

1 **Rapid Increase of CTX-M-Producing *Shigella sonnei* Isolates in Switzerland:**
2 **Spread of Common Plasmids and International Clones**

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26 **ABSTRACT**

27 The Swiss Centre for Antibiotic Resistance (ANRESIS) has recently noted an increase of
28 extended-spectrum cephalosporin-resistant (ESC-R) *S. sonnei* isolates nationwide (3.8% in
29 2016 vs. 37.5% in 2019). To understand this phenomenon, we analyzed 25 representative
30 isolates (of which 14 ESC-R) collected in Switzerland during 2016-2019. Whole-genome
31 sequencing was achieved using both Illumina and Nanopore platforms. Both ESC-R and
32 susceptible isolates belonged to ST152. The ESC-R isolates carried *bla*_{CTX-M-3} in IncI1-pST57
33 (n=5), *bla*_{CTX-M-15} in IncFII (F2:A-:B-) (n=5), *bla*_{CTX-M-15} in IncI1-pST16, and *bla*_{CTX-M-27},
34 *bla*_{CTX-M-55}, or *bla*_{CTX-M-134} in other IncFII plasmids (n=1 each). Plasmids having the same *bla*
35 and Inc group exhibited high genetic identity to each other, but also to plasmids previously
36 reported in other Enterobacterales. Core-genome analysis showed that there were 4 main
37 clusters, each of which included strains that differed by <58 SNVs, both *bla*_{CTX-M}-positive and
38 *bla*_{CTX-M}-negative isolates. Moreover, most isolates belonging to the same cluster shared an
39 identical cgST. For instance, cluster-1 included 4 isolates of cgST113036, of which only 3
40 harbored the IncI1-pST57 *bla*_{CTX-M-3}-positive plasmid. The 25 *S. sonnei* isolates were also
41 subjected to phylogenetic comparison with deposited international strains. As a result,
42 matching isolates (same cgST and differing by <8 SNVs) have been reported in the UK, USA,
43 France, and the Netherlands. Overall, our results suggest that some common *S. sonnei* clusters
44 can spread between continents and can be imported into other nations after international trips.
45 Such clusters include, in part, isolates that do not possess *bla*_{ESBL}-harboring plasmids,
46 indicating their tendency to acquire them from other Enterobacterales.

47 **INTRODUCTION**

48 *Shigella flexneri* is one of the most common causes of diarrhea in low-/middle-income
49 countries and is associated with high morbidity and mortality rates. In contrast, *Shigella*
50 *sonnei* is the leading species in high-income nations with the majority of cases described in
51 returning travelers, men who have sex with men (MSM), and young children (1-3).

52 The emergence of antibiotic-resistant *S. sonnei* isolates is nowadays a matter of concern
53 (4). The high resistance rates to first-line options (e.g., ciprofloxacin and azithromycin) have
54 made ceftriaxone the drug of choice for empirical treatment. However, there has also been a
55 significant recent increase in extended-spectrum cephalosporin-resistant (ESC-R) isolates,
56 especially in Asia (1, 5).

57 Usually, ESC-R *S. sonnei* (ESC-R-*Ss*) isolates produce extended-spectrum β -lactamases
58 (ESBL) of the CTX-M-type, of which CTX-M-3, CTX-M-14, CTX-M-15, CTX-M-27, and
59 CTX-M-55 are the most common (6). However, only a few studies have implemented whole-
60 genome sequencing (WGS) to characterize the *bla*_{CTX-M}-carrying plasmids in detail. So far, a
61 *bla*_{CTX-M-3}- IncI1 from Italy (7), a *bla*_{CTX-M-14}- IncB/O/K/Z from China (8), a *bla*_{CTX-M-15}- IncI1
62 from South Korea (9), a *bla*_{CTX-M-27}- IncFII from the UK (10), and a *bla*_{CTX-M-55}-harboring
63 IncI2 plasmid from China have been described in *S. sonnei* (11). For Switzerland, we note
64 that the first two ESC-R-*Ss* strains (CTX-M-14 and CTX-M-15 producers) were isolated in
65 2009, but no WGS analyses were performed on the 8 strains detected during 2009-2014 (12).

66 Due to the ability of *S. sonnei* to acquire multidrug-resistant (MDR) plasmids and the fact
67 that it shows a higher prevalence in industrialized countries compared to *S. flexneri* (3),
68 attention needs to be focused on the clonality of ESC-R-*Ss*. Just recently (2018), in the
69 European Union, 17 outbreaks due to *S. sonnei* have been documented (13).

70 Several authors have implemented the multilocus sequence typing (MLST), which has
71 revealed that sequence type (ST) 152 is the most frequent lineage among ESC-susceptible *S.*

72 *sonnei* (ESC-S-*Ss*) isolates (14-19). Other recent studies have also used core-genome analyses
73 to investigate epidemiological events (2, 15, 20, 21), although only one UK survey analyzed
74 exclusively ESBL-producing *S. sonnei* strains (10). Overall, these studies have shown that
75 single nucleotide variant (SNV) analysis represents a high-resolution tool for determining
76 clonality and tracking outbreaks at the community and global levels.

77 In this study, therefore, we used WGS to characterize the plasmids of ESC-R-*Ss* isolates
78 detected in Switzerland. Moreover, to investigate the hidden epidemiological profile of
79 contemporary circulating isolates, we implemented a core-genome analysis to determine the
80 clonality of ESC-R- and ESC-S-*Ss* strains.

81 **RESULTS AND DISCUSSION**

82 **Rate of ESC-R-Ss and analyzed strains.** According to the ANRESIS database, 53, 39, 85,
83 and 56 *S. sonnei* isolates were identified nationwide in 2016, 2017, 2018 and 2019 by
84 participating laboratories, respectively. Of them, 2 (3.8%), 5 (12.8%), 12 (14.1%) and 21
85 (37.5%) were reported as ESC-R, respectively.

86 Unfortunately, such results could not be compared to those of other countries. In fact,
87 though the spread of ESC-R *Shigella* spp. is of concern, recent studies analyzing their trends
88 are lacking (1, 4). Nevertheless, we note that in China the rate of ESC-R-Ss increased from
89 31.6% in 2012 to 64.3% in 2015 (22), whereas lower rates were recorded in other nations
90 during point-prevalence surveys (e.g., in 2015, 12% in England/Wales and 0% in Nepal; in
91 2015-2016, 7.1% in New Zealand) (10, 23, 24). It is therefore difficult to interpret our data on
92 the persistent increase in resistance to ESCs. In this context, we emphasize that the Swiss
93 population is at greater risk of acquiring and importing MDR *Shigella* spp. from endemic
94 areas due to its high propensity for international travel (12, 25). For this reason, in order to
95 better understand this general epidemiological phenomenon, a molecular characterization of
96 the strains is essential.

97 In the present study, we analyzed 14 ESC-R-Ss and 11 ESC-S-Ss collected in Switzerland
98 during 2016-2019. Species identification (ID) and antibiotic resistance phenotypes of all
99 strains were confirmed by appropriate methods before further molecular analyses (see
100 Material and Methods section and Tab. S1).

101 **Antimicrobial resistance genes (ARGs).** As shown in Tab. 1, both ESC-R- and ESC-S-Ss
102 isolates carried numerous ARGs conferring resistance to different classes of antibiotics,
103 including quinolones (e.g., *qnrS1*) and macrolides [e.g., *erm(B)* and *mph(A)*] (6). Those
104 phenotypically resistant to ESCs mainly possessed *bla*_{CTX-M-3} (n=5) or *bla*_{CTX-M-15} (n=6) ESBL

105 genes, but unique isolates harboring *bla*_{CTX-M-27}, *bla*_{CTX-M-55} and *bla*_{CTX-M-134} were also
106 detected.

107 Although studies analyzing the prevalence of specific *bla*_{ESBLs} in *S. sonnei* are lacking, *bla*_{CTX-}
108 _{M-15} and *bla*_{CTX-M-3} appear to be the most frequent worldwide (6). In particular, CTX-M-3
109 producers were described in Turkey, Switzerland and Italy (7, 12, 26, 27), while those with
110 CTX-M-15 have been found in various countries, including South Korea, where an outbreak
111 was described (9). With regard to the other ESBLs, a CTX-M-27-producing *S. sonnei* clone
112 was responsible for an outbreak in 2015 among MSM in England (10), CTX-M-55 was
113 reported in *S. sonnei* isolates from China and South Korea (11, 28), while CTX-M-134 was
114 only recently described in *E. coli* (29).

115 Since *bla*_{CTX-Ms} are usually carried by plasmids that can be exchanged between different
116 species of enterobacteria (e.g., from *E. coli* to *S. sonnei* in the human gut) (30), their
117 characterization is crucial for understanding the expansion of ESC-R-Ss isolates.

118 ***IncII bla*_{CTX-M-3}-*carrying plasmids.*** As shown in [Fig. 1A](#), 5 *S. sonnei* isolates harbored 86-
119 87kb IncII-pST57 *bla*_{CTX-M-3} carrying plasmids with a high genetic identity to each other and
120 to the Italian pLC1477_18_1 that we recently described (conjugation frequency, 1.2×10^{-4})
121 (7).

122 In all of these plasmids, *bla*_{CTX-M-3} was associated with a truncated *ISEcpI* in the same
123 element reported in the Italian plasmid. Considering the strong genetic similarity of the five
124 IncII-pST57 *bla*_{CTX-M-3} carrying plasmids collected in Switzerland, it is quite possible that
125 other plasmids with similar genetic characteristics exist outside the country. For instance, an
126 Enterobase *S. sonnei* strain from the UK in 2019 (named 811053; BioSample:
127 [SAMN12881824](#)) of the same cgST as our 509-1022 and 19-0822-3296 isolates
128 (cgST115537) was found to harbor *bla*_{CTX-M-3} and contained at least one pST57 plasmid (CGE
129 analysis; data not shown). Similarly, an *E. coli* strain from a 2013-2015 study in the

130 Netherlands was reported to be carrying an IncII-pST57 plasmid that possessed *bla*_{CTX-M-3}
131 (31).

132 ***IncFII bla*_{CTX-M-15}-harboring plasmids.** As depicted in [Fig. 1B](#), 5 other ESC-R-*Ss* harbored
133 IncFII (F2:A-:B-) *bla*_{CTX-M-15}-carrying plasmids (83-89kb) with high genetic identity to each
134 other and to pF93-2_1 (GenBank: [CP026158](#)) from *K. pneumoniae* found in China in 2014.
135 Globally, the F2: A-: B- is the predominant F plasmid type carrying *bla*_{CTX-Ms} among
136 Enterobacteriaceae and is highly conjugative (32).

137 The 5 *bla*_{CTX-M-15}-carrying plasmids identified in the present work shared an identical genetic
138 environment around *bla*_{CTX-M-15}, including both full (IS26 and IS*Kpn19*) and partial (Δ Tn3
139 and Δ IS3) transposable element coding sequences (CDS), along with the *qnrS1* ([Fig. 1B](#)). The
140 plasmid p19-0820-1561 also contained additional ARGs [*mph(A)*, *sul1*, *aadA5*, and *dfrA17*]
141 that were only present in pF93-2_1 in the form of the IS26-*mph(A)*-MFS transporter-
142 *tetR/acrR*-IS6100-*sul1-aadA5-dfrA17-int11* Δ -IS26-Tn3 Δ -IS1R Δ unit. This element has been
143 reported in multiple species such as *E. coli* and *K. pneumoniae* (BLAST analysis; data not
144 shown).

145 ***Other bla*_{CTX-M}-carrying plasmids.** The remaining 4 ESC-R-*Ss* isolates possessed unique
146 *bla*_{CTX-M}-positive plasmids ([Tab. 1](#)). In particular, a *bla*_{CTX-M-15} associated with IS*Ecp1* was
147 carried in an 89kb IncII-pST16 plasmid (p6607-69), but without further ARGs. This plasmid
148 showed a high identity with others found in both *S. sonnei* and *E. coli* isolates (mostly from
149 Asia), including some expressing the CTX-M-55 that is a single amino acid variant of CTX-
150 M-15 ([Fig. S1](#)). Likewise, one of our *S. sonnei* isolates carried the *bla*_{CTX-M-55}, but in this case
151 the gene was located in a 74kb IncFII (F2:A-:B-) plasmid (p0401952027) and was flanked by
152 two IS26. This plasmid showed a high genetic identity with others possessing *bla*_{CTX-M-55} or
153 *bla*_{CTX-M-15} that came from *E. coli* or *K. pneumoniae* isolates detected in Europe or North

154 America, but, interestingly, none of them co-carried the *tetR/acrR*-MFS trans-*mph(A)* unit
155 between *IS6100* and *IS1RA* (Fig. S2).

156 Another ESC-R-*Ss* carried a 67kb IncFII (F2:A-:B-) plasmid (p09163633) that harbored
157 only *bla*_{CTX-M-27} and showed a high identity with the backbone of plasmids from *E. coli* and *S.*
158 *flexneri* isolates. Nevertheless, only p09163633 possessed the *IS26-IS903BΔ-bla*_{CTX-M-27}-
159 *ISEcp1Δ-IS26* unit (Fig. S3), which has been reported in multiple *E. coli* and *K. pneumoniae*
160 isolates from Vietnam and China (BLAST analysis; data not shown), and also described in a
161 Japanese epidemic ST131 *E. coli* (33). Moreover, it was also present in the 69kb IncFII
162 (F35:A-:B-) plasmid (p3123885) found in our last ESC-R-*Ss* (Fig. S4), though this mobile-
163 genetic element (MGE) encoded the single amino acid variant CTX-M-134 instead of the
164 CTX-M-27 (29).

165 **Co-resistance to azithromycin.** Besides the specific ESBLs identified, 6 of the 25 *S. sonnei*
166 isolates were macrolide-resistant due to the presence of *erm(B)* and/or *mph(A)* ARGs (Tab. 1
167 and Tab. S1). As mentioned above, 3 ESC-R isolates carried *mph(A)* in different IncFII
168 plasmids co-harboring *bla*_{CTX-M-15}, *bla*_{CTX-M-55}, or *bla*_{CTX-M-134}. Of note, two of these plasmids
169 carried the element *IS26-*mph(A)*-MFS trans-*tetR/acrR-IS6100** (Fig. 1B and S4) and the other
170 one carried, with a slightly different arrangement, *IS6100-*tetR/acrR*-MFS trans-*mph(A)*-*
171 *IS1RA* (Fig. S2). These two very similar elements have been found in many plasmids carried
172 by *E. coli*, *K. pneumoniae*, *Salmonella enterica* (BLAST analysis; data not shown), and also
173 identified in the chromosome of a CTX-M-15-producing *Salmonella* Haardt isolated from
174 Japanese food workers (34).

175 Overall, these findings are epidemiologically relevant, since co-resistance to
176 azithromycin and ESCs makes the treatment of shigellosis difficult (1, 5). Such MDR
177 plasmids have been rarely reported in *S. sonnei*, though an IncFII (F2:A-:B-) plasmid
178 possessing *bla*_{CTX-M-27}, *mph(A)*, and *erm(B)* was associated with the outbreak among MSM in

179 England (10), while an IncB/O/K/Z co-harboring *bla*_{CTX-M-14} and *mph(A)* was linked to a
180 waterborne outbreak in China in 2015 (8). Having observed that at least three of our IncFII
181 plasmids carried very similar macrolide resistance elements, we speculate that under a certain
182 antibiotic selective pressure (e.g., azithromycin), *mph(A)* can be acquired *via* integration of
183 transposable elements (e.g., IS26-*mph(A)*-MFS trans- *tetR/acrR-IS6100*) (35).

184 **MLST and cgMLST.** Regardless of the presence of *bla*_{CTX-M} genes, 24 *S. sonnei* isolates were
185 of ST152, while one was of its single allele variant ST1503 (Tab. 1). ST152 has been
186 previously reported in ESC-S-Ss in many countries (e.g., California, China, Germany and
187 Iran) (15-19). Recently, we also described the ST152 CTX-M-3-producing strain LC-1477-18
188 isolated in Italy from a girl who acquired the infection in Albania (7). Overall, since our *S.*
189 *sonnei* isolates were acquired in different periods and/or in diverse geographic areas (Tab. 1),
190 one could speculate that a unique clone (ST152) is spreading worldwide.

191 To better investigate the clonality of our *S. sonnei* isolates, we performed a cgMLST
192 analysis according to the *E. coli* scheme. The higher resolution of cgMLST resulted in
193 multiple cgSTs: *i*) three *bla*_{CTX-M-3}-possessing isolates and one ESC-S of cgST113036; *ii*) two
194 *bla*_{CTX-M-3}-positives of cgST115537; *iii*) four *bla*_{CTX-M-15}-harboring of cgST112958; and *iv*)
195 two ESC-S isolates carrying *mph(A)* and *erm(B)* were of cgST107674. The remaining isolates
196 showed different cgSTs, but overall maintaining high allele matches among the 2513
197 analyzed (i.e., >98.5% for ESBL producers and >98.6% for those ESC-S) (Tab. 1). These
198 results support the hypothesis that common ESC-R clones may spread in different countries
199 and could be imported to other nations (e.g., Switzerland) after international trips. Based on
200 the identification of clones including both ESBL producers and not producers, it can be also
201 speculated that some ESC-S-Ss may be well-predisposed to acquire MDR plasmids from
202 other Enterobacterales.

203 **Core-genome analyses.** To determine the distance and clonality between our *S. sonnei* strains,
204 a high-resolution SNV analysis was performed (87% core-genome coverage among all
205 strains). As shown in [Fig. 2](#), the 4 *S. sonnei* clones identified by cgMLST were also confirmed
206 as 4 independent SNV clusters, though several additional isolates were grouped within
207 cluster-2 and cluster-4. To summarize: cluster-1 and cluster-2 included CTX-M-3-producing
208 or ESC-S-*Ss*; cluster-3 encompassed CTX-M-15 producers; and cluster-4 included the CTX-
209 M-134 producer and ESC-S-*Ss*. Notably, strains belonging to the same cluster differed by
210 only a limited number of SNVs (i.e., cluster-1: $\Delta=7-26$ SNVs; cluster-2: $\Delta=12-34$ SNVs;
211 cluster-3: $\Delta=5-19$ SNVs; and cluster-4: $\Delta=2-58$ SNVs).

212 Together, these results corroborate the above hypothesis on the dissemination of CTX-M-
213 producing hyperepidemic *S. sonnei* clones. This is consistent with what has been observed by
214 other authors for the fluoroquinolone-resistant international clones (e.g., the global lineage III,
215 GIII) (36, 37). However, our data also indicate that MLST analysis alone has a limited
216 resolution for studying the spread of such MDR pathogens. In fact, although almost all of our
217 *S. sonnei* isolates were identified as ST152, several clusters with different ARG and plasmid
218 patterns could be differentiated using cgMLST and/or core-genome SNV analyses.

219 We also note that isolates included in cluster-1 and cluster-2 carried the same IncII-
220 pST57 *bla*_{CTX-M-3} plasmid ([Fig. 1](#)). This finding was surprising, as the two bacterial groups
221 were genetically different (i.e., $\Delta=235$ SNVs; [Fig. 2](#)). We do not have a clear explanation for
222 the independent clustering, but it can be hypothesized that ESC-S *S. sonnei* isolates belonging
223 to cluster-1 and cluster-2 acquired the pST57 plasmid from a common enterobacterial
224 ancestor, including other *Shigella* spp.

225 **Link with international isolates.** To explore the lineage origins of our Swiss *S. sonnei*
226 isolates, we performed a database search in Enterobase for strains of global lineage and
227 matching cgST. A core-genome analysis was then performed using the results of the

228 Enterobase phylogenetic analysis as reference. Overall, we compared our collection (n=25) to
229 a subset of 114 strains of global lineage (38), 16 of matching cgST, and the Italian LC-1477-
230 18 isolate (7). The alignment of all strains (n=156) resulted in 4551 SNVs and 42% core-
231 genome coverage among all isolates.

232 As depicted in Fig. 3 and Tab. S2, all *S. sonnei* isolates included in cluster-1 were related
233 to an Iranian GIII strain detected in 2003, and were almost identical to another one found in
234 the UK in 2016 (same cgST and $\Delta=2-3$ SNVs). We also note that one of our isolates (7111-
235 69) had its clinical origin in Turkey (Tab. 1), the area where the first CTX-M-3-producing *S.*
236 *sonnei* was described (2001) and subsequently caused epidemic events (26, 27).

237 The cluster-2 and cluster-3 isolates were part of a large group shared by two GIII strains
238 of Egyptian origin reported in 2005-2006, LC-1477-18, and four detected in the UK: one
239 CTX-M-3 producer isolated in 2019, two CTX-M-15 producers detected in 2015-2016, and
240 one ESC-S found in 2015 (Fig. 3). These UK strains showed ≤ 5 Δ SNVs when compared to
241 cgST- matching isolates from Switzerland, indicating their commonality. Further evidence of
242 their possible origin could be seen in isolates 6105-15, 0401930105, 19-0821-3486, and
243 0401952027 where Egypt was their origin (Tab. 1), suggesting that these strains may have
244 originated in that geographic area.

245 *S. sonnei* cluster-4 isolates were grouped with recently detected ESC-S strains in the UK,
246 the USA, and France. Two additional CTX-M-15 producers, one from the UK (821179) and
247 one from the Netherlands (IBESS820), were also highly related with the cluster-4 isolates
248 (Fig. 3). The latter was identified in 2017 during a cross-sectional multicenter study (39), and
249 was genetically identical to our ESC-S-*Ss* 6412-75 strain (same cgST and $\Delta=0$ SNPs). In that
250 study, the patient of strain IBESS820 was reported to have a history of travel to India, as was
251 the Swiss patient with an infection caused by 6412-75 (Tab. 1). Despite these similarities, the

252 Swiss *S. sonnei* was ESBL-negative; nevertheless, this finding highlights the great capacity of
253 certain clones to acquire *bla*_{CTX-M}-harboring plasmids.

254 In total, 12 of the 16 international *S. sonnei* strains that have the same cgST as our
255 Swiss isolates were detected in the UK, while the remaining 4 were isolated in the USA,
256 France, and the Netherlands. This indicates that common *S. sonnei* lineages have been
257 circulating in Europe at least since 2015, and are now expanding in Switzerland. In fact, we
258 note that all 10 Swiss ESC-R-*Ss* detected in 2019 were linked to isolates detected in the same
259 year in the UK and France (Tab. 1 and Fig. 3), most of which were producers of CTX-M-3 or
260 CTX-M-15, as in the case of those in Switzerland.

261 It can be speculated that the same common plasmids described in the present work (e.g., the
262 Inc11-pST57 *bla*_{CTX-M-3}-positive) are also carried by contemporary non-Swiss ESC-R-*Ss*
263 isolates (as demonstrated for LC-1477-18). However, since the matching cgST isolates
264 identified from the Enterobase database are in the form of whole genome shotgun assemblies
265 generated from short-read data, without the full characterization of *bla*_{CTX-M}-carrying
266 plasmids with long-read sequencing data as in our study, this hypothesis cannot be fully
267 corroborated.

268 **Conclusions.** In this work, we presented the first detailed molecular investigation of *S. sonnei*
269 isolates detected in Switzerland. Hybrid WGS assemblies were implemented to accurately
270 describe the *bla*_{CTX-M}-harboring plasmids, while core-genome and phylogenetic analyses were
271 used to study the clonality of the strains.

272 Based on our results, we conclude that most of the contemporary Swiss ESBL-producing
273 *S. sonnei* isolates carry identical *bla*_{CTX-M}-positive plasmids that often have their counterparts
274 in other reported Enterobacterales worldwide. More importantly, due to transnational travel,
275 common international clones of MDR *S. sonnei* are emerging in Switzerland and this limits
276 our therapeutic armamentarium. Overall, our findings underline the importance of

277 continuously conducting epidemiological surveys using the WGS approach and linking the
278 results with other countries (40).

279 **MATERIALS AND METHODS**

280 **Epidemiological data.** Phenotypic data regarding the *S. sonnei* isolates detected in
281 Switzerland during 2016-2019 were retrieved from the Swiss Centre for Antibiotic Resistance
282 (ANRESIS) database (<http://www.anresis.ch/>) that collects information from 30 Swiss clinical
283 laboratories. Strains were categorized as ESC-R when non-susceptible (i.e., intermediate or
284 resistant) to ceftazidime, ceftriaxone, and/or cefepime according to the criteria implemented
285 for Enterobacterales by the routine clinical laboratories during the corresponding years. The
286 research project was exempted from the requirement for ethical approval because no health-
287 related personal data were used, while age, gender, and trip-related information (if available)
288 were retrieved from the laboratory databases.

289 **Strains, ID and antimicrobial susceptibility tests.** All ESC-R- and ESC-S-Ss isolates
290 available at -80°C and collected during 2016-2019 at the Institute for Infectious Diseases,
291 MCL Medizinische Laboratorien, and labormedizinisches zentrum Dr. Risch were analyzed.
292 The initial ID obtained by implementing the MALDI-TOF MS (Bruker) was confirmed with
293 the Type Strain Genome Server (TYGS) tools using Genome BLAST Distance Phylogeny
294 (<https://tygs.dsmz.de/>) based on genome data (see below). MICs were obtained by
295 implementing the Sensititre GNX2F microdilution panels (ThermoFisher). For isolates
296 possessing *erm(B)* and/or *mph(A)* genes, MICs for azithromycin were obtained using the Etest
297 (bioMérieux). Results were interpreted according to the EUCAST 2019 criteria (41).

298 **Whole-genome sequencing (WGS).** WGS was performed using both NovaSeq-6000
299 (Illumina) and MinION (Oxford Nanopore) sequencing platforms as previously described
300 (42-45). In brief, Illumina raw reads were quality-filtered with Trimmomatic (v0.36),
301 followed by whole-genome shotgun assembly with SPAdes (v3.12.0). Adaptors from
302 Nanopore raw reads were trimmed with Porechop (v0.2.4), and quality filtered with Filtrlong
303 (v0.2.0). Long-read assemblies were done with Canu (v1.7). The final hybrid assemblies were

304 generated by aligning the paired-end Illumina reads to the Canu assemblies with Bowtie2
305 (v2.3.4.1), and followed by multiple rounds of polishing with Pilon (v1.22).
306 Illumina SPAdes assemblies were used for: whole genome ID, analysis with the tools of the
307 Center for Genomic Epidemiology (CGE; <http://www.genomicepidemiology.org/>):
308 ResFinder, MLST with the *E. coli* scheme #1, PlasmidFinder, and pMLST. Hybrid assemblies
309 were used to characterize the *bla*_{ESBL}-carrying plasmids.
310 Annotations of both Illumina and hybrid assemblies were carried out by the NCBI
311 Prokaryotic Genome Annotation Pipeline. All annotated features presented in [Fig. 1](#) and [Fig.](#)
312 [S1-S4](#) were manually curated with UniProt (<https://www.uniprot.org/blast/>) and ISfinder
313 (<https://isfinder.biotoul.fr/>), and annotated accordingly.
314 **Core-genome analyses.** All *S. sonnei* isolates underwent cgMLST with CGE cgMLSTFinder
315 (v1.1) using Illumina raw reads as input and species' database set to *E. coli* Enterobase. These
316 isolates also undertook core-genome SNV analysis as previously done (46). Briefly, the core-
317 genome alignment was performed with Parsnp v1.2. All strains were treated as curated
318 genomes (-c parameter), and the Italian ST152 CTX-M-3-producing *S. sonnei* strain (LC-
319 1477-18; GenBank: [JAATWD000000000](https://www.ncbi.nlm.nih.gov/nuccore/JAATWD000000000)) was used as reference (7). The -C parameter was
320 set to 200, and other parameters were let as default. Variants with no flags (PASS) were
321 determined as reliable, and used for downstream SNV analysis with a custom R (v3.6.2)
322 script. The Parsnp-generated core-genome SNV phylogenetic tree was visualized with
323 FigTree (v1.4.4), and set to midpoint-rooted, and nodes by decreasing order ([Fig. 2](#)).

324 A SNV tree dendrogram of the Swiss *S. sonnei* collection vs. global lineage and
325 matching cgST strains was created in the Enterobase *Escherichia/Shigella* database
326 (<https://enterobase.warwick.ac.uk/species/index/ecoli>) ([Fig. 3](#)). The analyzed strains consisted
327 of 156 total strains of which 114 were of global lineage (38), 16 of matching cgST, 25 from
328 Switzerland, and the Italian LC-1477-18 as reference. The following search queries were used

329 to find the global lineage strains (date: April 10, 2020) in Enterobase: Species equals
330 "*Shigella sonnei*"; Comment contains "Holt Lineage"; and to find matching cgST strains
331 (date: April 21, 2020) : Experiment type= cgMLST V1 + HierCC V1; ST=108909, 108083,
332 64457, 108763, 117387, 101592, 108068, 114011, 107674, 113036, 67380, 109254, 98334,
333 20888, 37499, 118753, 115537, 112958. The Illumina raw reads of our 25 *S. sonnei* isolates
334 were uploaded to Enterobase for processing. The resulting assembled genomes by Enterobase
335 were used to create a SNV project of 156 strains with default settings (min % sites present:
336 95). The tree was visualized with the web-based browser.

337 An independent core genome analysis ([Tab. S2](#)) with Parsnp was used to analyze the strain
338 clusters identified in [Fig. 3](#), which also included the 156 *S. sonnei* assemblies. As described
339 above, the Italian LC-1477-18 was used as reference genome, the Parsnp -C parameter was
340 set to 300, and the rest as default.

341 **Data availability.** Illumina SPAdes assemblies were deposited under BioProject number
342 [PRJNA578838](#). Hybrid assemblies (*bla*_{ESBL}-carrying plasmids and corresponding
343 chromosomes) were deposited under BioProject number [PRJNA578858](#).

344

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517

Strain	Year	Age / Sex ^a	Sample	Origin of infection ^a	Group ^b	Co-resistance ^b	ST ^c	Antimicrobial resistance genes / plasmid replicons (pMLST, approximated size) ^c	Called alleles (%) among a total of 2513 ^d	Allele matches in cgST (%) ^d	cgST ^d
L4094	2018	na/na	na	na	ESC-R	-	ST152	bla _{CTX-M-3} , <i>aadA1</i> , <i>mdf(A)</i> , <i>dfrA1</i> / II (pST57, 86kb) , Col156, Col(BS512)	2503 (99.60)	2496 (99.32)	cgST113036
1205-3131	2018	35/M	Stool	Unknown	ESC-R	-	ST152	bla _{CTX-M-3} , <i>aadA1</i> , <i>mdf(A)</i> , <i>dfrA1</i> / II (pST57, 86kb) , Col156, Col(BS512)	2500 (99.48)	2492 (99.16)	cgST113036
7111-69	2019	20/M	Stool	Turkey	ESC-R	-	ST152	bla _{CTX-M-3} , <i>aadA1</i> , <i>mdf(A)</i> , <i>dfrA1</i> / II (pST57, 86kb) , Col156, Col(BS512)	2499 (99.44)	2491 (99.12)	cgST113036
LC-1477-18 ^e	2018	10/F	Stool	Albania	ESC-R	SXT	ST152	bla _{CTX-M-3} , <i>aadA1</i> , <i>aph(3'')-lb</i> , <i>aph(6)-ld</i> , <i>mdf(A)</i> , <i>dfrA1</i> , <i>sul2</i> , <i>tet(A)</i> / II (pST57, 85kb) , Col156, Col(BS512)	2505 (99.68)	2498 (99.40)	cgST118753
509-1022	2019	50/F	Stool	Unknown	ESC-R	SXT	ST152	bla _{CTX-M-3} , <i>aadA1</i> , <i>aph(3'')-lb</i> , <i>aph(6)-ld</i> , <i>mdf(A)</i> , <i>dfrA1</i> , <i>sul2</i> , <i>tet(A)</i> / II (pST57, 88kb) , Col156, Col(BS512)	2503 (99.60)	2495 (99.28)	cgST115537
19-0822-3296	2019	5/F	Stool	Unknown	ESC-R	SXT	ST152	bla _{CTX-M-3} , <i>aadA1</i> , <i>aph(3'')-lb</i> , <i>aph(6)-ld</i> , <i>mdf(A)</i> , <i>dfrA1</i> , <i>sul2</i> , <i>tet(A)</i> / II (pST57, 88kb) , Col156, Col(BS512)	2504 (99.64)	2495 (99.28)	cgST115537
6607-69	2017	60/F	Stool	Sri Lanka	ESC-R	SXT	ST1503 ^f	bla _{CTX-M-15} , <i>aph(3'')-lb</i> , <i>aph(6)-ld</i> , <i>mdf(A)</i> , <i>dfrA1</i> , <i>tet(A)</i> , <i>sul2</i> / II (pST16, 90kb) , FII, Col156, Col(BS512)	2504 (99.64)	2496 (99.32)	cgST64457
19-0821-3486	2019	45/M	Stool	Egypt	ESC-R	SXT	ST152	bla _{CTX-M-15} , <i>aadA1</i> , <i>aph(3'')-lb</i> , <i>aph(6)-ld</i> , <i>mdf(A)</i> , <i>dfrA1</i> , <i>qnrS1</i> , <i>sul2</i> , <i>tet(A)</i> / FII (F2:A-:B-, 83kb) , Col156, Col(BS512)	2500 (99.48)	2493 (99.20)	cgST112958
0401930105	2019	50/M	Stool	Egypt	ESC-R	SXT	ST152	bla _{CTX-M-15} , <i>aadA1</i> , <i>aph(3'')-lb</i> , <i>aph(6)-ld</i> , <i>mdf(A)</i> , <i>dfrA1</i> , <i>qnrS1</i> , <i>tet(A)</i> , <i>sul2</i> / FII (F2:A-:B-, 83kb) , Col156, Col(BS512)	2502 (99.56)	2496 (99.32)	cgST112958
6904-27	2018	35/M	Stool	Local	ESC-R	SXT	ST152	bla _{CTX-M-15} , <i>aadA1</i> , <i>aph(3'')-lb</i> , <i>aph(6)-ld</i> , <i>mdf(A)</i> , <i>dfrA1</i> , <i>qnrS1</i> , <i>tet(A)</i> , <i>sul2</i> / FII (F2:A-:B-, 83kb) , Col156, Col(BS512)	2502 (99.56)	2497 (99.36)	cgST112958
19-1125-3493	2019	40/F	Stool	Unknown	ESC-R	SXT	ST152	bla _{CTX-M-15} , <i>aadA1</i> , <i>aph(3'')-lb</i> , <i>aph(6)-ld</i> , <i>mdf(A)</i> , <i>dfrA1</i> , <i>qnrS1</i> , <i>tet(A)</i> , <i>sul2</i> / FII (F2:A-:B-, 83kb) , Col156, Col(BS512)	2496 (99.32)	2489 (99.04)	cgST112958
19-0820-1561	2019	15/F	Stool	Nepal	ESC-R	SXT, CIP, AZT	ST152	bla _{CTX-M-15} , <i>aadA5</i> , <i>mdf(A)</i> , <i>mph(A)</i> , <i>dfrA1</i> , <i>dfrA17</i> , <i>qnrS1</i> , <i>sul1</i> / FII (F2: A-:B-, 83kb) , Col156, Col(BS512)	2493 (99.20)	2477 (98.57)	cgST117387
0401952027	2019	45/M	Stool	Egypt	ESC-R	SXT, AZT	ST152	bla _{CTX-M-55} , <i>aadA1</i> , <i>aph(3'')-lb</i> , <i>aph(6)-ld</i> , <i>mdf(A)</i> , <i>mph(A)</i> , <i>dfrA1</i> , <i>sul2</i> , <i>tet(A)</i> / II, FII (F2:A-:B-, 74kb) , Col156, Col(BS512)	2498 (99.40)	2489 (99.04)	cgST20888
09163633	2019	50/M	Stool	Unknown	ESC-R	SXT	ST152	bla _{CTX-M-27} , <i>aadA1</i> , <i>aph(3'')-lb</i> , <i>aph(6)-ld</i> , <i>mdf(A)</i> , <i>dfrA1</i> , <i>sul2</i> , <i>tet(A)</i> / FII (F2:A-:B-, 68kb) , B/O/K/Z, Col156, Col(BS512)	2497 (99.36)	2489 (99.04)	cgST67380
3123885	2019	30/M	Stool	Israel	ESC-R	SXT, CIP, AZT	ST152	bla _{CTX-M-134} , <i>aph(6)-ld</i> , <i>mdf(A)</i> , <i>mph(A)</i> , <i>dfrA1</i> , <i>sul2</i> , <i>tet(A)</i> / FII (F35:A-:B-, 69kb) , B/O/K/Z, Col156, Col(BS512)	2489 (99.04)	2480 (98.69)	cgST114011
7103-58 ^g	2018	10/M	Stool	Romania	ESC-S	SXT, CIP, AZT	ST152	<i>bla</i> _{TEM-1B} , <i>aadA5</i> , <i>aph(3'')-lb</i> , <i>aph(6)-ld</i> , <i>mdf(A)</i> , <i>mph(A)</i> , <i>erm(B)</i> , <i>dfrA1/A17</i> , <i>sul1/2</i> , <i>tet(A)</i> / Col156, Col(BS512)	2489 (99.04)	2482 (98.77)	cgST107674
7103-28 ^g	2018	50/M	Stool	Romania	ESC-S	SXT, CIP, AZT	ST152	<i>bla</i> _{TEM-1B} , <i>aadA5</i> , <i>aph(3'')-lb</i> , <i>aph(6)-ld</i> , <i>mdf(A)</i> , <i>mph(A)</i> , <i>erm(B)</i> , <i>dfrA1/A17</i> , <i>sul1/2</i> , <i>tet(A)</i> / Col156, Col(BS512)	2490 (99.08)	2481 (98.73)	cgST107674
6407-57	2017	40/M	Stool	Local	ESC-S	SXT, CIP, AZT	ST152	<i>bla</i> _{TEM-1B} , <i>aadA5</i> , <i>aph(3'')-lb</i> , <i>aph(6)-ld</i> , <i>mdf(A)</i> , <i>mph(A)</i> , <i>erm(B)</i> , <i>dfrA1/A17</i> , <i>sul1/2</i> , <i>tet(A)</i> / II , Col156, Col(BS512)	2492 (99.16)	2484 (98.85)	cgST108068
6110-62	2016	60/F	Stool	Brazil	ESC-S	SXT	ST152	<i>bla</i> _{TEM-1B} , <i>aph(3'')-lb</i> , <i>aph(6)-ld</i> , <i>mdf(A)</i> , <i>dfrA8</i> , <i>sul2</i> / FII , Col156, Col(BS512)	2499 (99.44)	2492 (99.16)	cgST108083
6105-15	2016	35/M	Stool	Egypt	ESC-S	SXT	ST152	<i>aadA1</i> , <i>aph(3'')-lb</i> , <i>aph(6)-ld</i> , <i>mdf(A)</i> , <i>dfrA1</i> , <i>sul2</i> , <i>tet(A)</i> / FII , Col156, Col(BS512)	2497 (99.36)	2487 (98.97)	cgST37499
6101-40	2016	40/F	Stool	Western Africa	ESC-S	SXT	ST152	<i>aadA1</i> , <i>aph(3'')-lb</i> , <i>aph(6)-ld</i> , <i>mdf(A)</i> , <i>dfrA1</i> , <i>sul2</i> , <i>tet(A)</i> / FII , Col156, Col(BS512)	2492 (99.16)	2479 (98.65)	cgST98334
6412-75	2017	50/M	Blood, stool	India	ESC-S	CIP	ST152	<i>mdf(A)</i> , <i>dfrA1</i> / Col156, Col(BS512)	2495 (99.28)	2487 (98.97)	cgST101592
6502-32	2017	40/F	Stool	Dominican Republic	ESC-S	SXT	ST152	<i>aph(3'')-lb</i> , <i>aph(6)-ld</i> , <i>mdf(A)</i> , <i>dfrA1</i> , <i>sul2</i> , <i>tet(A)</i> / II , Col156, Col(BS512)	2494 (99.24)	2484 (98.85)	cgST108763
7111-23	2019	25/F	Stool	Philippines	ESC-S	-	ST152	<i>sul2</i> , <i>dfrA14</i> , <i>aph(3'')-lb</i> , <i>aph(6)-ld</i> , <i>mdf(A)</i> / II, FII , Col156	2495 (99.28)	2481 (98.73)	cgST108909
7109-28	2019	30/M	Stool	Colombia	ESC-S	SXT	ST152	<i>aadA1</i> , <i>aph(3'')-lb</i> , <i>aph(6)-ld</i> , <i>sul2</i> , <i>mdf(A)</i> , <i>dfrA1</i> , <i>qnrB19/B5/B81</i> , <i>tet(A)</i> / II , Col156	2499 (99.44)	2486 (98.93)	cgST109254
7001-38	2018	50/F	Stool	Local	ESC-S	SXT	ST152	<i>aadA1</i> , <i>mdf(A)</i> , <i>dfrA1</i> / Col156, Col(BS512)	2503 (99.60)	2494 (99.24)	cgST113036

Table 1. *S. sonnei* strains analyzed in the present study: summary of the demographic and travel-related (if any) data along with the results for the whole-genome sequencing (WGS) analyses

Note. M, male; F, female; ESC-R, extended-spectrum cephalosporin-resistant; ESC-S, ESC-susceptible; SXT, trimethoprim-sulfamethoxazole; CIP, ciprofloxacin; AZT, azithromycin; ST, sequence type; cgST, core-genome sequence type; na, not available

^a Based on the information provided to the clinical laboratory analyzing routine samples. Age has been approximated at ± 5 years. The origin of infection has been attributed to a foreign country if symptoms (i.e., diarrhea) occurred during or after returning from a specific country)

^b Based on the MICs obtained with the Sensititre GNX2F panel and interpreted according to the EUCAST 2019 criteria. Only key antibiotics showing non-susceptibility have been reported in the "co-resistance" column (see Tab. S1 for full MIC results)

^c Performed implementing the tools of the Center for Genomic Epidemiology (CGE). Specifically, MLST (v2.0; *E. coli* #1 configuration), ResFinder (v3.2), PlasmidFinder (v2.0), and pMLST (v2.0) when available. In bold the main *bla* genes and their associated carrying plasmids

^d Core-genome results obtained with cgMLSTFinder (v1.1)

^e This strain was detected in Italy (7). It was added to the analysis as control

[†] ST1503 is a single allele variant of ST152

[‡] These two patients are relatives

527 **LEGEND TO THE FIGURES**

528 **Figure 1.** BLAST comparisons of *S. sonnei* *bla*_{CTX-M}-carrying plasmids against reference
529 sequences. **A)** Five *S. sonnei* *bla*_{CTX-M-3}-carrying IncII plasmids against *S. sonnei* IncII
530 plasmid: pLC1477_18-1 (GenBank: [CP035009](#)) reference sequence. **B)** Five *S. sonnei* *bla*_{CTX-}
531 *M-15*-carrying IncFII (F2:A-:B-) plasmids against *K. pneumoniae* IncFII (F2:A-:B-) plasmid:
532 pF93-2_1 (GenBank: [CP026158](#)) reference sequence. Rings were constructed using BRIG
533 (BLAST Ring Image Generator) v0.95 software. Similarities with the reference plasmid are
534 represented by the colored rings. Genome accession numbers are indicated in the legend. Red
535 and blue arrows above the rings correspond to gene features of interest. Delta symbol (Δ) next
536 to feature label corresponds to partial/incomplete gene CDS. For each plasmid, we report
537 GenBank accession, species of isolation, tree cluster from Fig. 2, year, plasmid name, and
538 plasmid size.

539

540 **Figure 2.** Analysis of the core genome phylogeny of 25 *S. sonnei* isolates together with the
541 Italian strain LC-1477-18. For each strain, we show: strain, collection year, main β -lactamase
542 (if present), and cgST. Assembled WGS of strains is presented in a core-genome SNV tree.
543 The Δ SNVs value (e.g., $\Delta=1$ SNV) corresponds to the number of non-identical SNVs of the
544 core-genome between two strains. The four main clusters (grey boxes) were defined when the
545 nucleotide identity across two or more strains was $\geq 97.5\%$ of shared SNVs ($\Delta \leq 65$ SNVs).
546 The cluster matrix shows the maximum nucleotide identity (%) between all strains across two
547 clusters (top right corner), and the number of SNVs not shared among all compared strains.
548 The scale bar (0.05) represents the average number of nucleotide substitutions per site.
549 Asterisks (*) represent identical cgST as determined by CGE's cgMLSTFinder (v1.1).

550 ^a Core-genome represents the maximum total coverage (87%) of the alignment among all 26
551 *S. sonnei* conserved sequences, which corresponded to 2'608 SNVs.

552 ^b Cluster-1: Strains shared 98.77% SNVs

553 ^c Cluster-2: Strains shared 98.01% SNVs

554 ^d Cluster-3: Strains shared 99.08% SNVs

555 ^e Cluster-4: Strains shared 97.54% SNVs

556 ^f In cluster-1 and cluster-2, the *bla*_{CTX-M-3} was consistently carried by the same Inc11-pST57
557 plasmid (see Fig. 1A)

558

559 **Figure 3.** Enterobase SNV tree dendrogram of the Swiss *S. sonnei* (n=25), global lineage
560 (n=114), matching cgST strains (n=16), and the Italian LC-1477-18 as reference. The
561 combined SNP profiles of all 156 strains mapped to the reference are represented in a
562 RAxML tree, corresponding to a total of 9'850 SNVs.

563 Country labels are represented by colored circles (missing country labels correspond to strains
564 IBESS820 from the Netherlands, Ss046 from China, and 53G from Korea). Holt lineages I, II,
565 III, GIII, and IV are presented in color boxes. Dashed black braces with lines correspond to
566 zoom-in sections of the tree where the present study's strains are clustered. For the Swiss
567 isolates we show cluster (if any) / ESBL (if any), while for international strains with matching
568 cgST we show only the ESBL (if any). Among these strains, those detected in 2019 are
569 indicated with (*). The scale bar represents the average number of nucleotide substitutions per
570 site. See Tab. S2 for more information regarding the Parsnp SNV analysis results for zoom-in
571 sections 1-7:

572 ¹ Compared to the Swiss isolates possessing the same cgST, strains 811053, 266979, 175609,
573 152507, CFSAN091705, and 795376 show 0-1, 0-4, 1-5, 2, 5, and 4 SNVs, respectively

574 ² Compared to the Swiss isolate, strain 526163 has the same cgST and shows 2 SNVs

575 ³ Compared to the Swiss isolates, strain 299890 has the same cgST and shows 2-3 SNVs

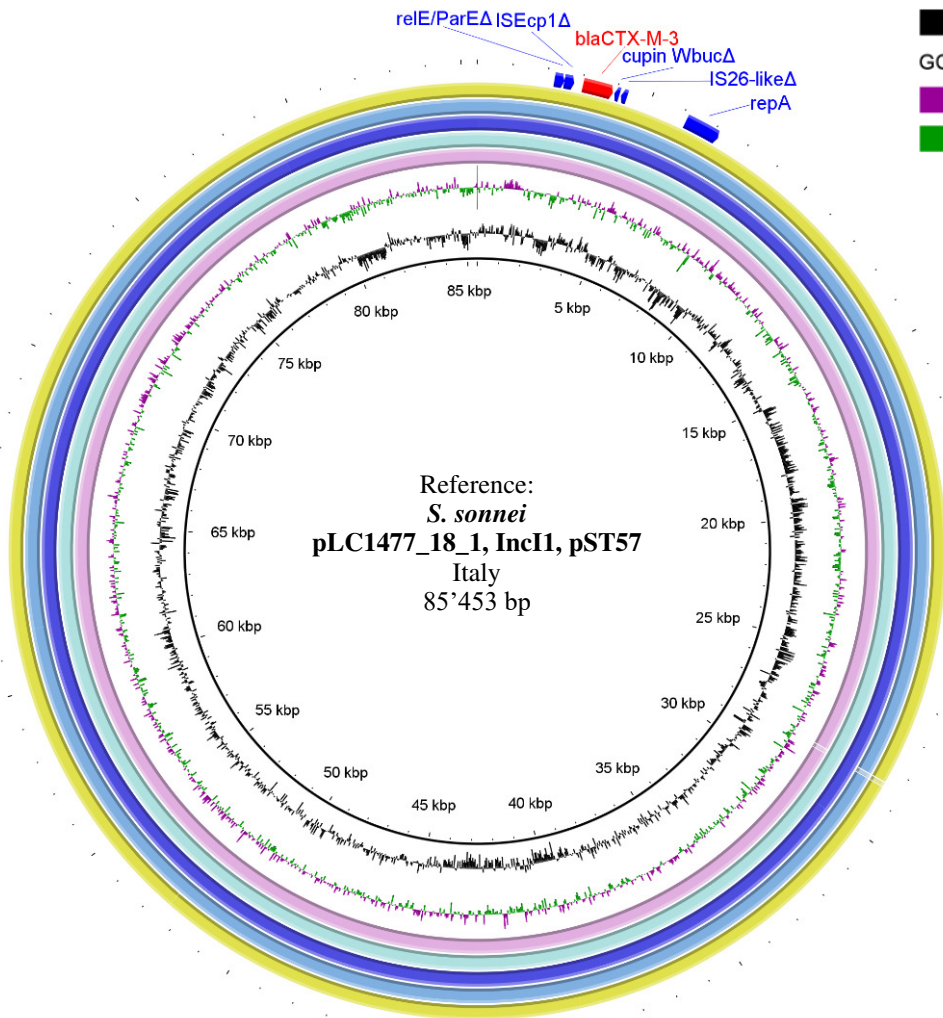
576 ⁴ Compared to the Swiss isolates possessing the same cgST, strains 190807, 201907857,
577 638735, IBESS820, 821179, and PNUSAE013040 show 6, 1, 1, 0, 8, and 1 SNVs,
578 respectively

579 ⁵ 61 SNVs between the Swiss isolate and the one found in Egypt

580 ⁶ Compared to the Swiss isolate, strain 191891 has the same cgST and shows 2 SNVs

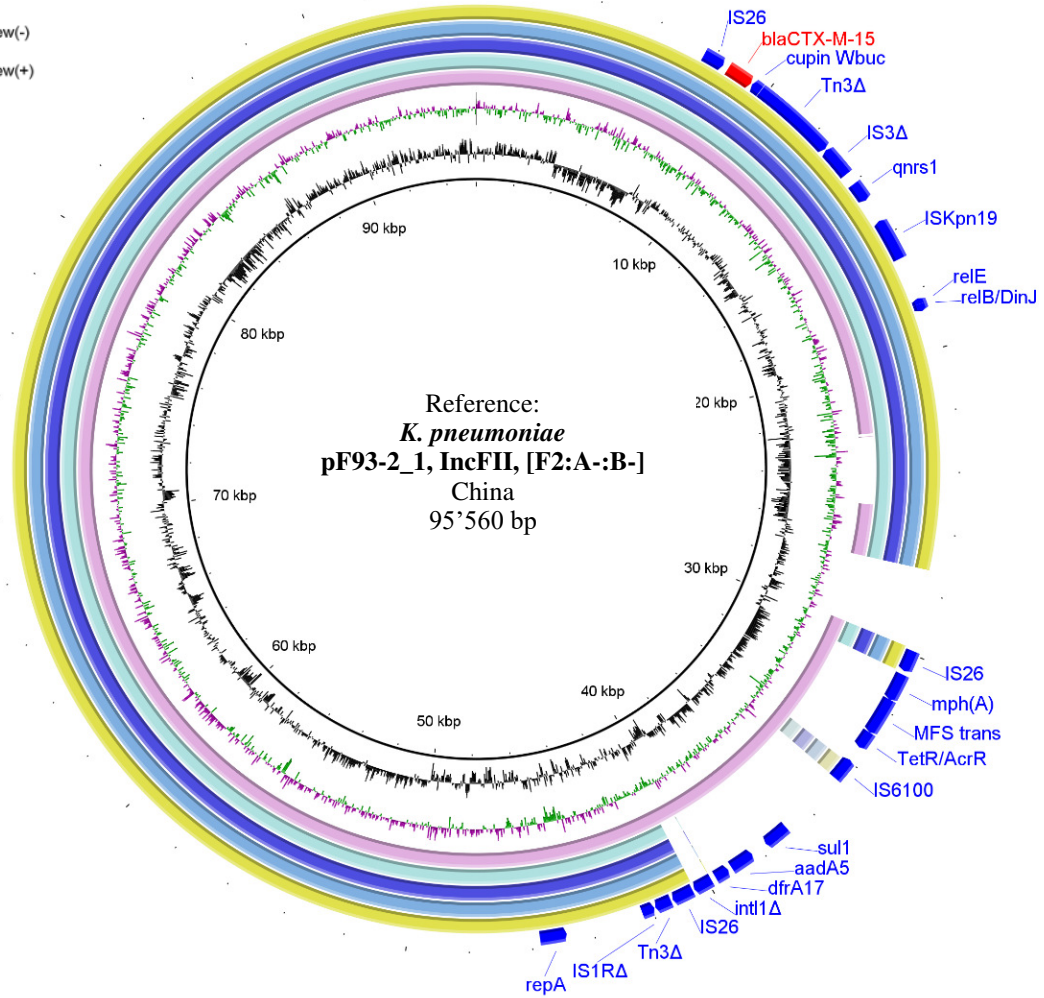
581 ⁷ Compared to the Swiss isolate, strain 524350 has the same cgST and shows 4 SNVs

Figure 1A



GC Content
GC Skew
GC Skew(-)
GC Skew(+)

Figure 1B



CP049178 <i>S. sonnei</i> , 2018 Cluster 1 p1205-3131 (86'039 bp)	CP049184 <i>S. sonnei</i> , 2019 Cluster 2 p19-0822-3296 (87'623 bp)	CP049182 <i>S. sonnei</i> , 2019 Cluster 2 p509-1022 (87'619 bp)	CP049176 <i>S. sonnei</i> , 2019 Cluster 1 p7111-69 (86'143 bp)	CP049180 <i>S. sonnei</i> , 2019 Cluster 1 pL4094 (86'136 bp)
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CP049174 <i>S. sonnei</i> , 2019 None p19-0820-1561 (89'387 bp)	CP049186 <i>S. sonnei</i> , 2019 Cluster 3 p19-0821-3486 (83'249 bp)	CP049172 <i>S. sonnei</i> , 2019 Cluster 3 p0401930105 (83'337 bp)	CP049170 <i>S. sonnei</i> , 2019 Cluster 3 p19-1125-3493 (83'333 bp)	CP045525.2 <i>S. sonnei</i> , 2018 Cluster 3 p6904-27 (83'273 bp)
---	--	--	--	---

100% identity	100% identity	100% identity	100% identity	100% identity
70% identity	70% identity	70% identity	70% identity	70% identity
50% identity	50% identity	50% identity	50% identity	50% identity

100% identity	100% identity	100% identity	100% identity	100% identity
70% identity	70% identity	70% identity	70% identity	70% identity
50% identity	50% identity	50% identity	50% identity	50% identity

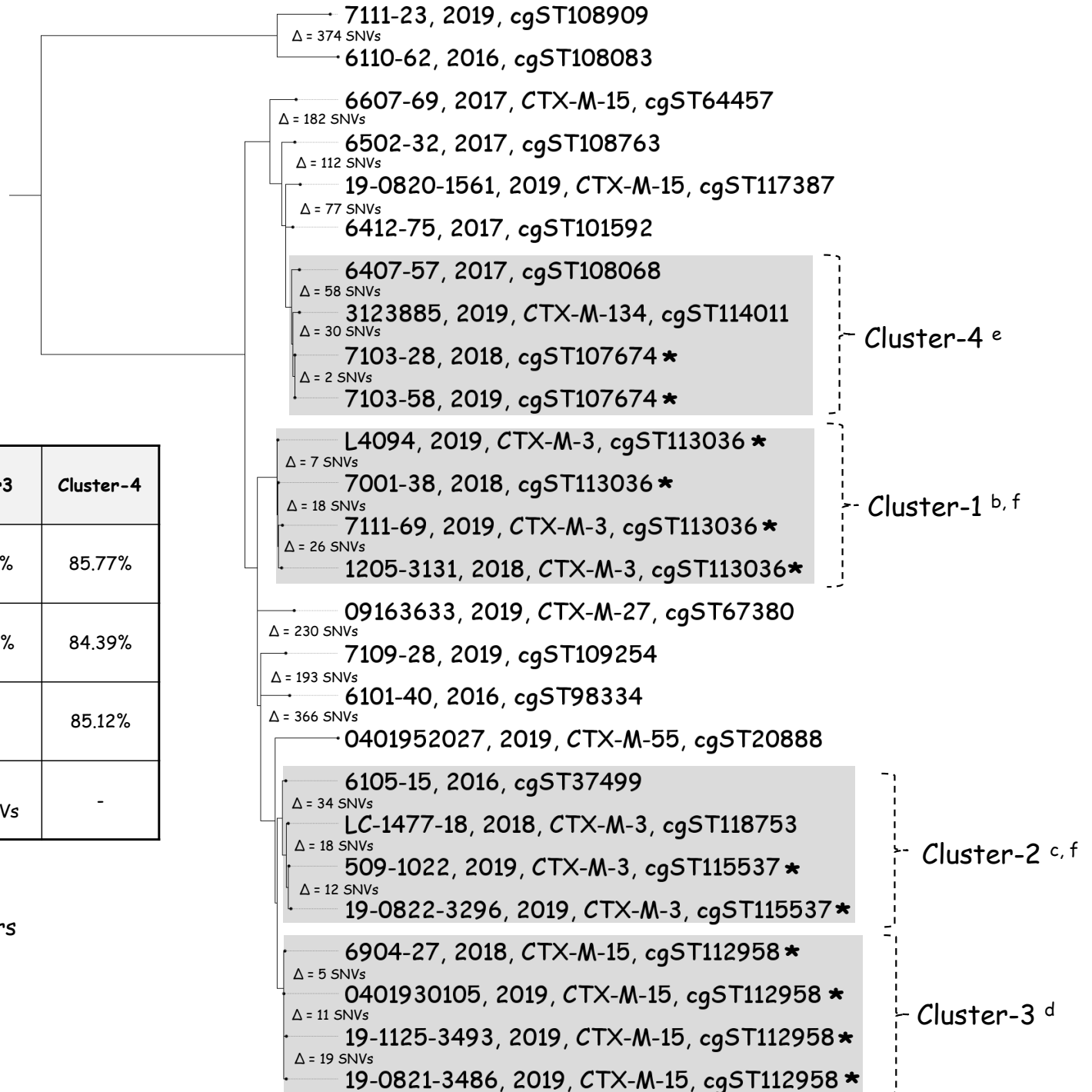
Figure 2

87% CORE-GENOME ^a
2'608 SNVs

	Cluster-1	Cluster-2	Cluster3	Cluster-4
Cluster-1	-	90.98%	91.56%	85.77%
Cluster-2	235 Δ SNVs	-	95.66%	84.39%
Cluster-3	220 Δ SNVs	113 Δ SNVs	-	85.12%
Cluster-4	371 Δ SNVs	407 Δ SNVs	388 Δ SNVs	-

■ = cgSNV clusters

* = cgST clones



Country

- France [26]
- Switzerland [25]
- South Korea [19]
- United Kingdom [19]
- Vietnam [18]
- Pakistan [7]
- Brazil [6]
- Sweden [6]
- Egypt [3]
- Madagascar [3]
- Morocco [3]
- Nigeria [3]
- United States [2]
- Burkina Faso [1]
- Cuba [1]
- Denmark [1]
- Haiti [1]
- Iran [1]
- Israel [1]
- Italy [1]
- Kenya [1]
- Mexico [1]
- Nepal [1]
- Peru [1]
- Senegal [1]
- Tanzania [1]
- Missing [3]

