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

Edgar I. Campos-Madueno, [a] Odette J. Bernasconi, [a] Aline I. Moser, [a] Peter M. Keller, [a] Francesco Luzzaro, [b] Carola Maffioli, [c] Thomas Bodmer, [d] Andreas Kronenberg, [a, e] and Andrea Endimiani [a*]

[a] Institute for Infectious Diseases, University of Bern, Bern, Switzerland; [b] Clinical Microbiology and Virology Unit, A. Manzoni Hospital, Lecco, Italy; [c] MCL Medizinische Laboratorien, Niederwangen, Switzerland; [d] labormedizinisches zentrum Dr. Risch, Bern-Liebefeld, Switzerland; [e] Swiss Centre for Antibiotic Resistance (ANRESIS)

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***Corresponding author:**
Prof. Andrea Endimiani, MD, PhD, FAMH
Institute for Infectious Diseases, University of Bern
Friedbühlstrasse 51, CH-3001, Bern, Switzerland
Phone: +41-31-632 8 632; Fax: +41-31-632 8 766
Emails: andrea.endimiani@ifik.unibe.ch; aendimiani@gmail.com

ABSTRACT

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

INTRODUCTION

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

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

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

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

Several authors have implemented the multilocus sequence typing (MLST), which has 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.

RESULTS AND DISCUSSION

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

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

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

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

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

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

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

IncI1 bla*$_{\textit{CTX-M-3}}$*-carrying plasmids. As shown in Fig. 1A, 5 *S. sonnei* isolates harbored 86-87kb IncI1-pST57 *bla*$_{\text{CTX-M-3}}$ carrying plasmids with a high genetic identity to each other and to the Italian pLC1477_18_1 that we recently described (conjugation frequency, 1.2 x 10^{-4}) (7).

In all of these plasmids, *bla*$_{\text{CTX-M-3}}$ was associated with a truncated IS*Ecp1* in the same element reported in the Italian plasmid. Considering the strong genetic similarity of the five IncI1-pST57 *bla*$_{\text{CTX-M-3}}$ carrying plasmids collected in Switzerland, it is quite possible that other plasmids with similar genetic characteristics exist outside the country. For instance, an Enterobase *S. sonnei* strain from the UK in 2019 (named 811053; BioSample: SAMN12881824) of the same cgST as our 509-1022 and 19-0822-3296 isolates (cgST115537) was found to harbor *bla*$_{\text{CTX-M-3}}$ and contained at least one pST57 plasmid (CGE analysis; data not shown). Similarly, an *E. coli* strain from a 2013-2015 study in the

Netherlands was reported to be carrying an IncI1-pST57 plasmid that possessed *bla*$_{\text{CTX-M-3}}$ (31).

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

The 5 *bla*$_{\text{CTX-M-15}}$-carrying plasmids identified in the present work shared an identical genetic environment around *bla*$_{\text{CTX-M-15}}$, including both full (IS*26* and IS*Kpn19*) and partial (ΔTn*3* and ΔIS*3*) transposable element coding sequences (CDS), along with the *qnrS1* (Fig. 1B). The plasmid p19-0820-1561 also contained additional ARGs [*mph(A)*, *sul1*, *aadA5*, and *dfrA17*] that were only present in pF93-2_1 in the form of the IS*26*-*mph(A)*-MFS transporter-*tetR/acrR*-IS*6100*-*sul1*-*aadA5*-*dfrA17*-*intl1*Δ-IS*26*-*Tn3*Δ-IS*1R*Δ unit. This element has been reported in multiple species such as *E. coli* and *K. pneumoniae* (BLAST analysis; data not shown).

Other bla*$_{\text{CTX-M}}$*-carrying plasmids. The remaining 4 ESC-R-*Ss* isolates possessed unique *bla*$_{\text{CTX-M}}$-positive plasmids (Tab. 1). In particular, a *bla*$_{\text{CTX-M-15}}$ associated with IS*Ecp1* was carried in an 89kb IncI1-pST16 plasmid (p6607-69), but without further ARGs. This plasmid showed a high identity with others found in both *S. sonnei* and *E. coli* isolates (mostly from Asia), including some expressing the CTX-M-55 that is a single amino acid variant of CTX-M-15 (Fig. S1). Likewise, one of our *S. sonnei* isolates carried the *bla*$_{\text{CTX-M-55}}$, but in this case the gene was located in a 74kb IncFII (F2:A-:B-) plasmid (p0401952027) and was flanked by two IS*26*. This plasmid showed a high genetic identity with others possessing *bla*$_{\text{CTX-M-55}}$ or *bla*$_{\text{CTX-M-15}}$ that came from *E. coli* or *K. pneumoniae* isolates detected in Europe or North

America, but, interestingly, none of them co-carried the *tetR*/*acrR*-MFS trans-*mph(A)* unit between IS*6100* and IS*1R*Δ (Fig. S2).

Another ESC-R-*Ss* carried a 67kb IncFII (F2:A-:B-) plasmid (p09163633) that harbored only $bla_{\text{CTX-M-27}}$ and showed a high identity with the backbone of plasmids from *E. coli* and *S. flexneri* isolates. Nevertheless, only p09163633 possessed the IS*26*-IS*903B*Δ-$bla_{\text{CTX-M-27}}$-IS*Ecp1*Δ-IS*26* unit (Fig. S3), which has been reported in multiple *E. coli* and *K. pneumoniae* isolates from Vietnam and China (BLAST analysis; data not shown), and also described in a Japanese epidemic ST131 *E. coli* (33). Moreover, it was also present in the 69kb IncFII (F35:A-:B-) plasmid (p3123885) found in our last ESC-R-*Ss* (Fig. S4), though this mobile-genetic element (MGE) encoded the single amino acid variant CTX-M-134 instead of the CTX-M-27 (29).

Co-resistance to azithromycin. Besides the specific ESBLs identified, 6 of the 25 *S. sonnei* isolates were macrolide-resistant due to the presence of *erm(B)* and/or *mph(A)* ARGs (Tab. 1 and Tab. S1). As mentioned above, 3 ESC-R isolates carried *mph(A)* in different IncFII plasmids co-harboring $bla_{\text{CTX-M-15}}$, $bla_{\text{CTX-M-55}}$, or $bla_{\text{CTX-M-134}}$. Of note, two of these plasmids carried the element IS*26-mph(A)*-MFS trans- *tetR*/*acrR*-IS*6100* (Fig. 1B and S4) and the other one carried, with a slightly different arrangement, IS*6100-tetR*/*acrR*-MFS trans-*mph(A)*-IS*1RΔ* (Fig. S2). These two very similar elements have been found in many plasmids carried by *E. coli*, *K. pneumoniae*, *Salmonella enterica* (BLAST analysis; data not shown), and also identified in the chromosome of a CTX-M-15-producing *Salmonella* Haardt isolated from Japanese food workers (34).

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

England (10), while an IncB/O/K/Z co-harboring *bla*$_{\text{CTX-M-14}}$ and *mph(A)* was linked to a waterborne outbreak in China in 2015 (8). Having observed that at least three of our IncFII plasmids carried very similar macrolide resistance elements, we speculate that under a certain antibiotic selective pressure (e.g., azithromycin), *mph(A)* can be acquired *via* integration of transposable elements (e.g., IS*26*-*mph(A)*-MFS trans- *tetR*/*acrR*-IS*6100*) (35).

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

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

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

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

We also note that isolates included in cluster-1 and cluster-2 carried the same IncI1-pST57 $bla_{\text{CTX-M-3}}$ plasmid (Fig. 1). This finding was surprising, as the two bacterial groups were genetically different (i.e., Δ=235 SNVs; Fig. 2). We do not have a clear explanation for the independent clustering, but it can be hypothesized that ESC-S *S. sonnei* isolates belonging to cluster-1 and cluster-2 acquired the pST57 plasmid from a common enterobacterial ancestor, including other *Shigella* spp.

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

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

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

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

S. sonnei cluster-4 isolates were grouped with recently detected ESC-S strains in the UK, the USA, and France. Two additional CTX-M-15 producers, one from the UK (821179) and one from the Netherlands (IBESS820), were also highly related with the cluster-4 isolates (Fig. 3). The latter was identified in 2017 during a cross-sectional multicenter study (39), and was genetically identical to our ESC-S-*Ss* 6412-75 strain (same cgST and Δ=0 SNPs). In that study, the patient of strain IBESS820 was reported to have a history of travel to India, as was 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*$_{\text{CTX-M}}$-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 IncI1-pST57 *bla*$_{\text{CTX-M-3}}$-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*$_{\text{CTX-M}}$-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*$_{\text{CTX-M}}$-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*$_{\text{CTX-M}}$-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).

MATERIALS AND METHODS

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

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

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

generated by aligning the paired-end Illumina reads to the Canu assemblies with Bowtie2 (v2.3.4.1), and followed by multiple rounds of polishing with Pilon (v1.22).

Illumina SPAdes assemblies were used for: whole genome ID, analysis with the tools of the Center for Genomic Epidemiology (CGE; http://www.genomicepidemiology.org/): ResFinder, MLST with the *E. coli* scheme #1, PlasmidFinder, and pMLST. Hybrid assemblies were used to characterize the bla_{ESBL}-carrying plasmids.

Annotations of both Illumina and hybrid assemblies were carried out by the NCBI Prokaryotic Genome Annotation Pipeline. All annotated features presented in Fig. 1 and Fig. S1-S4 were manually curated with UniProt (https://www.uniprot.org/blast/) and ISfinder (https://isfinder.biotoul.fr/), and annotated accordingly.

Core-genome analyses. All *S. sonnei* isolates underwent cgMLST with CGE cgMLSTFinder (v1.1) using Illumina raw reads as input and species' database set to *E. coli* Enterobase. These isolates also undertook core-genome SNV analysis as previously done (46). Briefly, the core-genome alignment was performed with Parsnp v1.2. All strains were treated as curated genomes (-c parameter), and the Italian ST152 CTX-M-3-producing *S. sonnei* strain (LC-1477-18; GenBank: JAATWD000000000) was used as reference (7). The –C parameter was set to 200, and other parameters were let as default. Variants with no flags (PASS) were determined as reliable, and used for downstream SNV analysis with a custom R (v3.6.2) script. The Parsnp-generated core-genome SNV phylogenetic tree was visualized with FigTree (v1.4.4), and set to midpoint-rooted, and nodes by decreasing order (Fig. 2).

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

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

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

Data availability. Illumina SPAdes assemblies were deposited under BioProject number PRJNA578838. Hybrid assemblies (bla_{ESBL}-carrying plasmids and corresponding chromosomes) were deposited under BioProject number PRJNA578858.

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REFERENCES

1. Kotloff KL, Riddle MS, Platts-Mills JA, Pavlinac P, Zaidi AKM. 2018. Shigellosis. Lancet 391:801-812.
2. Bardsley M, Jenkins C, Mitchell HD, Mikhail AFW, Baker KS, Foster K, Hughes G, Dallman TJ. 2020. Persistent Transmission of Shigellosis in England Is Associated with a Recently Emerged Multidrug-Resistant Strain of *Shigella sonnei*. J Clin Microbiol 58.
3. Thompson CN, Duy PT, Baker S. 2015. The Rising Dominance of *Shigella sonnei*: An Intercontinental Shift in the Etiology of Bacillary Dysentery. PLoS Negl Trop Dis 9:e0003708.
4. Puzari M, Sharma M, Chetia P. 2018. Emergence of antibiotic resistant *Shigella* species: A matter of concern. J Infect Public Health 11:451-454.
5. Ingle DJ, Easton M, Valcanis M, Seemann T, Kwong JC, Stephens N, Carter GP, Goncalves da Silva A, Adamopoulos J, Baines SL, Holt KE, Chow EPF, Fairley CK, Chen MY, Kirk MD, Howden BP, Williamson DA. 2019. Co-circulation of Multidrug-resistant *Shigella* Among Men Who Have Sex With Men in Australia. Clin Infect Dis 69:1535-1544.
6. Ranjbar R, Farahani A. 2019. Shigella: Antibiotic-Resistance Mechanisms And New Horizons For Treatment. Infect Drug Resist 12:3137-3167.
7. Luzzaro F, Clement M, Principe L, Viaggi V, Bernasconi OJ, Endimiani A. 2019. Characterisation of the first extended-spectrum β-lactamase (ESBL)-producing *Shigella sonnei* clinical isolate in Italy. J Glob Antimicrob Resist 17:58-59.
8. Ma Q, Xu X, Luo M, Wang J, Yang C, Hu X, Liang B, Wu F, Yang X, Wang J, Liu H, Li W, Zhong Y, Li P, Xie J, Jia L, Wang L, Hao R, Du X, Qiu S, Song H, Sun Y. 2017. A Waterborne Outbreak of *Shigella sonnei* with Resistance to Azithromycin and Third-Generation Cephalosporins in China in 2015. Antimicrob Agents Chemother 61.
9. Kim JS, Kim J, Jeon SE, Kim SJ, Kim NO, Hong S, Kang YH, Han S, Chung GT. 2014. Complete nucleotide sequence of the IncI1 plasmid pSH4469 encoding CTX-M-15 extended-spectrum β-lactamase in a clinical isolate of *Shigella sonnei* from an outbreak in the Republic of Korea. Int J Antimicrob Agents 44:533-7.
10. Mook P, McCormick J, Bains M, Cowley LA, Chattaway MA, Jenkins C, Mikhail A, Hughes G, Elson R, Day M, Manuel R, Dave J, Field N, Godbole G, Dallman T,

Crook P. 2016. ESBL-Producing and Macrolide-Resistant *Shigella sonnei* Infections among Men Who Have Sex with Men, England, 2015. Emerg Infect Dis 22:1948-1952.

11. Qu F, Ying Z, Zhang C, Chen Z, Chen S, Cui E, Bao C, Yang H, Wang J, Liu C, Mao Y, Zhou D. 2014. Plasmid-encoding extended-spectrum β-lactamase CTX-M-55 in a clinical *Shigella sonnei* strain, China. Future Microbiol 9:1143-50.
12. Nuesch-Inderbinen M, Heini N, Zurfluh K, Althaus D, Hachler H, Stephan R. 2016. *Shigella* Antimicrobial Drug Resistance Mechanisms, 2004-2014. Emerg Infect Dis 22:1083-5.
13. Anonymous. European Food Safety Authority and European Centre for Disease Prevention and Control (EFSA and ECDC). The European Union One Health 2018 Zoonoses Report. https://www.ecdc.europa.eu/sites/default/files/documents/zoonoses-EU-one-health-2018-report.pdf. doi:doi: 10.2903/j.efsa.2019.5926.
14. Wirth T, Falush D, Lan R, Colles F, Mensa P, Wieler LH, Karch H, Reeves PR, Maiden MC, Ochman H, Achtman M. 2006. Sex and virulence in *Escherichia coli*: an evolutionary perspective. Mol Microbiol 60:1136-51.
15. Kozyreva VK, Jospin G, Greninger AL, Watt JP, Eisen JA, Chaturvedi V. 2016. Recent Outbreaks of Shigellosis in California Caused by Two Distinct Populations of *Shigella sonnei* with either Increased Virulence or Fluoroquinolone Resistance. mSphere 1.
16. Shahsavan S, Nobakht M, Rastegar-Lari A, Owlia P, Bakhshi B. 2016. Multi-locus sequence type analysis of *Shigellas* pp. isolates from Tehran, Iran. Iran J Microbiol 8:298-306.
17. Shokoohizadeh L, Kaydani GA, Ekrami A. 2017. Molecular characterization of *Shigella* spp. isolates from a pediatric hospital in Southwestern Iran. Gastroenterol Hepatol Bed Bench 10:319-322.
18. Cao Y, Wei D, Kamara IL, Chen W. 2012. Multi-Locus Sequence Typing (MLST) and Repetitive Extragenic Palindromic Polymerase Chain Reaction (REP-PCR), characterization of shigella spp. over two decades in Tianjin China. Int J Mol Epidemiol Genet 3:321-32.
19. Inouye M, Conway TC, Zobel J, Holt KE. 2012. Short read sequence typing (SRST): multi-locus sequence types from short reads. BMC Genomics 13:338.

20. Dallman TJ, Chattaway MA, Mook P, Godbole G, Crook PD, Jenkins C. 2016. Use of whole-genome sequencing for the public health surveillance of *Shigella sonnei* in England and Wales, 2015. J Med Microbiol 65:882-884.
21. Abelman RL, M'Ikanatha N M, Figler HM, Dudley EG. 2019. Use of whole genome sequencing in surveillance for antimicrobial-resistant *Shigella sonnei* infections acquired from domestic and international sources. Microb Genom 5.
22. Qian H, Liu G, Chen Y, Ma P, Kong X, Zhou L, Hong J, Bao C, Gu B. 2018. Increasing clinical resistance rate of *Shigella sonnei* to cefotaxime in Jiangsu Province, China, between 2012 and 2015. Ann Transl Med 6:207.
23. Shakya G, Acharya J, Adhikari S, Rijal N. 2016. Shigellosis in Nepal: 13 years review of nationwide surveillance. J Health Popul Nutr 35:36.
24. Heffernan H, Woodhouse R, Hewison C, Sherwood J. 2018. Antimicrobial resistance among *Shigella* in New Zealand. N Z Med J 131:56-62.
25. Buhler S, Ruegg R, Steffen R, Hatz C, Jaeger VK. 2014. A profile of travelers--an analysis from a large swiss travel clinic. J Travel Med 21:324-31.
26. Acikgoz ZC, Eser OK, Kocagoz S. 2008. CTX-M-3 type β-lactamase producing *Shigella sonnei* isolates from pediatric bacillary dysentery cases. Jpn J Infect Dis 61:135-7.
27. Acikgoz ZC, Gulay Z, Bicmen M, Gocer S, Gamberzade S. 2003. CTX-M-3 extended-spectrum β-lactamase in a *Shigella sonnei* clinical isolate: first report from Turkey. Scand J Infect Dis 35:503-5.
28. Kim JS, Kim S, Park J, Shin E, Yun YS, Lee DY, Kwak HS, Seong WK, Chung GT, Kim J. 2017. Plasmid-mediated transfer of CTX-M-55 extended-spectrum β-lactamase among different strains of *Salmonella* and *Shigella* spp. in the Republic of Korea. Diagn Microbiol Infect Dis 89:86-88.
29. Castanheira M, Costello S, Jones R. 2015. Molecular Characterization of Genes Encoding CTX-M-134, TEM-207 and TEM-212 Detected among Clinical Isolates from USA Hospitals. Poster P0982. European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), Copenhagen, Denmark
30. Rashid H, Rahman M. 2015. Possible transfer of plasmid mediated third generation cephalosporin resistance between *Escherichia coli* and *Shigella sonnei* in the human gut. Infect Genet Evol 30:15-18.

31. Liakopoulos A, van den Bunt G, Geurts Y, Bootsma MCJ, Toleman M, Ceccarelli D, van Pelt W, Mevius DJ. 2018. High Prevalence of Intra-Familial Co-colonization by Extended-Spectrum Cephalosporin Resistant Enterobacteriaceae in Preschool Children and Their Parents in Dutch Households. Front Microbiol 9:293.
32. Rozwandowicz M, Brouwer MSM, Fischer J, Wagenaar JA, Gonzalez-Zorn B, Guerra B, Mevius DJ, Hordijk J. 2018. Plasmids carrying antimicrobial resistance genes in Enterobacteriaceae. J Antimicrob Chemother 73:1121-1137.
33. Matsumura Y, Johnson JR, Yamamoto M, Nagao M, Tanaka M, Takakura S, Ichiyama S, Kyoto-Shiga Clinical Microbiology Study G, Kyoto-Shiga Clinical Microbiology Study G. 2015. CTX-M-27- and CTX-M-14-producing, ciprofloxacin-resistant *Escherichia coli* of the H30 subclonal group within ST131 drive a Japanese regional ESBL epidemic. J Antimicrob Chemother 70:1639-49.
34. Shigemura H, Sakatsume E, Sekizuka T, Yokoyama H, Hamada K, Etoh Y, Carle Y, Mizumoto S, Hirai S, Matsui M, Kimura H, Suzuki M, Onozuka D, Kuroda M, Inoshima Y, Murakami K. 2020. Food workers as a reservoir of extended-spectrum cephalosporin-resistant *Salmonella* in Japan. Appl Environ Microbiol doi:10.1128/AEM.00072-20.
35. Darton TC, Tuyen HT, The HC, Newton PN, Dance DAB, Phetsouvanh R, Davong V, Campbell JI, Hoang NVM, Thwaites GE, Parry CM, Thanh DP, Baker S. 2018. Azithromycin Resistance in *Shigella* spp. in Southeast Asia. Antimicrob Agents Chemother 62.
36. Chung The H, Rabaa MA, Pham Thanh D, De Lappe N, Cormican M, Valcanis M, Howden BP, Wangchuk S, Bodhidatta L, Mason CJ, Nguyen Thi Nguyen T, Vu Thuy D, Thompson CN, Phu Huong Lan N, Voong Vinh P, Ha Thanh T, Turner P, Sar P, Thwaites G, Thomson NR, Holt KE, Baker S. 2016. South Asia as a Reservoir for the Global Spread of Ciprofloxacin-Resistant *Shigella sonnei*: A Cross-Sectional Study. PLoS Med 13:e1002055.
37. Chung The H, Boinett C, Pham Thanh D, Jenkins C, Weill FX, Howden BP, Valcanis M, De Lappe N, Cormican M, Wangchuk S, Bodhidatta L, Mason CJ, Nguyen TNT, Ha Thanh T, Voong VP, Duong VT, Nguyen PHL, Turner P, Wick R, Ceyssens PJ, Thwaites G, Holt KE, Thomson NR, Rabaa MA, Baker S. 2019. Dissecting the molecular evolution of fluoroquinolone-resistant *Shigella sonnei*. Nat Commun 10:4828.

38. Holt KE, Baker S, Weill FX, Holmes EC, Kitchen A, Yu J, Sangal V, Brown DJ, Coia JE, Kim DW, Choi SY, Kim SH, da Silveira WD, Pickard DJ, Farrar JJ, Parkhill J, Dougan G, Thomson NR. 2012. *Shigella sonnei* genome sequencing and phylogenetic analysis indicate recent global dissemination from Europe. Nat Genet 44:1056-9.
39. Hendriks ACA, Reubsaet FAG, Kooistra-Smid A, Rossen JWA, Dutilh BE, Zomer AL, van den Beld MJC, group I. 2020. Genome-wide association studies of Shigella spp. and Enteroinvasive *Escherichia coli* isolates demonstrate an absence of genetic markers for prediction of disease severity. BMC Genomics 21:138.
40. Anonymous. European Centre for Disease Prevention and Control. Expert opinion on whole genome sequencing for public health surveillance. Stockholm: ECDC; 2016. doi:10.2900/12442
41. Anonymous. European Committee on Antimicrobial Susceptibility Testing (EUCAST). Breakpoints tables for interpretation of MICs and zone diameters. Version 9.0, valid from 2019-01-01.
42. Clement M, Ramette A, Bernasconi OJ, Principe L, Luzzaro F, Endimiani A. 2018. Whole-Genome Sequence of the First Extended-Spectrum β-Lactamase-Producing Strain of *Salmonella enterica* subsp. enterica Serovar Napoli. Microbiology resource announcements 7.
43. Bernasconi OJ, Dona V, Pires J, Kuenzli E, Hatz C, Luzzaro F, Perreten V, Endimiani A. 2018. Deciphering the complete deletion of the *mgrB* locus in an unusual colistin-resistant *Klebsiella pneumoniae* isolate colonising the gut of a traveller returning from India. International journal of antimicrobial agents 51:529-531.
44. Clement M, Keller PM, Bernasconi OJ, Stirnimann G, Frey PM, Bloemberg GV, Sendi P, Endimiani A. 2019. First Clinical Case of In Vivo Acquisition of DHA-1 Plasmid-Mediated AmpC in a *Salmonella enterica* subsp. enterica Isolate. Antimicrob Agents Chemother 63.
45. Endimiani A, Bernasconi OJ, Budel T, Campos-Madueno EI, Kuenzli E, Hatz C, Carattoli A. 2020. Whole-genome characterization of a *Shewanella algae* strain co-harboring $bla_{\text{CTX-M-15}}$ and *armA* genes on a novel IncC plasmid. Antimicrob Agents Chemother 64.
46. Büdel T, Kuenzli E, Campos-Madueno EI, Mohammed AH, Hassan NK, Zinsstag J, Hatz C, Endimiani A. 2020. On the Island of Zanzibar People in the Community are Frequently Colonized with the Same Multidrug-Resistant Enterobacterales Found in

Poultry and Retailed Chicken Meat. Journal of Antimicrobial Chemotherapy
doi:10.1093/jac/dkaa198

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*$_{CTX-M-3}$**, *aadA1*, *mdf(A)*, *dfrA1* / **I1 (pST57, 86kb)**, Col156, Col(BS512)	2503 (99.60)	2496 (99.32)	cgST113036
1205-3131	2018	35/M	Stool	Unknown	ESC-R	-	ST152	***bla*$_{CTX-M-3}$**, *aadA1*, *mdf(A)*, *dfrA1* / **I1 (pST57, 86kb)**, Col156, Col(BS512)	2500 (99.48)	2492 (99.16)	cgST113036
7111-69	2019	20/M	Stool	Turkey	ESC-R	-	ST152	***bla*$_{CTX-M-3}$**, *aadA1*, *mdf(A)*, *dfrA1* / **I1 (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*$_{CTX-M-3}$**, *aadA1*, *aph(3")-Ib*, *aph(6)-Id*, *mdf(A)*, *dfrA1*, *sul2*, *tet(A)* / **I1 (pST57, 85kb)**, Col156, Col(BS512)	2505 (99.68)	2498 (99.40)	cgST118753
509-1022	2019	50/F	Stool	Unknown	ESC-R	SXT	ST152	***bla*$_{CTX-M-3}$**, *aadA1*, *aph(3")-lb*, *aph(6)-ld*, *mdf(A)*, *dfrA1*, *sul2*, *tet(A)* / **I1 (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*$_{CTX-M-3}$**, *aadA1*, *aph(3")-lb*, *aph(6)-ld*, *mdf(A)*, *dfrA1*, *sul2*, *tet(A)* / **I1 (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*$_{CTX-M-15}$**, *aph(3")-lb*, *aph(6)-ld*, *mdf(A)*, *dfrA1*, *tet(A)*, *sul2* / **I1 (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*$_{CTX-M-15}$**, *aadA1*, *aph(3")-lb*, *aph(6)-ld*, *mdf(A)*, *dfrA1*, *qnrS1*, *sul2*, *tet(A)* / **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*$_{CTX-M-15}$**, *aadA1*, *aph(3")-lb*, *aph(6)-ld*, *mdf(A)*, *dfrA1*, *qnrS1*, *tet(A)*, *sul2* / **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*$_{CTX-M-15}$**, *aadA1*, *aph(3")-lb*, *aph(6)-ld*, *mdf(A)*, *dfrA1*, *qnrS1*, *tet(A)*, *sul2* / **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*$_{CTX-M-15}$**, *aadA1*, *aph(3")-lb*, *aph(6)-ld*, *mdf(A)*, *dfrA1*, *qnrS1*, *tet(A)*, *sul2* / **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*$_{CTX-M-15}$**, *aadA5*, *mdf(A)*, *mph(A)*, *dfrA1*, *dfrA17*, *qnrS1*, *sul1* / **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*$_{CTX-M-55}$**, *aadA1*, *aph(3")-lb*, *aph(6)-ld*, *mdf(A)*, *mph(A)*, *dfrA1*, *sul2*, *tet(A)* / I1, **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*$_{CTX-M-27}$**, *aadA1*, *aph(3")-lb*, *aph(6)-ld*, *mdf(A)*, *dfrA1*, *sul2*, *tet(A)* / **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*$_{CTX-M-134}$**, *aph(6)-ld*, *mdf(A)*, *mph(A)*, *dfrA1*, *sul2*, *tet(A)* / **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	*bla*$_{TEM-1B}$, *aadA5*, *aph(3")-lb*, *aph(6)-ld*, *mdf(A)*, *mph(A)*, *erm(B)*, *dfrA1/A17*, *sul1/2*, *tet(A)* / Col156, Col(BS512)	2489 (99.04)	2482 (98.77)	cgST107674
7103-28 [g]	2018	50/M	Stool	Romania	ESC-S	SXT, CIP, AZT	ST152	*bla*$_{TEM-1B}$, *aadA5*, *aph(3")-lb*, *aph(6)-ld*, *mdf(A)*, *mph(A)*, *erm(B)*, *dfrA1/A17*, *sul1/2*, *tet(A)* / Col156, Col(BS512)	2490 (99.08)	2481 (98.73)	cgST107674
6407-57	2017	40/M	Stool	Local	ESC-S	SXT, CIP, AZT	ST152	*bla*$_{TEM-1B}$, *aadA5*, *aph(3")-lb*, *aph(6)-ld*, *mdf(A)*, *mph(A)*, *erm(B)*, *dfrA1/A17*, *sul1/2*, *tet(A)* / I1, Col156, Col(BS512)	2492 (99.16)	2484 (98.85)	cgST108068
6110-62	2016	60/F	Stool	Brazil	ESC-S	SXT	ST152	*bla*$_{TEM-1B}$, *aph(3")-lb*, *aph(6)-ld*, *mdf(A)*, *dfrA8*, *sul2* / FII, Col156, Col(BS512)	2499 (99.44)	2492 (99.16)	cgST108083
6105-15	2016	35/M	Stool	Egypt	ESC-S	SXT	ST152	*aadA1*, *aph(3")-lb*, *aph(6)-ld*, *mdf(A)*, *dfrA1*, *sul2*, *tet(A)* / FII, Col156, Col(BS512)	2497 (99.36)	2487 (98.97)	cgST37499
6101-40	2016	40/F	Stool	Western Africa	ESC-S	SXT	ST152	*aadA1*, *aph(3")-lb*, *aph(6)-ld*, *mdf(A)*, *dfrA1*, *sul2*, *tet(A)* / FII, Col156, Col(BS512)	2492 (99.16)	2479 (98.65)	cgST98334
6412-75	2017	50/M	Blood, stool	India	ESC-S	CIP	ST152	*mdf(A)*, *dfrA1* / Col156, Col(BS512)	2495 (99.28)	2487 (98.97)	cgST101592
6502-32	2017	40/F	Stool	Dominican Republic	ESC-S	SXT	ST152	*aph(3")-lb*, *aph(6)-ld*, *mdf(A)*, *dfrA1*, *sul2*, *tet(A)* / I1, Col156, Col(BS512)	2494 (99.24)	2484 (98.85)	cgST108763
7111-23	2019	25/F	Stool	Philippines	ESC-S	-	ST152	*sul2*, *dfrA14*, *aph(3")-lb*, *aph(6)-ld*, *mdf(A)* / I1, FII, Col156	2495 (99.28)	2481 (98.73)	cgST108909
7109-28	2019	30/M	Stool	Colombia	ESC-S	SXT	ST152	*aadA1*, *aph(3")-lb*, *aph(6)-ld*, *sul2*, *mdf(A)*, *dfrA1*, *qnrB19/B5/B81*, *tet(A)* / I1, Col156	2499 (99.44)	2486 (98.93)	cgST109254
7001-38	2018	50/F	Stool	Local	ESC-S	SXT	ST152	*aadA1*, *mdf(A)*, *dfrA1* / 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

[f] ST1503 is a single allele variant of ST152
[g] These two patients are relatives

LEGEND TO THE FIGURES

Figure 1. BLAST comparisons of *S. sonnei* $bla_{CTX\text{-}M}$-carrying plasmids against reference sequences. **A)** Five *S. sonnei* $bla_{CTX\text{-}M\text{-}3}$-carrying IncI1 plasmids against *S. sonnei* IncI1 plasmid: pLC1477_18-1 (GenBank: CP035009) reference sequence. **B)** Five *S. sonnei* $bla_{CTX\text{-}M\text{-}15}$-carrying IncFII (F2:A-:B-) plasmids against *K. pneumoniae* IncFII (F2:A-:B-) plasmid: pF93-2_1 (GenBank: CP026158) reference sequence. Rings were constructed using BRIG (BLAST Ring Image Generator) v0.95 software. Similarities with the reference plasmid are represented by the colored rings. Genome accession numbers are indicated in the legend. Red and blue arrows above the rings correspond to gene features of interest. Delta symbol (Δ) next to feature label corresponds to partial/incomplete gene CDS. For each plasmid, we report GenBank accession, species of isolation, tree cluster from Fig. 2, year, plasmid name, and plasmid size.

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

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

[b] Cluster-1: Strains shared 98.77% SNVs

[c] Cluster-2: Strains shared 98.01% SNVs

[d] Cluster-3: Strains shared 99.08% SNVs

[e] Cluster-4: Strains shared 97.54% SNVs

[f] In cluster-1 and cluster-2, the *bla*$_{\text{CTX-M-3}}$ was consistently carried by the same IncI1-pST57 plasmid (see Fig. 1A)

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

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

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

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

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

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

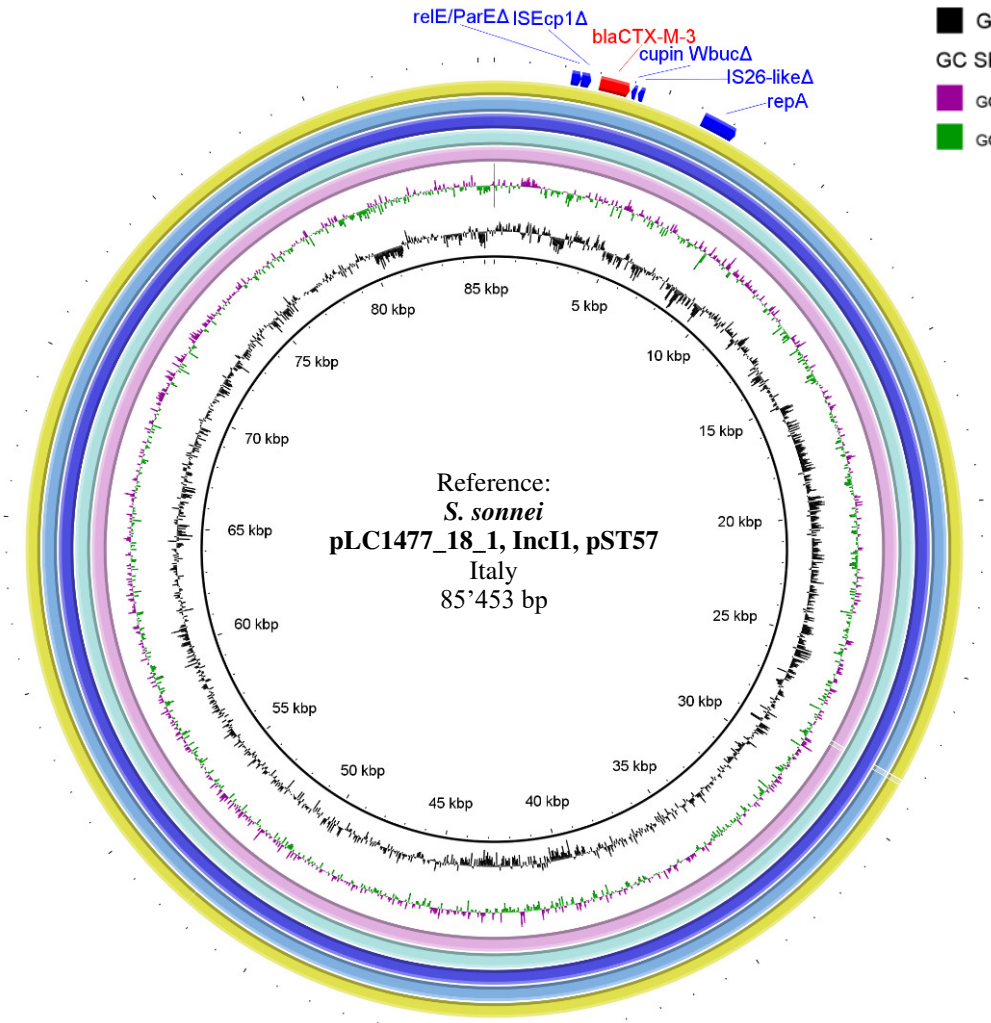
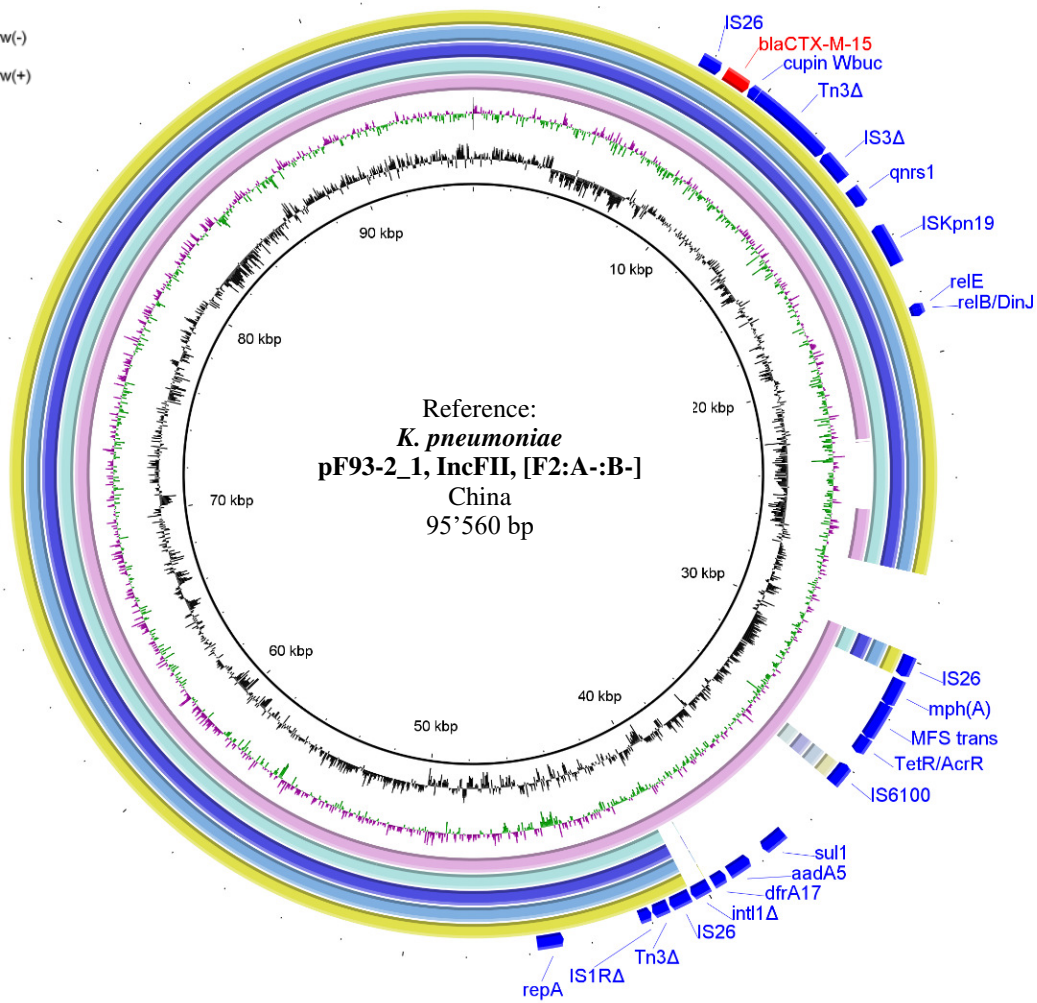


Figure 1B



CP049178
S. sonnei, 2018
Cluster 1
p1205-3131
(86'039 bp)

CP049184
S. sonnei, 2019
Cluster 2
p19-0822-3296
(87'623 bp)

CP049182
S. sonnei, 2019
Cluster 2
p509-1022
(87'619 bp)

CP049176
S. sonnei, 2019
Cluster 1
p7111-69
(86'143 bp)

CP049180
S. sonnei, 2019
Cluster 1
pL4094
(86'136 bp)

CP049174
S. sonnei, 2019
None
p19-0820-1561
(89'387 bp)

CP049186
S. sonnei, 2019
Cluster 3
p19-0821-3486
(83'249 bp)

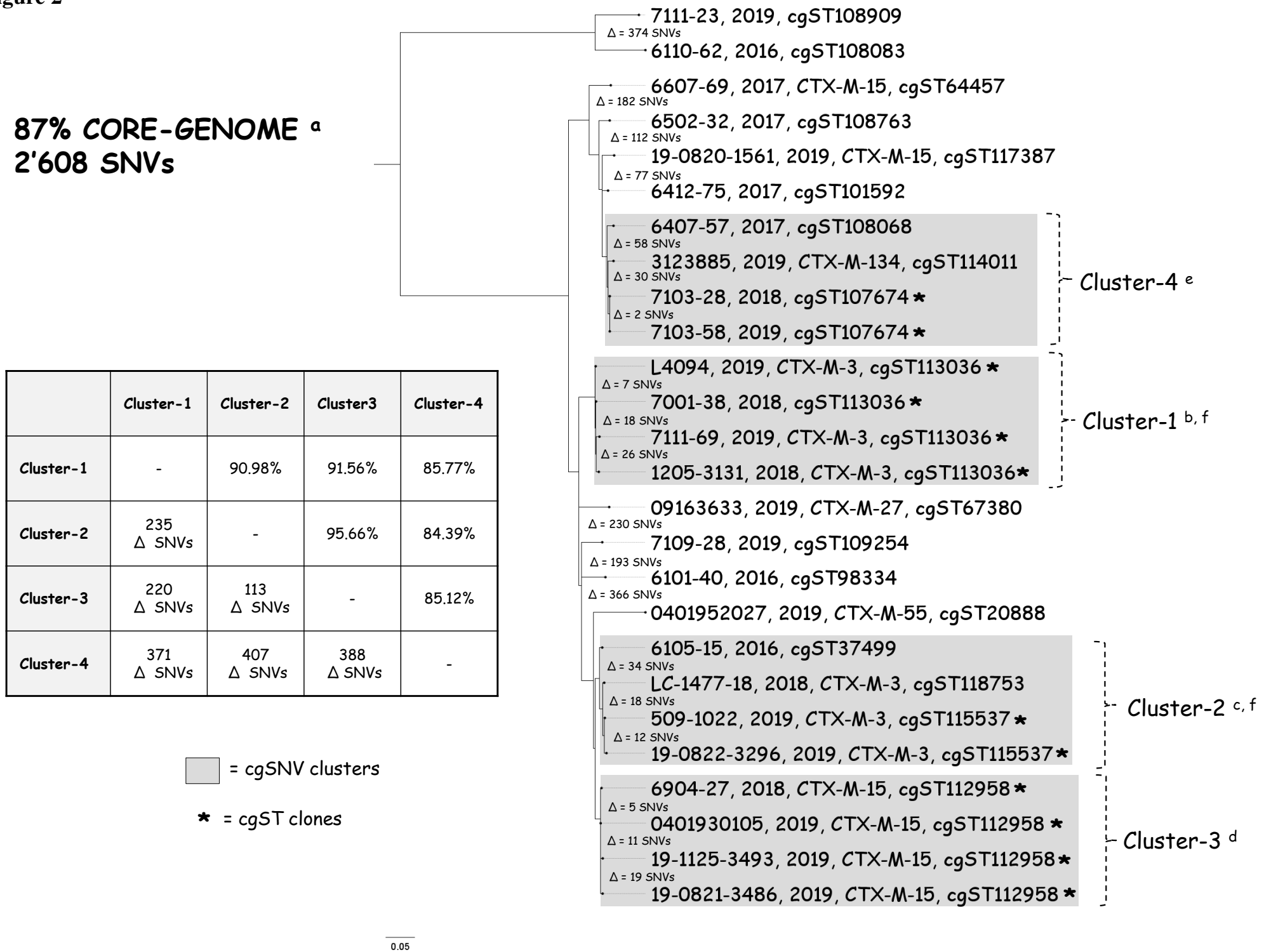
CP049172
S. sonnei, 2019
Cluster 3
p0401930105
(83'337 bp)

CP049170
S. sonnei, 2019
Cluster 3
p19-1125-3493
(83'333 bp)

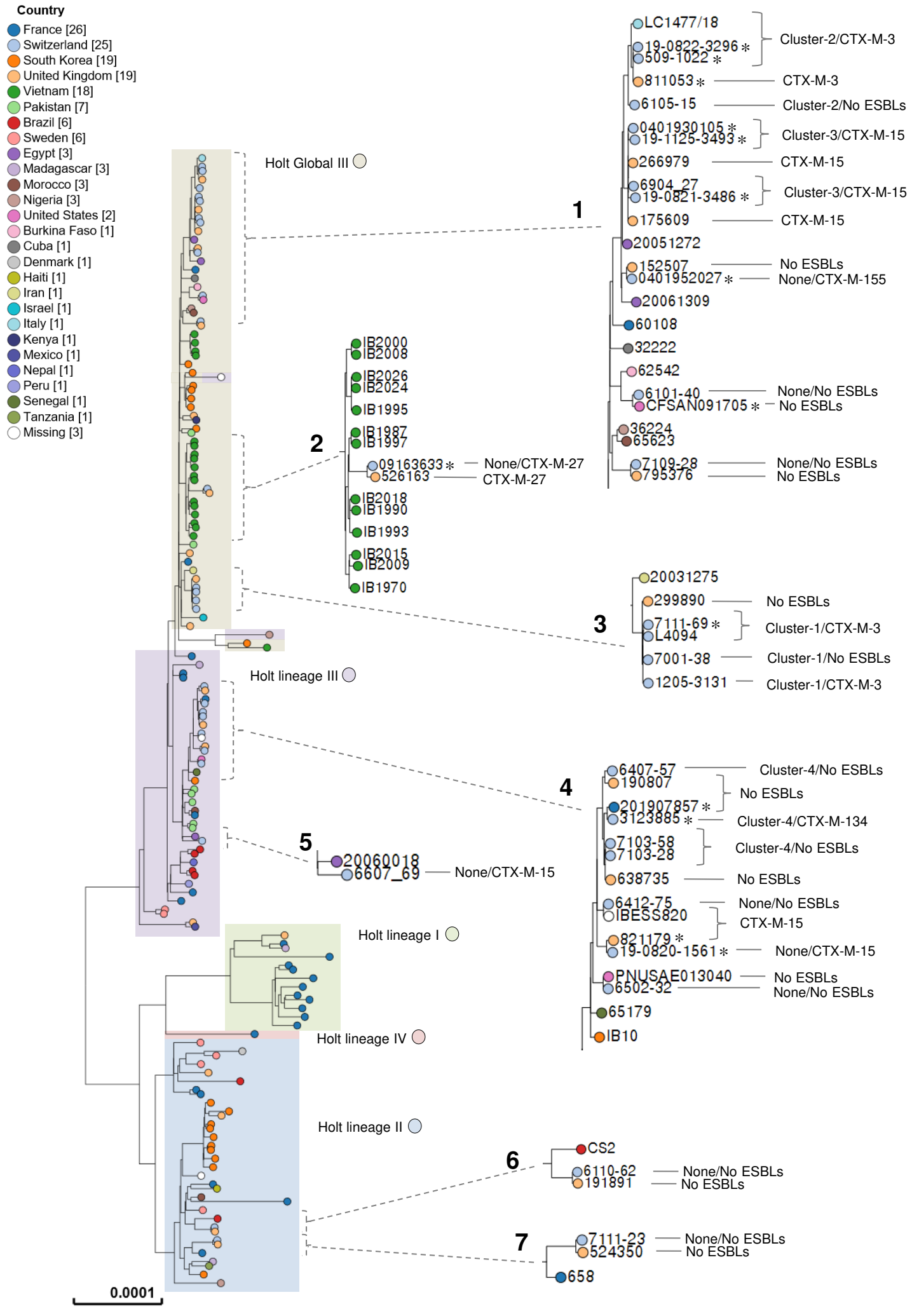
CP045525.2
S. sonnei, 2018
Cluster 3
p6904-27
(83'273 bp)

100% identity
70% identity
50% identity

Figure 2



	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	-


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]
Holt Global III
Holt lineage III
Holt lineage I
Holt lineage IV
Holt lineage II
1
LC1477/18
19-0822-3296 *
509-1022 *
Cluster-2/CTX-M-3
811053 *
CTX-M-3
6105-15
Cluster-2/No ESBLs
0401930105 *
19-1125-3493 *
Cluster-3/CTX-M-15
266979
CTX-M-15
6904_27
19-0821-3486 *
Cluster-3/CTX-M-15
175609
CTX-M-15
20051272
152507
No ESBLs
0401952027 *
None/CTX-M-155
20061309
60108
32222
62542
6101-40
None/No ESBLs
CFSAN091705 *
No ESBLs
36224
65623
7109-28
None/No ESBLs
795376
No ESBLs
2
IB2000
IB2008
IB2026
IB2024
IB1995
IB1987
IB1997
09163633 *
None/CTX-M-27
526163
CTX-M-27
IB2018
IB1990
IB1993
IB2015
IB2009
IB1970
3
20031275
299890
No ESBLs
7111-69 *
L4094
Cluster-1/CTX-M-3
7001-38
Cluster-1/No ESBLs
1205-3131
Cluster-1/CTX-M-3
4
6407-57
190807
Cluster-4/No ESBLs
No ESBLs
201907857 *
3123885 *
Cluster-4/CTX-M-134
7103-58
7103-28
Cluster-4/No ESBLs
638735
No ESBLs
6412-75
IBESS820
None/No ESBLs
CTX-M-15
821179 *
19-0820-1561 *
None/CTX-M-15
PNUSAE013040
No ESBLs
6502-32
None/No ESBLs
65179
IB10
5
20060018
6607_69
None/CTX-M-15
6
CS2
6110-62
191891
None/No ESBLs
No ESBLs
7
7111-23
524350
None/No ESBLs
No ESBLs
658
0.0001