most commonly used  
482 antimicrobials worldwide due to its low cost and clinical effectiveness against a wide range of  
483 Gram-positive and Gram-negative bacterial infections. The extensive use of ciprofloxacin in  
484 humans and animals inevitably led to a rapid increase in reduced susceptibility to ciprofloxacin in  
485 both Gram-negative and Gram-positive bacteria during the 1990s<sup>55</sup>. Since the turn of the century,  
486 multiple cipR clones in various pathogenic species/serotypes have emerged and spread  
487 successfully in various countries, with many eventually disseminating internationally. Organisms  
488 with internationally successful cipR clones include *Salmonella* Typhi<sup>56</sup> and *Shigella dysenteriae*  
489 type 1<sup>57</sup> in South Asia, and methicillin-resistant *Staphylococcus aureus* ST22<sup>58</sup>, ST131-H30  
490 clone of *E. coli*<sup>58</sup>, *Salmonella* Kentucky ST198<sup>60</sup>, *Clostridium difficile* 027<sup>61</sup>, *Shigella sonnei*<sup>22</sup>  
491 and *E. coli* ST1193<sup>62-65</sup>. Tracking the global transmission and local establishment of these

492 clinically important clones through routine surveillance, particularly with the integration of  
493 genomics, has become essential for guiding public health control strategy and clinical practice.  
494 Here, by decoding the genomic sequences of *S. sonnei* isolated in Vietnam between 2014 and  
495 2016, we provide unparalleled insight into the local clonal establishment and AMR dynamics of  
496 *cipR S. sonnei* as it entered a new human population. Our work outlines the progression and co-  
497 circulation of multiple XDR *S. sonnei* clones in Vietnam, some of which have gained resistance  
498 to all antimicrobial therapies currently recommended by WHO for the treatment of *Shigella*.

499

500 Although the *cipR S. sonnei* sublineage Central Asia III has spread internationally, the  
501 development of XDR within this lineage has not been reported previously. *S. sonnei* are highly  
502 efficient at spreading internationally; therefore, the identification and pervasiveness of AMR in  
503 these organisms means that future investigations should monitor their international circulation to  
504 provide early warning for public health authorities and healthcare providers. More specifically,  
505 the emergence and expansion of *cipR XDR S. sonnei* clones associated with *ISecp1-bla<sub>CTX-M-55</sub>*  
506 raises a major concern regarding the epidemic potential of this novel CTX-M ESBL variant in *S.*  
507 *sonnei*<sup>66-68</sup>. A significant burden of shigellosis, high prevalence of AMR among Gram-negative  
508 commensal bacteria, and the purchasing of antimicrobials in the community may be factors  
509 contributing to the emergence and maintenance of XDR *S. sonnei* in Vietnam.

510

511 We describe multiple different plasmid structures in the *cipR S. sonnei* population, distinguishing  
512 their dynamics from those of the resident *S. sonnei* population, which underwent a clonal  
513 expansion characterized by a single plasmid structure<sup>47</sup>. In most cases when a *mphA/bla<sub>CTX-M</sub>*  
514 plasmid was acquired, the plasmids appeared not to become established in the population. This  
515 observation suggests that these structures could have a sustained fitness disadvantage in the  
516 absence of antimicrobial pressure. Conversely, the successful maintenance of four independent  
517 XDR *S. sonnei* clones warrants further investigation into their potential fitness, plasmid stability,

518 and future evolutionary trajectories. Additionally, the selected IncB/O and IncI ESBL-encoding  
519 conjugative plasmids acquired and maintained by *Shigella sonnei* suggest plasmid preferences in  
520 this species. IncI and IncB/O plasmids belong to the IncI-complex (IncI, IncB/O, IncK, IncZ),  
521 which have comparable antisense RNA plasmid replication control mechanisms<sup>69</sup>. Our results  
522 concur with previous reports that proposed a commonality of IncI-complex plasmids associated  
523 with the *bla*<sub>CTX-M</sub> element in *S. sonnei* from countries at various stages of economic development  
524 <sup>21,47,70–74</sup>. The reasons for these specific plasmid-host combinations remain elusive; however, we  
525 found that IncI and IncB/O plasmids displayed the highest *in vitro* conjugation efficiencies  
526 compared to other ESBL-encoding plasmids. Moreover, the IncB/O plasmid group was found to  
527 be the second most commonly identified plasmid group in commensal and pathogenic *E. coli*  
528 from humans and animals<sup>75</sup>, while the conjugative IncI group was can also be highly prevalent in  
529 commensal bacteria from infants<sup>76</sup>. The regular sampling of these plasmids by *Shigella sonnei*  
530 could be attributed to several factors, including the close genetic relatedness between *Shigella* and  
531 *E. coli*, the propensity of *S. sonnei* to acquire AMR plasmids, and the circulation of highly  
532 transmissible AMR plasmids in commensal *E. coli*. To combat the emergence and circulation of  
533 AMR Gram-negative bacteria, a better understanding of plasmid-host interactions, plasmid  
534 stability, and the role of plasmids in the fitness of *Shigella sonnei* are now critical.

535

536 The routine acquisition of a wide variety of ESBL-encoding plasmids by *Shigella sonnei* reflects  
537 extensive interspecies gene flow from a substantial local gene pool, possibly as a result of  
538 bacterial response to selective pressures exerted by widespread and largely uncontrolled  
539 antimicrobial use. These plasmids appear to originate from bacterial hosts that share the same  
540 ecological niche as *S. sonnei*. We performed WGS of selected commensal bacteria from fecal  
541 samples infected with *S. sonnei* and from healthy children and identified an extensive range of  
542 AMR genes/plasmids that conferred resistance to all antimicrobial classes in these commensal  
543 organisms. This diversity included the IncI and IncB/O plasmids that had been routinely acquired

544 by cipR *S. sonnei*. We also provide evidence for *in vivo* IncI/*bla*<sub>CTX-M-15</sub> plasmid transfer between  
545 commensal *E. coli* and cipR *S. sonnei* in a single patient; however, we cannot resolve the  
546 directionality of plasmid movement or discount the role of other components of the human  
547 microbiome as the original donor of this plasmid. Potential plasmid transfer between commensal  
548 Gram-negative bacteria and *Shigella* spp. in the human gut has been suggested previously<sup>77,78</sup>.  
549 The large biomass of Enterobacteriaceae in the human gastrointestinal tract and the apparent  
550 common circulation of IncI and IncB/O plasmids in commensal bacteria in the gastrointestinal  
551 tracts of Vietnamese children suggests that the direction of plasmid transfer is more likely to be  
552 from commensal bacteria (potentially *E. coli*) to *S. sonnei*.

553

554 We additionally aimed to assess the role of ciprofloxacin in facilitating the transfer of ESBL-  
555 encoding plasmids from commensal bacteria to cipR *S. sonnei*. Our data show that exposure to  
556 sub-[inhibitory concentrations to ciprofloxacin may facilitate the transfer of ESBL-encoding  
557 plasmids between commensal *E. coli* and cipR *S. sonnei*. As the majority of commensal *E. coli*  
558 were cipR, our results suggest that horizontal plasmid transfer between cipR Gram-negative  
559 organisms and *Shigella* may occur at higher frequencies in the presence of increasing  
560 ciprofloxacin concentrations. Our observations question the effect of ciprofloxacin on the  
561 composition of commensal bacterial and transfer dynamics of AMR determinants in the human  
562 gut during and after treatment. In the clinical study in which the *S. sonnei* described here were  
563 isolated, the majority of *S. sonnei* infected children (85%, 67/79) were treated empirically with  
564 ciprofloxacin. We found that the clinical outcomes (duration of hospitalization) between children  
565 infected with cipR (51 cases) versus cipS *S. sonnei* (16 cases) were comparable (median 4 days  
566 (IQR: 3-6.5 days) versus 3 days (IQR: 2-4))<sup>79</sup>. Additionally, four cases (three infected with cipR  
567 *S. sonnei*) did not receive antimicrobial treatment but still recovered in a similar time period.  
568 These supporting data call for a re-evaluation of the necessity and benefit of treating children  
569 with *S. sonnei* dysentery with ciprofloxacin. Treatment with fluoroquinolones in the absence of

570 appropriate diagnostics and susceptibility testing could potentially select for the maintenance and  
571 transmission of XDR *S. sonnei* and promote horizontal plasmid transfer between commensal  
572 bacteria and *S. sonnei*.

573

574 In conclusion, multiple XDR clones of *S. sonnei* have emerged and are co-circulating in Vietnam.  
575 Commensal *E. coli* in the gastrointestinal tract of Vietnamese children display an exceptionally  
576 high degree of diversity in AMR genes and plasmid composition, and our evidence suggests these  
577 are the most likely reservoir for the maintenance and transfer of MDR plasmids to cipR *S. sonnei*.  
578 Our data further suggest *in vivo* plasmid transfer between commensal *E. coli* and cipR *S. sonnei*  
579 during infection, which is likely facilitated by the presence of sub-MIC concentrations of  
580 ciprofloxacin. We advocate for the continued surveillance of XDR *S. sonnei* in Vietnam and a  
581 suggest a urgent re-evaluation of the empirical use of ciprofloxacin for a range of gastrointestinal  
582 infections.

583

#### 584 **References**

- 585 1 Kotloff KL, Winickoff JP, Ivanoff B, *et al.* Global burden of Shigella infections:  
586 Implications for vaccine development and implementation of control strategies. *Bull*  
587 *World Health Organ* 1999; **77**: 651–66.
- 588 2 Lanata CF, Fischer-Walker CL, Olascoaga AC, *et al.* Global causes of diarrheal disease  
589 mortality in children. *PLoS One* 2013; **8**: e72788.
- 590 3 Kotloff KL, Nataro JP, Blackwelder WC, *et al.* Burden and aetiology of diarrhoeal disease  
591 in infants and young children in developing countries (the Global Enteric Multicenter  
592 Study, GEMS): a prospective, case-control study. *Lancet* 2013; **382**: 209–22.
- 593 4 Thompson CN, Duy PT, Baker S. The rising dominance of Shigella sonnei: An  
594 intercontinental shift in the etiology of bacillary dysentery. *PLoS Negl. Trop. Dis.* 2015; **9**.  
595 DOI:10.1371/journal.pntd.0003708.

- 596 5 Baker KS, Campos J, Pichel M, *et al.* Whole genome sequencing of *Shigella sonnei*  
597 through PulseNet Latin America and Caribbean: advancing global surveillance of  
598 foodborne illnesses. *Clin Microbiol Infect* 2017; **23**: 845–53.
- 599 6 Holt KE, Baker S, Weill F-X, *et al.* *Shigella sonnei* genome sequencing and phylogenetic  
600 analysis indicate recent global dissemination from Europe. 2012; **44**.  
601 DOI:10.1038/ng.2369.
- 602 7 D. Legros, D. Legros. Guidelines for the control of shigellosis, including epidemics due to  
603 *Shigella dysenteriae* type 1. *World Health* 2005; : 1–70.
- 604 8 Pazhani GP, Ramamurthy T, Mitra U, Bhattacharya SK, Niyogi SK. Species diversity and  
605 antimicrobial resistance of *Shigella* spp. isolated between 2001 and 2004 from  
606 hospitalized children with diarrhoea in Kolkata (Calcutta), India. *Epidemiol Infect* 2005;  
607 **133**: 1089–95.
- 608 9 Shakya G, Acharya J, Adhikari S, Rijal N. Shigellosis in Nepal: 13 years review of  
609 nationwide surveillance. *J Health Popul Nutr* 2016; **35**: 36.
- 610 10 Von Seidlein L, Deok RK, Ali M, *et al.* A multicentre study of *Shigella* diarrhoea in six  
611 Asian countries: Disease burden, clinical manifestations, and microbiology. *PLoS Med*  
612 2006; **3**: 1556–69.
- 613 11 Pazhani GP, Niyogi SK, Singh AK, *et al.* Molecular characterization of multidrug-  
614 resistant *Shigella* species isolated from epidemic and endemic cases of shigellosis in India.  
615 *J Med Microbiol* 2008; **57**: 856–63.
- 616 12 Rajpara N, Nair M, Chowdhury G, *et al.* Molecular analysis of multidrug resistance in  
617 clinical isolates of *Shigella* spp. from 2001–2010 in Kolkata, India: role of integrons,  
618 plasmids, and topoisomerase mutations. *Infect Drug Resist* 2018; **Volume 11**: 87–102.
- 619 13 De Lappe N, O’Connor J, Garvey P, McKeown P, Cormican M. Ciprofloxacin-resistant  
620 *Shigella sonnei* associated with travel to India. *Emerg Infect Dis* 2015; **21**: 894–6.
- 621



- 622 14 Bowen A, Hurd J, Hoover C, *et al.* Importation and Domestic Transmission of *Shigella*  
623 *sonnei* Resistant to Ciprofloxacin - United States, May 2014-February 2015. *MMWR*  
624 *Morb Mortal Wkly Rep* 2015; **64**: 318–20.
- 625 15 Nüesch-Inderbinnen M, Heini N, Zurfluh K, Althaus D, Hächler H, Stephan R. *Shigella*  
626 antimicrobial drug resistance mechanisms, 2004–2014. *Emerg Infect Dis* 2016; **22**: 1083–  
627 5.
- 628 16 Ruekit S, Wangchuk S, Dorji T, *et al.* Molecular characterization and PCR-based replicon  
629 typing of multidrug resistant *Shigella sonnei* isolates from an outbreak in Thimphu,  
630 Bhutan. *BMC Res Notes* 2014; **7**. DOI:10.1186/1756-0500-7-95.
- 631 17 Kim JS, Kim JJ, Kim SJ, *et al.* Outbreak of ciprofloxacin-resistant *Shigella sonnei*  
632 associated with travel to Vietnam, Republic of Korea. *Emerg Infect Dis* 2015; **21**: 1247–  
633 50.
- 634 18 Gaudreau C, Ratnayake R, Pilon PA, Gagnon S, Roger M, Levesque S. Ciprofloxacin-  
635 resistant *Shigella sonnei* among men who have sex with men, Canada, 2010. *Emerg Infect*  
636 *Dis* 2011; **17**: 1747–50
- 637 19 Chiou CS, Izumiya H, Kawamura M, *et al.* The worldwide spread of ciprofloxacin-  
638 resistant *Shigella sonnei* among HIV-infected men who have sex with men, Taiwan. *Clin*  
639 *Microbiol Infect* 2016; **22**: 383.e11–383.e16.
- 640 20 Baker KS, Dallman TJ, Field N, *et al.* Genomic epidemiology of *Shigella* in the United  
641 Kingdom shows transmission of pathogen sublineages and determinants of antimicrobial  
642 resistance. *Sci Rep* 2018; **8**: 1–8.
- 643 21 Bodhidatta L, Thanh TH, Thanh DP, *et al.* Introduction and establishment of  
644 fluoroquinolone-resistant *Shigella sonnei* into Bhutan. *Microb Genomics* 2015; **1**.  
645 DOI:10.1099/mgen.0.000042.
- 646 22 Chung The H, Rabaa MA, Pham Thanh D, *et al.* South Asia as a Reservoir for the Global  
647 Spread of Ciprofloxacin-Resistant *Shigella sonnei*: A Cross-Sectional Study. *PLoS Med*

- 648 2016; **13**: 1–12.
- 649 23 Duong VT, Tuyen HT, Van Minh P, *et al.* No Clinical benefit of empirical antimicrobial  
650 therapy for pediatric diarrhea in a high-usage, high-resistance setting. *Clin Infect Dis*  
651 2018; **66**: 504–11.
- 652 24 Magiorakos AP, Srinivasan A, Carey RB, *et al.* Multidrug-resistant, extensively drug-  
653 resistant and pandrug-resistant bacteria: An international expert proposal for interim  
654 standard definitions for acquired resistance. *Clin Microbiol Infect* 2012; **18**: 268–81.
- 655 25 Thiem VD, Sethabutr O, Von Seidlein L, *et al.* Detection of Shigella by a PCR Assay  
656 Targeting the ipaH Gene Suggests Increased Prevalence of Shigellosis in Nha Trang,  
657 Vietnam. *J Clin Microbiol* 2004; **42**: 2031–5.
- 658 26 Thompson CN, Anders KL, Nhi LTQ, *et al.* A cohort study to define the age-specific  
659 incidence and risk factors of Shigella diarrhoeal infections in Vietnamese children: A  
660 study protocol. *BMC Public Health* 2014; **14**. DOI:10.1186/1471-2458-14-1289.
- 661 27 Li H, Handsaker B, Wysoker A, *et al.* The Sequence Alignment/Map format and  
662 SAMtools. *Bioinformatics* 2009; **25**: 2078–9.
- 663 28 Croucher NJ, Page AJ, Connor TR, *et al.* Rapid phylogenetic analysis of large samples of  
664 recombinant bacterial whole genome sequences using Gubbins. *Nucleic Acids Res* 2015;  
665 **43**: e15.
- 666 29 Nguyen LT, Schmidt HA, Von Haeseler A, Minh BQ. IQ-TREE: A fast and effective  
667 stochastic algorithm for estimating maximum-likelihood phylogenies. *Mol Biol Evol* 2015;  
668 **32**: 268–74.
- 669 30 Rambaut A, Lam TT, Max Carvalho L, Pybus OG. Exploring the temporal structure of  
670 heterochronous sequences using TempEst (formerly Path-O-Gen). *Virus Evol* 2016; **2**:  
671 vew007.
- 672 31 Drummond AJ, Rambaut A. BEAST: Bayesian evolutionary analysis by sampling trees.  
673 *BMC Evol Biol* 2007; **7**. DOI:10.1186/1471-2148-7-214.

- 674 32 Drummond AJ, Rambaut A, Shapiro B, Pybus OG. Bayesian Coalescent Inference of Past  
675 Population Dynamics from Molecular Sequences. DOI:10.1093/molbev/msi103.
- 676 33 Drummond AJ, Ho SYW, Phillips MJ, Rambaut A. Relaxed Phylogenetics and Dating  
677 with Confidence. DOI:10.1371/journal.pbio.0040088.
- 678 34 Xie W, Lewis PO, Fan Y, Kuo L, Chen MH. Improving marginal likelihood estimation for  
679 bayesian phylogenetic model selection. *Syst Biol* 2011; **60**: 150–60.
- 680 35 Lartillot N, Philippe H. Computing Bayes factors using thermodynamic integration. *Syst*  
681 *Biol* 2006; **55**: 195–207.
- 682 36 Inouye M, Dashnow H, Raven L-A, *et al.* SRST2: Rapid genomic surveillance for public  
683 health and hospital microbiology labs. *Genome Med* 2014; **6**: 90.
- 684 37 Gupta SK, Padmanabhan BR, Diene SM, *et al.* ARG-annot, a new bioinformatic tool to  
685 discover antibiotic resistance genes in bacterial genomes. *Antimicrob Agents Chemother*  
686 2014; **58**: 212–20.
- 687 38 Carattoli A, Zankari E, García-Fernández A, *et al.* In Silico detection and typing of  
688 plasmids using plasmidfinder and plasmid multilocus sequence typing. *Antimicrob Agents*  
689 *Chemother* 2014; **58**: 3895–903.
- 690 39 García-Fernández A, Chiaretto G, Bertini A, *et al.* Multilocus sequence typing of IncII  
691 plasmids carrying extended-spectrum  $\beta$ -lactamases in Escherichia coli and Salmonella of  
692 human and animal origin. *J Antimicrob Chemother* 2008; **61**: 1229–33.
- 693 40 Zerbino DR, Birney E. Velvet: Algorithms for de novo short read assembly using de  
694 Bruijn graphs. *Genome Res* 2008; **18**: 821–9.
- 695 41 Seemann T. Prokka: Rapid prokaryotic genome annotation. *Bioinformatics* 2014; **30**:  
696 2068–9.
- 697 42 Assefa S, Keane TM, Otto TD, Newbold C, Berriman M. ABACAS: Algorithm-based  
698 automatic contiguation of assembled sequences. *Bioinformatics* 2009; **25**: 1968–9.
- 699 43 Carver T, Berriman M, Tivey A, *et al.* Artemis and ACT: Viewing, annotating and

- 700 comparing sequences stored in a relational database. *Bioinformatics* 2008; **24**: 2672–6.
- 701 44 Wood DE, Salzberg SL. Kraken: Ultrafast metagenomic sequence classification using  
702 exact alignments. *Genome Biol* 2014; **15**. DOI:10.1186/gb-2014-15-3-r46.
- 703 45 CI Kado AND ST. Liu. Rapid Procedure for Detection and Isolation of Large and Small  
704 Plasmids. *J Bacteriol* 1981; **145**: 1365–73.
- 705 46 Bankevich A, Nurk S, Antipov D, *et al.* SPAdes: A New Genome Assembly Algorithm  
706 and Its Applications to Single-Cell Sequencing. *J Comput Biol* 2012; **19**: 455–77.
- 707 47 Holt KE, Thieu Nga TV, Thanh DP, *et al.* Tracking the establishment of local endemic  
708 populations of an emergent enteric pathogen. *Proc Natl Acad Sci U S A* 2013; **110**:  
709 17522–7.
- 710 48 Nhi LTQ, Tuyen HT, Trung PD, *et al.* Excess body weight and age associated with the  
711 carriage of fluoroquinolone and third-generation cephalosporin resistance genes in  
712 commensal escherichia coli from a cohort of urban vietnamese children. *J Med Microbiol*  
713 2018; **67**: 1457–66.
- 714 49 Bearson BL, Brunelle BW. Fluoroquinolone induction of phage-mediated gene transfer in  
715 multidrug-resistant Salmonella. *Int J Antimicrob Agents* 2015; **46**: 201–4.
- 716 50 Hastings PJ, Rosenberg SM, Slack A. Antibiotic-induced lateral transfer of antibiotic  
717 resistance. *Trends Microbiol.* 2004; **12**: 401–4.
- 718 51 Qin T, Kang H, Ma P, Li P, Huang L, Gu B. SOS response and its regulation on the  
719 fluoroquinolone resistance. *Ann Transl Med* 2015; **3**: 358.
- 720 52 Baharoglu Z, Bikard D, Mazel D. Conjugative DNA transfer induces the bacterial SOS  
721 response and promotes antibiotic resistance development through integron activation.  
722 *PLoS Genet* 2010; **6**: 1–10.
- 723 53 Ubeda C, Maiques E, Knecht E, Lasa I, Novick RP, Penadés JR. Antibiotic-induced SOS  
724 response promotes horizontal dissemination of pathogenicity island-encoded virulence  
725 factors in staphylococci. *Mol Microbiol* 2005; **56**: 836–44.

- 726 54 Beaber JW, Hochhut B, Waldor MK. SOS response promotes horizontal dissemination of  
727 antibiotic resistance genes. *Nature* 2004; **427**: 72–4.
- 728 55 Dalhoff A. Global fluoroquinolone resistance epidemiology and implications for clinical  
729 use. *Interdiscip Perspect Infect Dis* 2012; **2012**. DOI:10.1155/2012/976273
- 730 56 Thanh DP, Karkey A, Dongol S, *et al.* A novel ciprofloxacin-resistant subclade of h58.  
731 *Salmonella typhi* is associated with fluoroquinolone treatment failure. *Elife* 2016; **5**.  
732 DOI:10.7554/eLife.14003.
- 733 57 Talukder KA, Khajanchi BK, Islam MA, *et al.* Genetic relatedness of ciprofloxacin-  
734 resistant *Shigella dysenteriae* type 1 strains isolated in south Asia. *J Antimicrob*  
735 *Chemother* 2004; **54**: 730–4.
- 736 58 Holden MTG, Hsu L-Y, Kurt K, *et al.* A genomic portrait of the emergence, evolution,  
737 and global spread of a methicillin-resistant *Staphylococcus aureus* pandemic. *Genome Res*  
738 2013; **23**: 653–64.
- 739 59 Banerjee R, Johnson JR. A new clone sweeps clean: The enigmatic emergence of  
740 *Escherichia coli* sequence type 131. *Antimicrob. Agents Chemother.* 2014; **58**: 4997–  
741 5004.
- 742 60 Le Hello S, Bekhit A, Granier SA, *et al.* The global establishment of a highly-  
743 fluoroquinolone resistant *Salmonella enterica* serotype Kentucky ST198 strain. *Front*  
744 *Microbiol* 2013; **4**. DOI:10.3389/fmicb.2013.00395.
- 745 61 He M, Miyajima F, Roberts P, *et al.* Emergence and global spread of epidemic healthcare-  
746 associated *Clostridium difficile*. *Nat Genet* 2013; **45**: 109–13
- 747 62 Xia L, Liu Y, Xia S, *et al.* Prevalence of ST1193 clone and IncII/ST16 plasmid in E-coli  
748 isolates carrying blaCTX-M-55 gene from urinary tract infections patients in China. *Sci*  
749 *Rep* 2017; **7**. DOI:10.1038/srep44866.
- 750 63 Kim Y, Oh T, Nam YS, Cho SY, Lee HJ. Prevalence of ST131 and ST1193 among  
751 bloodstream isolates of *Escherichia coli* not susceptible to ciprofloxacin in a tertiary care

- 752 university hospital in korea, 2013-2014. *Clin Lab* 2017; **63**: 1541–3.
- 753 64 Wu J, Lan F, Lu Y, He Q, Li B. Molecular characteristics of ST1193 clone among  
754 phylogenetic group B2 Non-ST131 fluoroquinolone-resistant *Escherichia coli*. *Front*  
755 *Microbiol* 2017; **8**. DOI:10.3389/fmicb.2017.02294.
- 756 65 Tchesnokova VL, Rechkina E, Larson L, *et al*. Rapid and Extensive Expansion in the U.S.  
757 of a New Multidrug-Resistant *Escherichia coli* Clonal Group, Sequence Type ST1193.  
758 *Clin Infect Dis* 2018; : 2016–9.
- 759 66 Zurita J, Ortega-Paredes D, Barba P. First description of *Shigella sonnei* harboring  
760 blaCTX-M-55 outside Asia. *J Microbiol Biotechnol* 2016; **26**: 2224–7.
- 761 67 Lee W, Chung HS, Lee H, *et al*. CTX-M-55-type extended-spectrum  $\beta$ -lactamase-  
762 producing *Shigella sonnei* isolated from a Korean patient who had travelled to China. *Ann*  
763 *Lab Med* 2013; **33**: 141–4.
- 764 68 Qu F, Ying Z, Zhang C, *et al*. Plasmid-encoding extended-spectrum  $\beta$ -lactamase CTX-M-  
765 55 in a clinical *Shigella sonnei* strain, China. *Future Microbiol* 2014; **9**: 1143–50.
- 766 69 Praszkie J, Pittard AJ. Control of replication in I-complex plasmids. *Plasmid*. 2005; **53**:  
767 97–112.
- 768 70 Allué-Guardia A, Koenig SSK, Quirós P, Muniesa M, Bono JL, Eppinger M. Closed  
769 genome and comparative phylogenetic analysis of the clinical multidrug resistant *Shigella*  
770 *sonnei* strain 866. *Genome Biol Evol* 2018. DOI:10.1093/gbe/evy168.
- 771 71 Folster JP, Pecic G, Krueger A, *et al*. Identification and characterization of CTX-M-  
772 producing *Shigella* isolates in the United States. *Antimicrob. Agents Chemother*. 2010;  
773 **54**: 2269–70.
- 774 72 Ma Q, Xu X, Luo M, *et al*. A waterborne outbreak of *shigella sonnei* with resistance to  
775 azithromycin and third-generation cephalosporins in China in 2015. *Antimicrob Agents*  
776 *Chemother* 2017; **61**. DOI:10.1128/AAC.00308-17.
- 777 73 Seral C, Rojo-Bezares B, Garrido A, Gude MJ, Sáenz Y, Castillo FJ. Characterisation of a

- 778 CTX-M-15-producing *Shigella sonnei* in a Spanish patient who had not travelled abroad.  
779 *Enferm Infecc Microbiol Clin* 2012; : 2011–3
- 780 74 Kim JS, Kim J, Jeon SE, *et al.* Complete nucleotide sequence of the IncI1 plasmid  
781 pSH4469 encoding CTX-M-15 extended-spectrum  $\beta$ -lactamase in a clinical isolate of  
782 *Shigella sonnei* from an outbreak in the Republic of Korea. *Int J Antimicrob Agents* 2014;  
783 **44**: 533–7.
- 784 75 Johnson TJ, Wannemuehler YM, Johnson SJ, *et al.* Plasmid replicon typing of commensal  
785 and pathogenic *Escherichia coli* isolates. *Appl Environ Microbiol* 2007; **73**: 1976–83.
- 786 76 Ravi A. Characterization of the infant gut microbiota mobilome. 2017.  
787 <http://hdl.handle.net/11250/2497973>.
- 788 77 Bratoeva MP, John JF. In vivo R–plasmid transfer in a patient with a mixed infection of  
789 shigella dysentery. *Epidemiol Infect* 1994; **112**: 247–52.
- 790 78 Rashid H, Rahman M. Possible transfer of plasmid mediated third generation  
791 cephalosporin resistance between *Escherichia coli* and *Shigella sonnei* in the human gut.  
792 *Infect Genet Evol* 2015; **30**: 15–8.
- 793 79 Duong VT, Tuyen HT, Van Minh P, *et al.* No Clinical benefit of empirical antimicrobial  
794 therapy for pediatric diarrhea in a high-usage, high-resistance setting. *Clin Infect Dis*  
795 2018; **66**: 504–11.

796

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800

#### 801 **Author contributions**

802 PTD, MAR and SB designed the study. PTD performed in data analysis and interpretation of the  
803 results under the scientific guidance of MAR and SB. PTD drafted and edited the paper, with

804 MAR and SB revising and structuring the paper. TNTN, HCT, CB, FA, HNDD, and HTT  
805 performed laboratory work and generated the data for analysis. DVT recruited patients and  
806 performed the clinical work required for the study. HCT, CB, and GET contributed to the editing  
807 of the paper. All authors read and approved the final draft.

808

809 **Additional information**

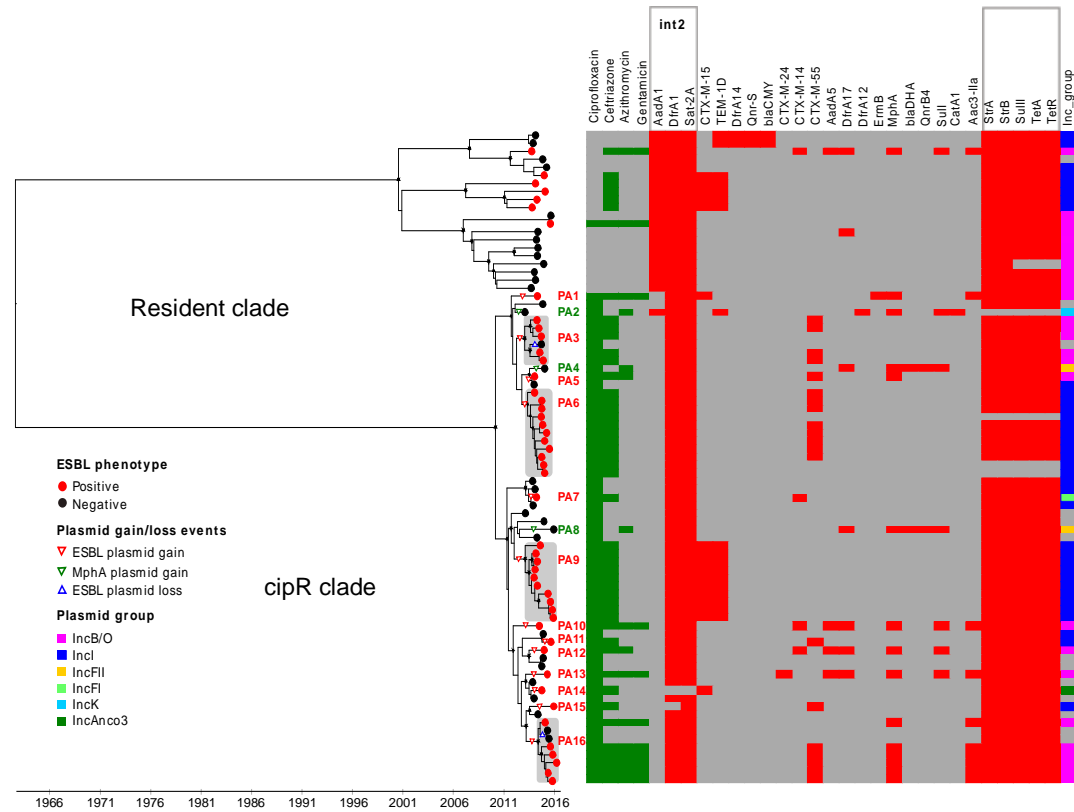
810 None

811

812 **Competing interests**

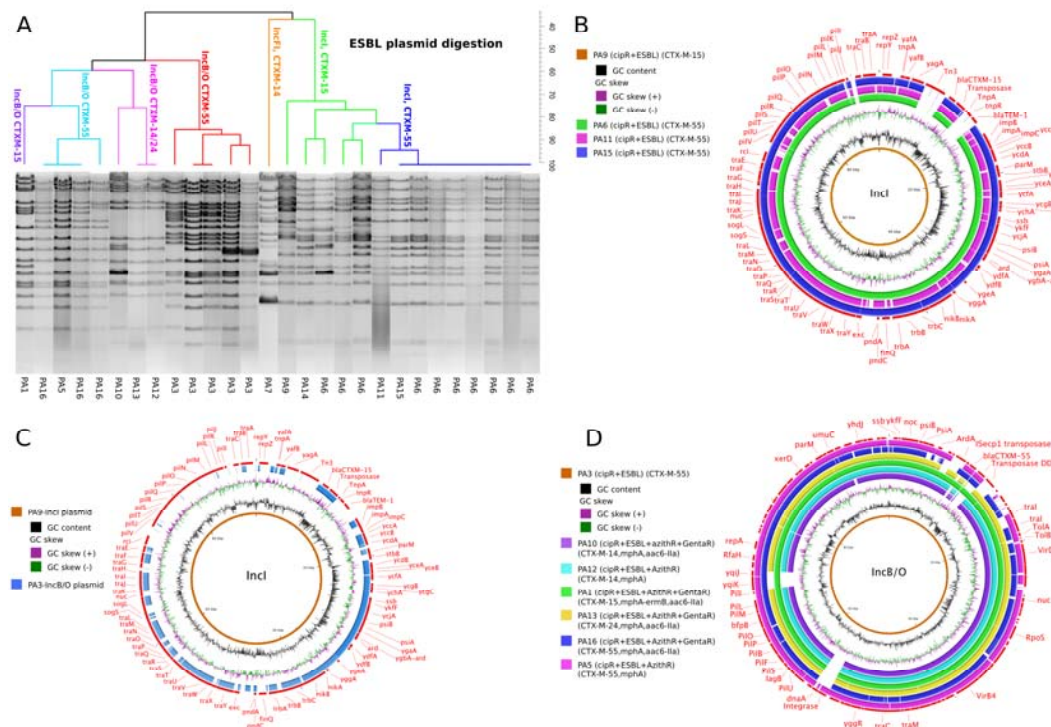
813 None





**Figure 1.** The temporal phylogenetic structure of *Shigella sonnei* in Vietnam, 2014 and 2016

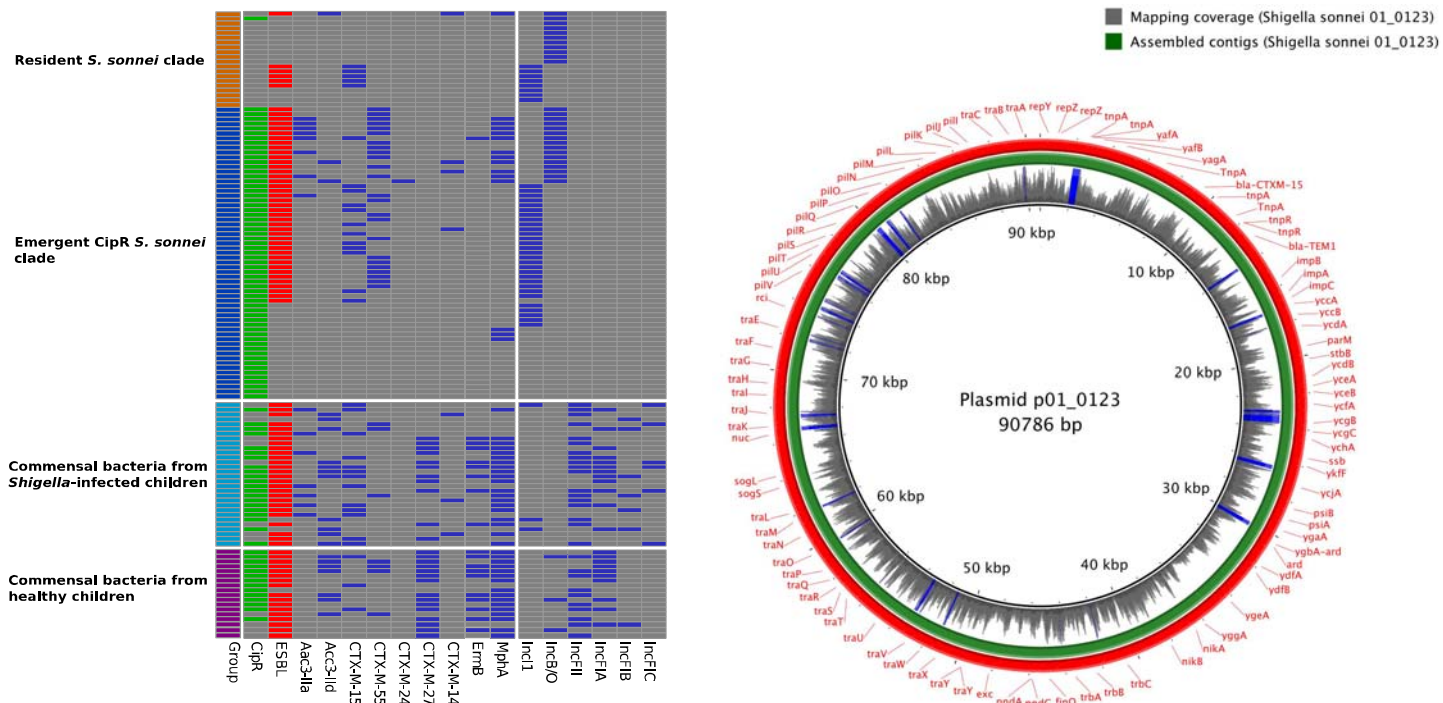
Maximum clade credibility phylogeny showing two distinct clades corresponding to the resident and the cipR *S. sonnei* populations. The black asterisks indicate posterior probability support  $\geq 70\%$  on internal nodes. The red circles at terminal leaves highlight the ESBL-positive isolates. Triangles indicate plasmid gain/loss events. Sixteen plasmid acquisitions (PAs) were reconstructed across the tree and designated as PA1-PA16. The columns on the right correspond to: the resistance phenotype to key antimicrobials (green), the presence of AMR genes (red), and the presence of different plasmid groups (multiple colors), respectively.

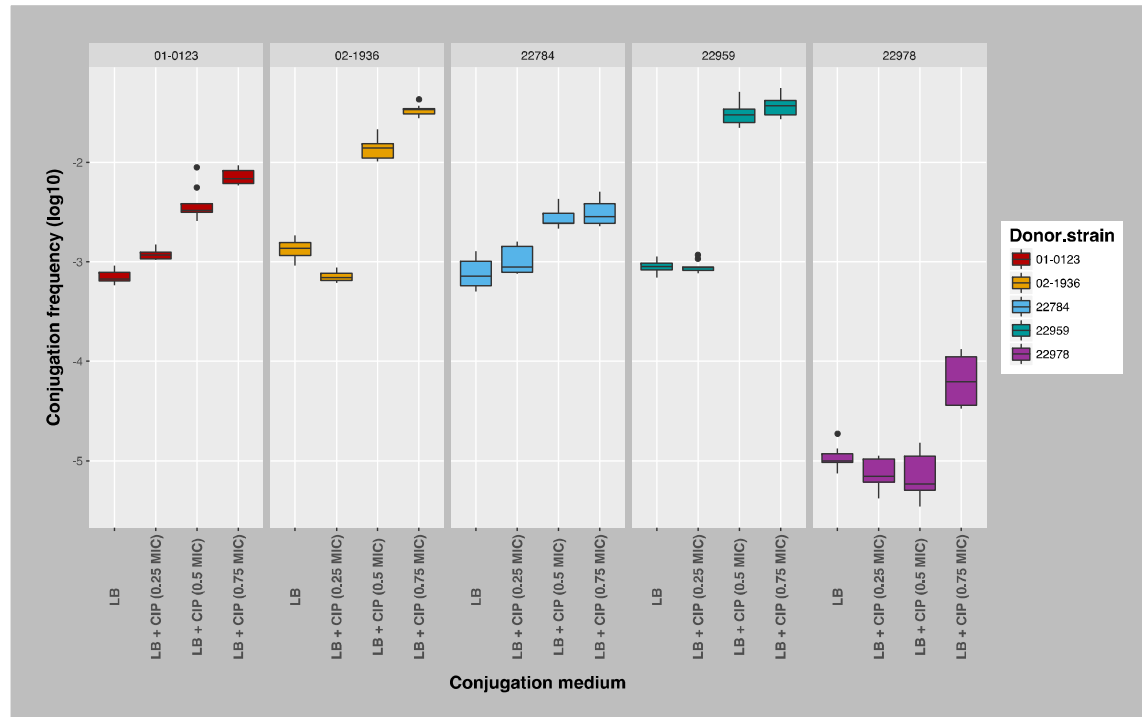


**Figure 2.** The Diversity of ESBL-encoding plasmids in ciprofloxacin-resistant *Shigella sonnei*

A) Dendrogram showing the similarities in restriction digestion patterns of ESBL-encoding plasmids associated with independent plasmid acquisitions.

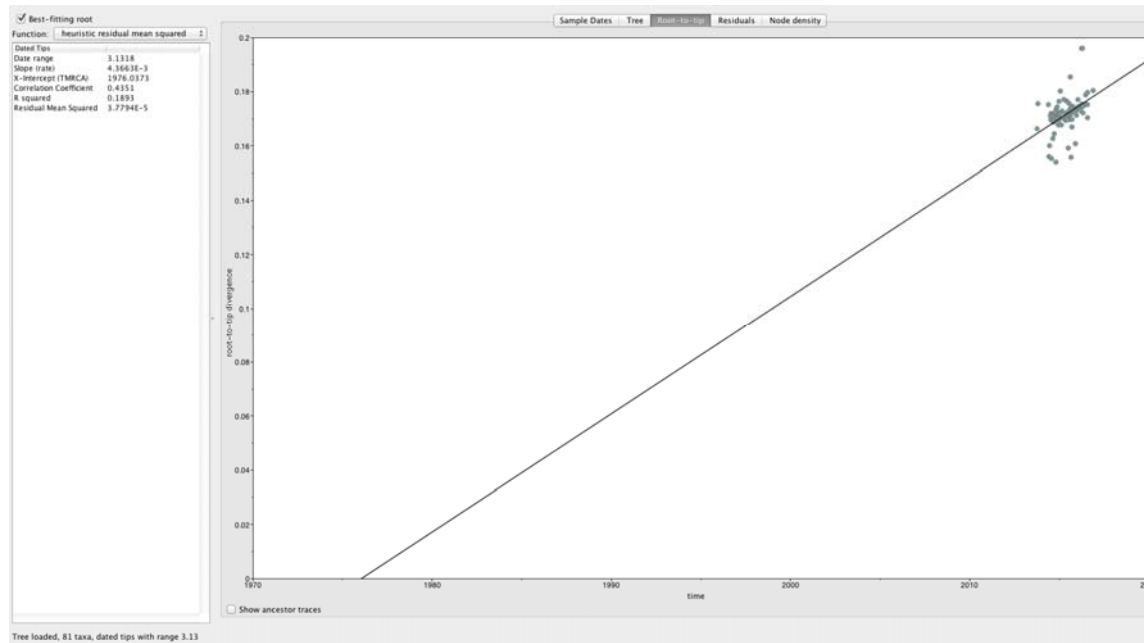
B) BLAST comparisons of IncI plasmids associated with four independent acquisitions (PA6, 9, 11, 15). The central circle is the full reference sequence of the IncI plasmid associated with PA9, with similarity between the reference sequence and other IncI plasmids shown as concentric rings. C) BLASTN comparison between IncI and IncB/O plasmid structures, in which the central circle is the IncI plasmid (PA9). D) BLAST comparisons of IncB/O plasmids associated with seven independent acquisitions (PA1, 3, 5, 10, 12, 13, 16). The central circle is the full reference sequence of the IncB/O plasmid associated with PA3, with similarity between the reference sequence and other IncB/O plasmids shown as concentric rings.





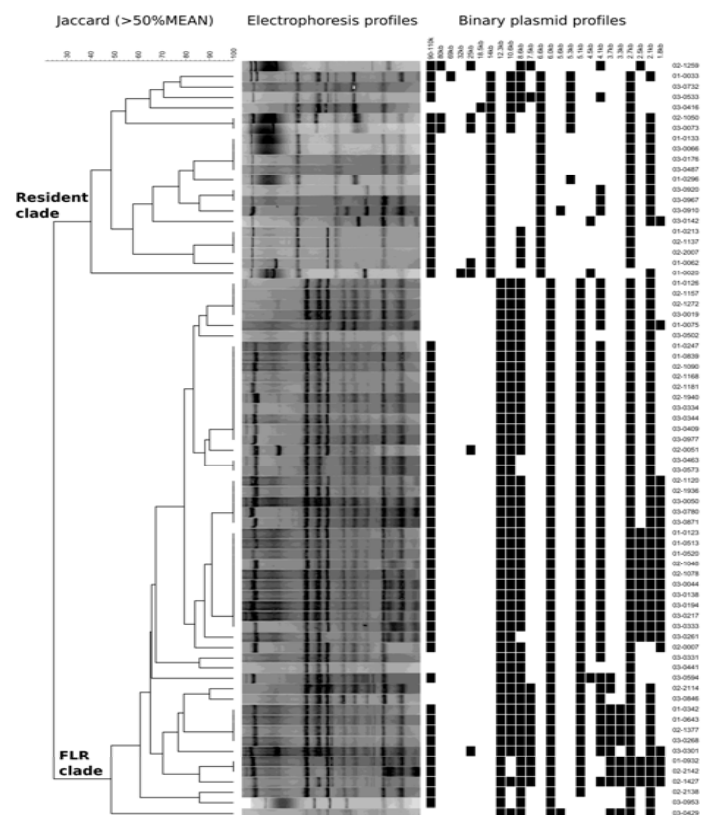
**Figure 4.** The Conjugation efficiency of ESBL-encoding plasmids from human commensal *E. coli* to ciprofloxacin-resistant *Shigella sonnei* with and without supplementation with ciprofloxacin

Boxplots showing the conjugation frequencies (log<sub>10</sub>) between five commensal *E. coli* isolates and cipR *S. sonnei* strain 03-0520 with and without supplementing conjugation media with 0.25x, 0.5x, and 0.75x MIC of the donor cipS commensal *E. coli*. Each conjugation experiment was performed in triplicate in each condition.



**Supplementary Figure 1.** Root-to-tip regression for the maximum likelihood tree of *Shigella sonnei* in Vietnam, 2014 and 2016

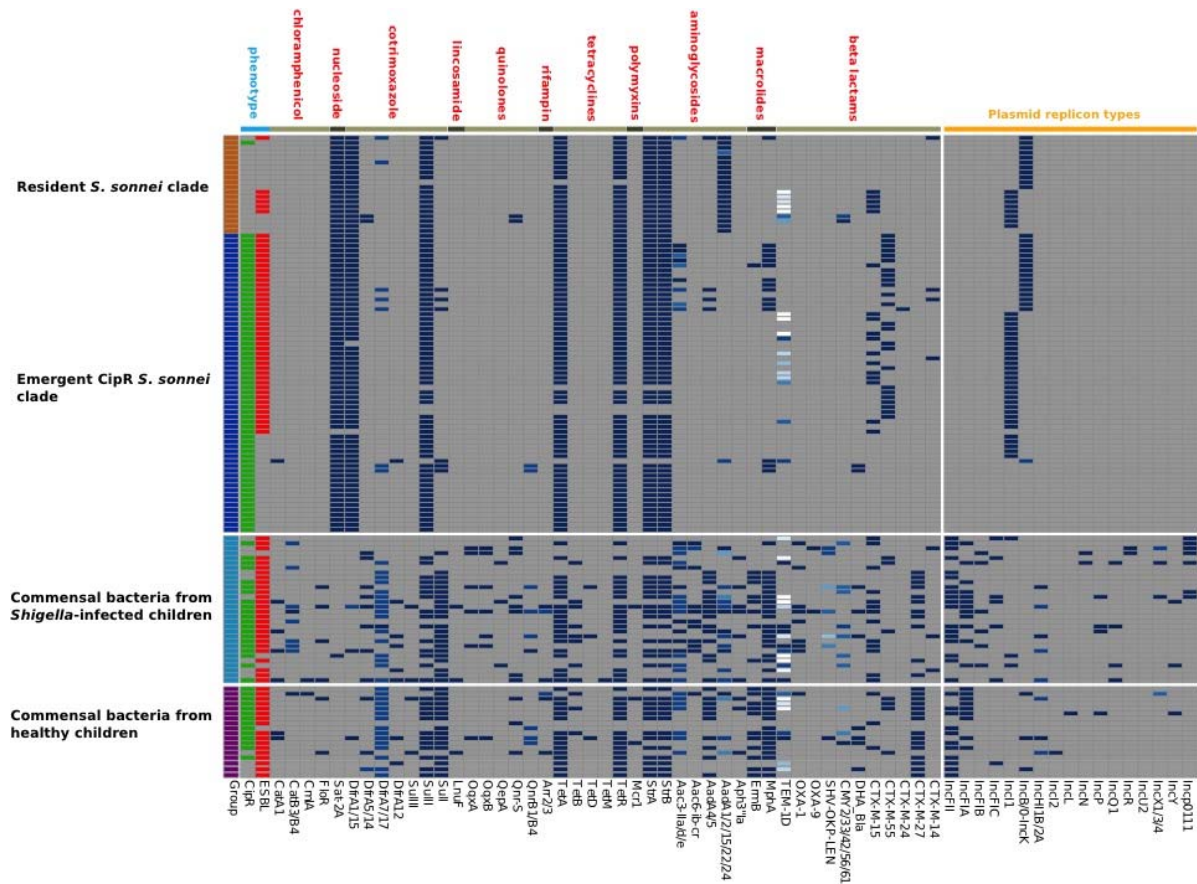
Each point on the plot corresponds to a measurement of genetic distance from the inferred root to each tip in the tree. The solid line is the regression line fitted using the ordinary least squares method. The slope of the line is a crude estimate of the evolutionary rate, the x-intercept corresponds to the time to the most recent common ancestor, and the  $R^2$  value measures the degree of clock-like behavior.



**Supplementary Figure 2.** Plasmid profiling of *Shigella sonnei* in Vietnam, 2014 and 2016

Dendrogram shows the difference in plasmid electrophoresis patterns between the resident *S. sonnei* clade and the cipR *S. sonnei* clade in Vietnam. Cluster analysis was performed with Bionumerics by using the Jaccard coefficient and the unweighted pair group mathematical average (UPGMA) clustering algorithm.





**Supplementary Figure 3.** Distribution of antimicrobial resistance genes and plasmid groups in *Shigella sonnei* and human commensal bacteria

The first column highlights the four different isolate types in different colors. Fecal/rectal swab cultures with ciprofloxacin-resistant and ESBL-producing isolates are highlighted in green and red (second and third columns, respectively). The remaining columns show the distribution of all antimicrobial resistance genes and plasmid groups identified in commensal bacteria and *S. sonnei*. AMR genes are grouped together based on the class of antimicrobial agents to which they are resistant, with different variants of an antimicrobial resistant gene shown in different shades of blue.