Real-time sampling of travelers shows intestinal colonization by multidrug-resistant bacteria to be a dynamic process with multiple transient acquisitions

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Abbreviations

AMR – Antimicrobial resistance

DEC – diarrheagenic *Escherichia coli*

ESBL – extended-spectrum beta-lactamase

ESBL-Ec – extended-spectrum beta-lactamase (ESBL)-producing *E. coli*

ESBL-GN – extended-spectrum beta-lactamase-producing gram-negative bacteria

ESBL-PE – extended-spectrum beta-lactamase-producing *Enterobacteriaceae*

IQR – inter quartile range

Laos – Lao People's Democratic Republic People's Democratic Republic

LMIC – low- and middle-income countries

mcr – mobile colistin resistance genes

MDR – multidrug-resistant

MDR-GN – multidrug-resistant gram-negative bacteria

TD – travelers' diarrhea

WGS – whole-genome sequencing

Abstract

Background

Antimicrobial resistance (AMR) is highly prevalent in low- and middle-income countries (LMIC). International travel contributes substantially to the global spread of intestinal multidrug-resistant gram-negative (MDR-GN) bacteria. Of the 100 million annual visitors to LMIC, 30–70% become colonized by MDR-GN bacteria. The phenomenon has been well documented, but since sampling has only been conducted after travelers' return home, data on the actual colonization process are scarce. We aimed to characterize colonization dynamics by exploring stool samples abroad on a daily basis while visiting LMIC.

Methods

A group of 20 European volunteers visiting Lao People's Democratic Republic for three weeks provided daily stool samples and filled in daily questionnaires. Acquisition of extended-spectrum beta-lactamase-producing gram-negative bacteria (ESBL-GN) was examined by selective stool cultures followed by whole-genome sequencing (WGS) of isolates.

Results

While colonization rates were 70% at the end of the study, daily sampling revealed that all participants had acquired ESBL-GN at some time point during their overseas stay, the colonization status varying day by day. WGS analysis ascribed the transient pattern of colonization to sequential acquisition of new strains, resulting in a loss of detectable colonization by the initial MDR-GN strains. All but one participant acquired multiple strains (n=2–7). Of the total of 83 unique strains identified (53 *E. coli*, 10 *Klebsiella*, 20 other ESBL-GN species), some were shared by as many as four subjects.

74

75 **Conclusions**

76 This is the first study to characterize in real time the dynamics of acquiring MDR-GN during

77 travel. Our data show multiple transient colonization events indicative of constant microbial

78 competition.

79

80 **Keywords:** antimicrobial resistance; AMR; MDR; ESBL; whole genome sequencing; genomics;

81 mcr; colistin; LMIC

82

83 **Background**

84 Antimicrobial resistance (AMR) poses a serious threat to human health worldwide [1]. The
85 rapid global spread of multidrug-resistant (MDR) clones of *Escherichia coli, Klebsiella*
86 *pneumoniae* and other Enterobacteriaceae raises an alarming public health concern [1].
87 Worldwide dissemination of successful clones such as *E. coli* ST131 has been the primary
88 driver in extended-spectrum beta-lactamase (ESBL)-producing *E. coli* (ESBL-Ec) becoming
89 prevalent among clinical isolates [2,3]. Correspondingly, the global spread of carbapenemase-
90 producing clones of *E. coli* such as ST410 [4] and ST167 [5], and *K. pneumoniae* clones such as
91 CG258 and ST11 [6,7] largely accounts for the rapid emergence of carbapenem resistance in
92 clinical isolates of gram-negative pathogens worldwide.

93

94 The literature shows international travel to be strongly associated with acquisition of MDR-
95 GN strains, mostly ESBL-*Ec* [8–15]. The carriage rate of intestinal MDR-GN bacteria is highest
96 among inhabitants of the Indian subcontinent and Southeast Asia, followed by Africa and
97 South America [16]. Not surprisingly, travelers visiting these high-risk regions are at
98 substantial risk of acquiring MDR-GN bacteria [15]. Colonization occurs even during short
99 visits [8–15] and without antimicrobial use [17] and can last for months or even over a year
100 [8,12,13] and lead to further spread after return home [8,13]. Genome-level analysis of MDR
101 strains colonizing travelers shows that newly acquired MDR strains tend to displace resident
102 intestinal commensal *E. coli* strains alongside new non-MDR strains, such that the pre-travel
103 population remains as a minority [18].

104

105 In previous studies of travelers' intestinal colonization by MDR-GN bacteria, samples have
106 been taken immediately prior to travel and upon return to home country [8–14]. As such the

107 dynamics of this competitive colonization process are unknown. Here we present a
108 longitudinal study carried out with volunteers visiting the Lao People's Democratic Republic
109 (Laos). Combining daily samples from these travelers during their stay and fine-scale genomic
110 analysis enabled us to characterize the colonization dynamics.
111
112 **Methods**
113 **Study aim, design and setting**
114 To obtain data on the dynamics of intestinal MDR bacteria acquisition, we characterized the
115 colonization process by ESBL-GN by sampling participants' stools on arrival, each day abroad,
116 and at departure. The study was conducted over 22 days. The specimens were examined for
117 presumptive ESBL-GN by culture, and the isolates were analyzed by whole-genome
118 sequencing. The study protocol was approved by the Ethics Committee of the Helsinki
119 University Hospital and the Ethikkommission Nordwest- und Zentralschweiz. All subjects
120 provided written informed consent.
121
122 **Characteristics of volunteers, samples, and travel destination**
123 Volunteers were recruited prospectively among participants at a medical course held 19
124 September – 9 October, 2015 in Vientiane, Laos. We also invited the recruitees' companions
125 to volunteer. While staying there, each volunteer was asked to provide daily stool samples.
126 Those collected within the first two days were considered as baseline samples and the final
127 stools as departure samples. Questionnaires assessing background information and travel-
128 related data were used at recruitment and before departure. During the stay, the volunteers
129 were asked to complete a health card to record gastrointestinal symptoms, food habits, and
130 medication use each day. Travelers' diarrhea (TD) was defined as passage of ≥ 3 loose or liquid

131 stools per day. Any ESBL-GN not found in the baseline samples but detected in one or more
132 stool samples taken later were defined as travel-acquired ESBL-GN. Only volunteers providing
133 at least five daily samples were included in the final subject group (Fig. 1).
134
135 **Stool cultures and phenotypic susceptibility testing**
136 The initial screening for presumptive ESBL-GN strains from stool samples was conducted in
137 Vientiane at the Microbiology Laboratory of Mahosot Hospital by culture on CHROMagar™
138 ESBL agar plates (CHROMagar, Paris France). Phenotypically distinct colonies were
139 subcultured and stored at -80°C in Protect tubes (Technical Service Consultants Ltd, Heywood,
140 UK) along with the original swabs. The samples were then transported by plane on dry ice
141 first to the Swiss Tropical and Public Health Institute in Basel, and then to the University of
142 Helsinki (Finland) for analyses. In Finland, the bacteria were re-cultured on an equivalent
143 chromogenic media, ChromID ESBL agar (BioMérieux, Marcy-l'Étoile, France), and a colony of
144 all morphotypes present on the ChromID plate was subcultured and cryopreserved. The
145 isolates were shipped on dry ice to the University of Birmingham for genome sequencing by
146 the MicrobesNG facility (http://microbesng.uk). Libraries were prepared using the Nextera XT
147 kit, and sequenced on the Illumina HiSeq platform.
148
149 **Genomic analyses**
150 Illumina genome sequence reads were trimmed using Trimmomatic (V 0.3) [19] with a sliding
151 window quality of Q15 and length of 20 base pairs. *De novo* assembled genomes were
152 produced using SPAdes (V 3.13.0) [20]. Resulting assembled genomes were annotated using
153 Prokka (V 1.11) [21]. Antibiotic resistance genes were detected in assembled and annotated
154 genomes using Abricate (V 0.8.7, https://github.com/tseemann/abricate) and the Resfinder

155 database. Prokka-annotated genomes were manually inspected to confirm the presence of
156 resistance genes identified. MLST (V 2.15, https://github.com/tseemann/mlst) was used to
157 verify species identification and assign classical sequence type designations to isolates. Where
158 isolates were suspected of sharing recent source or transmission events, Snippy (V 4.3.6,
159 https://github.com/tseemann/snippy) was used to map reads of isolates against the
160 assembled genome of the earliest relative isolate. The number of SNPs between strains was
161 determined using snp-dists (V 0.6.3, https://github.com/tseemann/snp-dists).
162
163 **Results**
164 **Description of participants, travel, and symptoms during stay**
165 A total of 23 volunteers were recruited, three of whom had to be later excluded for having
166 only provided two samples (of note, ESBL-GN was found in them all). The final study
167 population thus comprised 20 European volunteers. Of the volunteers, 50% were aged < 50
168 years, 55% were female, and 19 of them were medical doctors. The median age was 42.5
169 years (IQR 33.5−57.0), and the median duration of stay in Laos was 20 (IQR 12−21) days. Five
170 participants (25%) had used antimicrobial medication during the previous year, three (15%)
171 arrived directly from another tropical region, one (5%) had visited the tropics within the past
172 three months and seven (35%) within the last year (Table S1). The group provided a total of
173 236 stool samples.
174
175 Over the sampling period, the volunteers stayed at three separate hotels, visited various
176 restaurants either in small groups or all together, and participated in daily rounds at local
177 hospitals. Four of the twenty participants reported TD, and one took antibiotics (Table S1).
178

179 **All participants were colonized by ESBL-GN, dominated by *E. coli***

180 ESBL-GN strains were detected in the primary samples of seven of the 20 volunteers, including
181 three who had arrived directly from another Asian destination. For all volunteers at least one
182 stool sample tested positive for ESBL-GN in culture by day 10 of their visit (Fig 2). Of the 236
183 fecal samples collected, 73.7% (n=174) contained detectable ESBL-GN, yielding a total of 306
184 isolates. *E. coli* was the most abundant species isolated, accounting for 219 of the 306 isolates,
185 followed by *Citrobacter* spp. (28 isolates), *Klebsiella* spp. (16), *Acinetobacter* spp. (12),
186 *Enterobacter cloacae* (11), and a number of other low-prevalence species including
187 *Aeromonas* spp. and *Stenotrophomonas maltophilia* (Table S2). When allocating individual
188 isolates to the participants, colonization by any given ESBL-GN during the study period was
189 clearly transient in nature, with isolates detected in only one or a few samples obtained from
190 any given individual, sometimes with days between isolation.

191

192 **Genomic analyses of isolates identified high variability in colonization both at strain and**
193 **species level**

194 The genomes of all 306 isolates were found to contain at least one antimicrobial resistance
195 gene. The most prevalent ESBL gene type was $bla_{\text{CTX-M}}$, found in a total of 226 isolates (74%),
196 with CTX-M-55 (n=64), CTX-M-14 (n=58), CTX-M-159 (n=57), CTX-M-15 (n=30), and CTX-M-
197 102 (n=25) as the most common types (Table S3). Some isolates contained multiple CTX-M
198 types. Mobile colistin resistance genes (*mcr*) were found in 82 isolates (28%), all but two of
199 which were *E. coli* (one *Aeromonas* sp., the other *K. pneumoniae*). Superimposing MLST
200 designation (Figure 2, Figure S1) and *bla/mcr* gene type (Figure 3) onto each individual
201 traveler's isolates provided further insight into the transient nature of gut colonization. Whilst
202 participant 11 was found to have contracted a single ST2067 strain of *E. coli* carrying CTX-M-

203 15, the others were colonized by multiple STs of *E. coli* and co-colonized by other ESBL-GN
204 species. For example, participant 34, who took azithromycin for TD on 21–23 September, was
205 transiently colonized by five different *E. coli* strains over the study period, each with different
206 *bla* gene repertoires. Participant 16 showed a regular flux between isolation of an ST38, ST93,
207 and ST101 strain, whilst participant 40 had initial colonization by an ST48 strain later displaced
208 by an ST38. All these strains have unique signatures of carriage of multiple *bla* genes,
209 indicating that travelers are exposed to a large number of MDR-GN bacteria and MDR-
210 conferring genes during the initial colonization process. All but one participant acquired
211 multiple strains (n=2–7).
212
213 **Uncommon population structure of ESBL-GN isolates**
214 We analyzed the population of *E. coli* isolates at MLST designation level (Figure 4). The most
215 common *E. coli* sequence types identified were ST101, ST34, ST38, and ST195. These lineages
216 are very uncommon in surveys of ESBL and carbapenem-resistant *E. coli*, both in Europe [3]
217 and indeed in previous human isolates from Laos [22,23]. Superimposing the presence of
218 specific ESBL-Ec and *mcr* genes onto a phylogenetic tree of the *E. coli* isolates revealed bla_{CTX-}
219 $_{M}$ genes to be ubiquitous throughout the sampled population of isolates, with *mcr* genes also
220 widely distributed across the population (Figure S2). Analysis of the *K. pneumoniae* lineages
221 showed isolates belonging predominantly to ST2176 and ST37, none of which are well
222 characterized globally disseminated clones [6,24].
223
224 **Fine-scale genomic analysis identified common strains colonizing participants**
225 When analyzing the population structure of the *E. coli* isolates, very little diversity was found
226 in strains within each of the different lineages. To investigate relationships between isolates

227 within the lineages, we carried out a high-resolution SNP analysis using the first isolated strain
228 as a reference. Our data showed a number of common strains colonizing participants, often
229 with zero SNPs' difference between them (Figure 5). An identical ST515 strain colonized
230 participants 6, 17, 33, and 5, whilst an identical ST38 colonized participants 5, 40, and 13.
231 Participants 19 and 34 shared an identical ST34 strain, whilst participants 6 and 21 shared an
232 identical ST385 strain. Participant 11 was colonized on day 8 by an ST2067 isolate that was
233 also isolated from a companion of Participant 11 (11B) on the following day (Figure 5).
234

235 **Discussion**

236 Combining personal data of 20 European visitors to Laos with fine-scale genomic analyses of
237 the strains isolated from their daily fecal samples, we demonstrated that colonization by an
238 ESBL-GN strain during travel to endemic regions is an extremely dynamic process. We
239 identified a constant influx of newly acquired ESBL-GN strains in all but one of the twenty
240 participants. Over the duration of their visits, the volunteers were colonized by up to seven
241 different strains, and often acquired multiple ESBL-GN species. Few traveler studies have
242 employed genome-level analyses [18], but several have reported isolating more than one new
243 colonizing ESBL-GN strain from post-travel samples [10–14]. Our data reveal the true scale
244 and complexity at which drug-resistant bacteria colonize the intestinal tract during travel,
245 demonstrating that it has been seriously underestimated, both with respect to rates of
246 volunteers acquiring MDR-GN and number of individual strains contracted. In addition, our
247 data clearly show that several of our participants lost some of their travel-acquired ESBL-GN
248 strains while still abroad. This indicates that previous studies solely employing pre- and post-
249 travel sampling have under-reported the actual extent to which travelers are colonized by
250 ESBL-GN. The result is especially relevant if we assume that MDR-GN simply drop below

251 detection level but do not vanish completely, only to resurface once selection pressure
252 increases again. There is a potential caveat to our study in that the apparent cyclic
253 disappearance and re-appearance of strains may be related to the sensitivity of the culture
254 methods used, and colonizing strains may occasionally have been missed when picking
255 colonies for sequencing.
256
257 Our fine-scale genomic analysis enabled identification of a number of strains shared by our
258 volunteers. Some of the strains colonized up to four participants, often with zero SNPs'
259 difference across the entire genome, and with a maximum of five SNPs' difference between
260 shared strains. Whilst direct transmission cannot be confirmed, the clonality of the isolates
261 suggests that the two colonization events did not result from exposure to a common
262 environmental reservoir. Such reservoirs are generally colonized by bacteria for extended
263 periods of time, which leads to extensive diversity within the bacterial population [25,26].
264 Thus, direct transmission or acquisition through common exposure such as consumption of
265 food or water appears the most likely explanation.
266
267 The population of ESBL-Ec isolates in this study has an unexpected composition.
268 Epidemiological surveys of multidrug-resistant *E. coli*, especially those focusing on ESBL
269 strains, are dominated by *E. coli* ST131 [27]. Epidemiological investigations carried out on *E.*
270 *coli* in Laos have also shown ST131 to be the dominant drug-resistant lineage in the country
271 [22,23]. However, we isolated no ST131 strains, and ST38 was the only lineage in our study
272 that has been reported in studies previously conducted in Laos. These studies investigated
273 both intestinal colonization isolates [23] and clinical blood stream isolates [22], suggesting
274 that the ESBL-Ec population in Laos may be particularly dynamic and prone to frequent

275 fluctuation. Interestingly, ST101, ST34, and ST195, and other lineages frequently isolated in
276 our study have never been reported as clinical ESBL-Ec isolates in countries such as the United
277 Kingdom where high-quality longitudinal data are available [3]. Some reports describe ESBL-
278 Ec ST101 [28], but none report ST34 or ST195. *E. coli* ST38 has been extensively reported as
279 an ESBL-Ec strain isolated both from humans and animals [29–31].
280
281 Most previous studies among travelers have only described acquisition of ESBL-*E. coli.* A few
282 have reported multiple findings of ESBL-DEC (diarrheagenic *E. coli*) [32] or single findings of
283 ESBL-*Klebsiella* [11,14] or carbapenemase-producing *E. coli* [12,13]. Our data show, in
284 addition to ESBL- *E. coli* (219 of 306 strains, 72%) a substantial number of ESBL-producing non-
285 *E. coli* Gram-negative bacteria, such as *Citrobacter* (9%), *Klebsiella* (5%), *Acinetobacter* (4%),
286 and *Enterobacter cloacae* (4%) and even low numbers of *Aeromonas spp.* and
287 *Stenotrophomonas maltophilia*. This finding may be ascribed to an especially high rate of
288 exposure to a variety of MDR-GN bacteria, since 19 of our volunteers attended a course of
289 tropical medicine which included daily clinical rounds at local hospitals. High MDR-GN
290 colonization rates have been reported among travelers hospitalized in the tropics [33].
291
292 The complete absence of carbapenemase-producing *E. coli* in these samples is also
293 noteworthy. We screened for ESBL-producing strains, but carbapenemase producers would
294 also have grown on the selective plates used. However, WGS analysis showed a complete
295 absence of carbapenemase genes, a finding somewhat surprising in Southeast Asia where the
296 prevalence of carbapenem-resistant Enterobacteriaceae is increasing [34]. Such isolates have
297 only recently been reported in Laos [35], suggesting that carbapenem resistance has not yet

298 become a major problem in the country. Additionally, other traveler studies have shown a
299 low carriage rate of carbapenemase genes [36].
300
301 Even more striking were the extremely high levels of the mobile colistin resistance gene *mcr*
302 in our *E. coli* isolates. The ST101 lineage which dominated our isolate collection has been
303 identified as a driving lineage in the emergence of ESBL-Ec, and *mcr* positive *E. coli* in the
304 region [37], but we observed the *mcr* gene across a large number of lineages, a finding which
305 may be of substantial importance. This confirms earlier reports showing that travel
306 exacerbates the global spread of not only ESBL genes but also *E. coli* strains carrying mobile
307 colistin resistance genes [38].
308
309 **Conclusions**
310 By combining real-time sampling of travelers with genome-level analyses, we have
311 demonstrated that colonization by ESBL-GN during travel is an extremely dynamic process
312 characterized by competition between resistant strains and an individual's own microbiota.
313 We have also shown that prevalent strains can colonize multiple travelers via shared routes
314 such as transmission or acquisition from a common source. The challenge now lies in
315 unravelling the mechanisms that underlie this process and competition between the clones,
316 as well as finding tools to prevent colonization already at its initial stages.
317

Declarations

Ethics approval and consent to participate

The study protocol was approved by the Ethics Committee of the Helsinki University Hospital and the Ethikkommission Nordwest- und Zentralschweiz. All subjects provided written informed consent.

Consent for publication

Not applicable.

Availability of data and materials

Raw sequence data for all isolates is available via NCBI under the Bioproject accession number PRJNA558187.

Competing interests

A. K. has received honoraria from Valneva and Immuron, and investigator-initiated grants from Pfizer and Valneva, none of them relevant to the submitted work.

All other authors report no potential conflicts of interest.

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Authors' contributions

Authors AK, EK and SD contributed equally to this manuscript. Authors JC and AM contributed equally to this manuscript. Study concept, design and organization AK, EK; sample and epidemiological data collection: AK, EK, DABD, PNP, VD, SM, SHP, AN, CH; funding for WGS: AM, JC; genomic analyses: SD, AS, TK, JC, AM; data interpretation: SD, AS, JC, AM; data visualisation: SD, AS; drafting of manuscript AK, EK, SD, TK, JC, AM; critical comments on the manuscript: DABD, PNP, VD, SM, SHP, AN, CH, AS; all authors read and approved the final manuscript.

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492
493

494 **Figure 1.** Overview of sampling and analysis workflow with inclusion criteria.

495 This study recruited 23 participants who provided daily stool samples. These samples were
496 screened for gram-negative extended-spectrum beta-lactamase-producing gram-negative
497 (ESBL-GN) bacteria. Participants who produced less than four stool samples (due to
498 constipation, for example) were excluded from the final study. Following selective culture,
499 isolates were whole-genome sequenced and analysed to determine sequence type,
500 resistance profiles, and genomic homology.

501

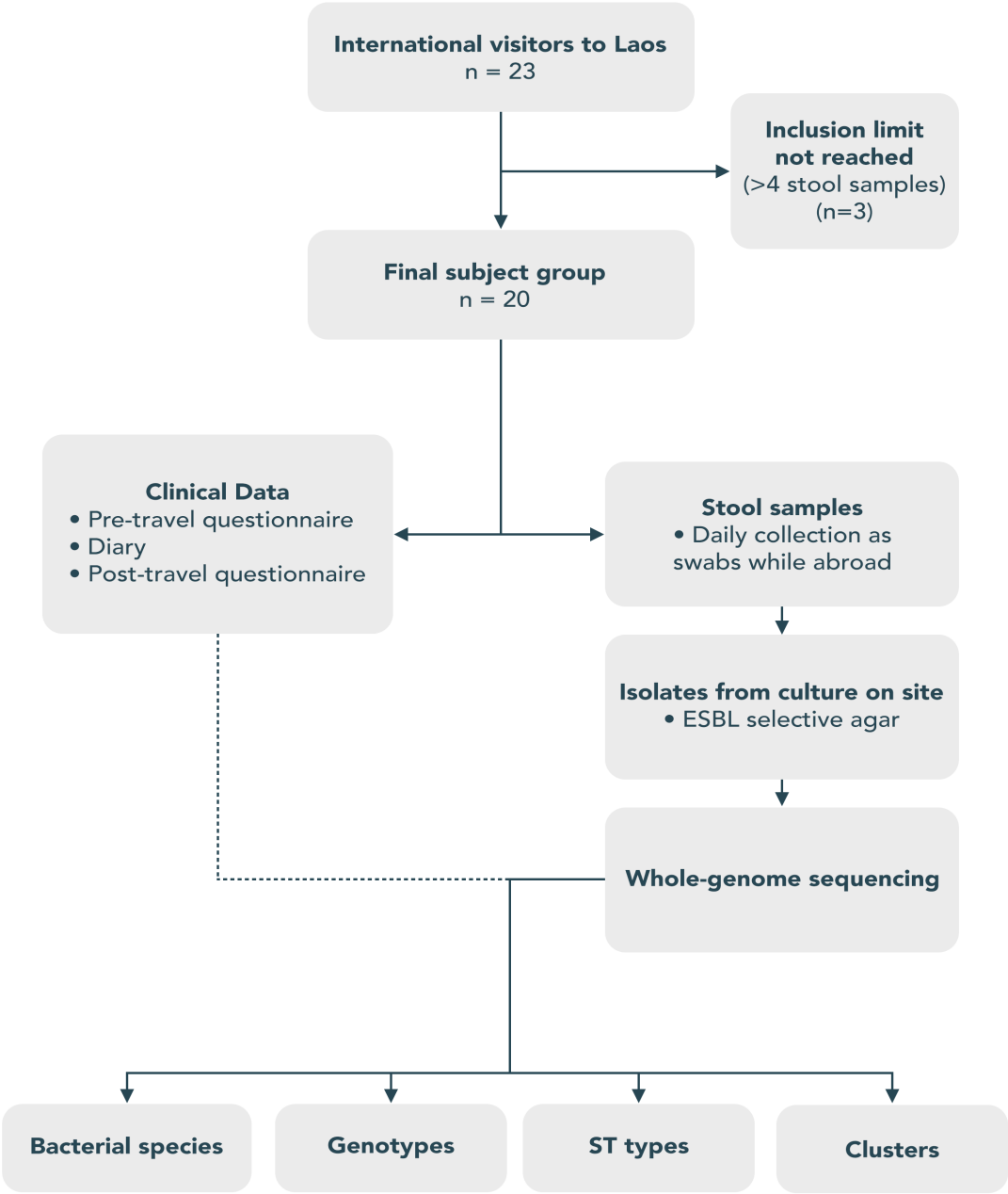
502 **Figure 2.** Colonization of participants by ESBL-producing gram-negative (ESBL-GN) over a 21-
503 day period in Lao PDR.

504 Patterns of colonization vary dramatically between participants. Some harbored a single
505 dominant strain, whilst more transient and frequent strains were detected in the stool
506 samples of others. Some participants appeared to have carried a considerably higher load of
507 ESBL bacteria (e.g. 21, 34), while others only had one or two samples with ESBL isolates
508 (e.g.18, 26). Solid lines represent uninterrupted and concurrent colonization by a single strain.
509 Dashed lines represent maintenance of a single strain over multiple days interrupted by
510 colonization by another strain of the same species or a period of no growth. Strains are shown
511 by participant, with a maximum of five differing strains identified from the samples of a single
512 individual. Due to the large number of constituent strains in the database, the color
513 designations do not represent the same strains across multiple volunteers.

514

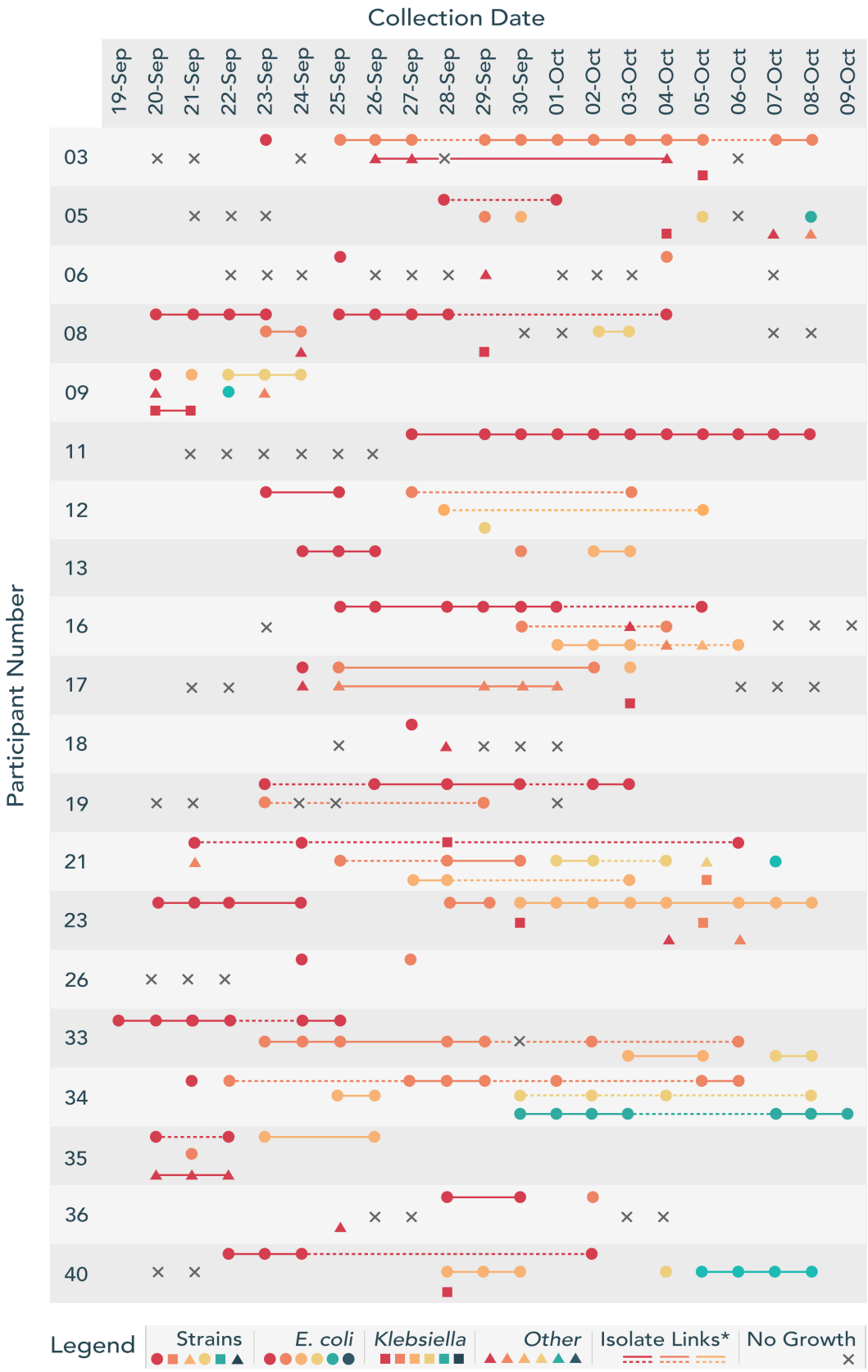
515 **Figure 3.** Resistance determinants identified in ESBL-GN isolates over a 21-day period in Lao
516 PDR.

517 We detected a highly diverse and abundant set of resistance determinants with numerous
518 CTX-M variants, and a surprising abundance of MCR (28% carriage rate). We also found less
519 common ESBL types, such as CMY, VEB, and CFE. Frequent patterns of resistance genes co-
520 occurred in the same patient, overlapping with instances where a single colonizing strain was
521 identified (detailed in Figure 2). Some of these also extended to other participants, (further
522 characterized in Figure 5). Beta-lactamase genes are encoded using symbols, MCR = square,
523 CMY = triangle, TEM = diamond, OXA = pentagon, SED = star, ACT = hexagon, VEB = inverted
524 triangle, CFE = kite. CTX-M enzymes are encoded in colored circles, with CTX-M subtype
525 encoded by different hues. CTX-M-14 = red, 15 = dark orange, 40 = pale orange, 55 = yellow,
526 65 = teal, and 102 = navy.
527
528 **Figure 4.** Abundance of unique sequence types amongst the traveler cohort over the 21-day
529 study period.
530 Isolates were examined using a high-resolution SNP analysis. Strains exhibiting genetic
531 heterogeneity were considered unique, and their sequence type was recorded. ST-101 was
532 the most abundant sequence type observed.
533
534 **Figure 5.** Linkage of isolates between participants.
535 High resolution SNP analysis identified several instances of a single strain colonizing multiple
536 participants. These strains differed by ≥5 SNPs, with the most prolifically shared strain (ST-
537 515) found in participants 06, 17 and 33. Participant 11B represents a contact of participant
538 11, who was found to be colonised by a shared strain. Solid lines represent isolates that
539 were identical (i.e. 0 SNP's difference). Dashed lines represent isolates that were found to
540 contain between 1-5 SNPs.


International visitors to Laos
n = 23
Inclusion limit not reached
(>4 stool samples)
(n=3)
Final subject group
n = 20
Clinical Data
• Pre-travel questionnaire
• Diary
• Post-travel questionnaire
Stool samples
• Daily collection as swabs while abroad
Isolates from culture on site
• ESBL selective agar
Whole-genome sequencing
Bacterial species
Genotypes
ST types
Clusters

541

542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19-Sep
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21-Sep
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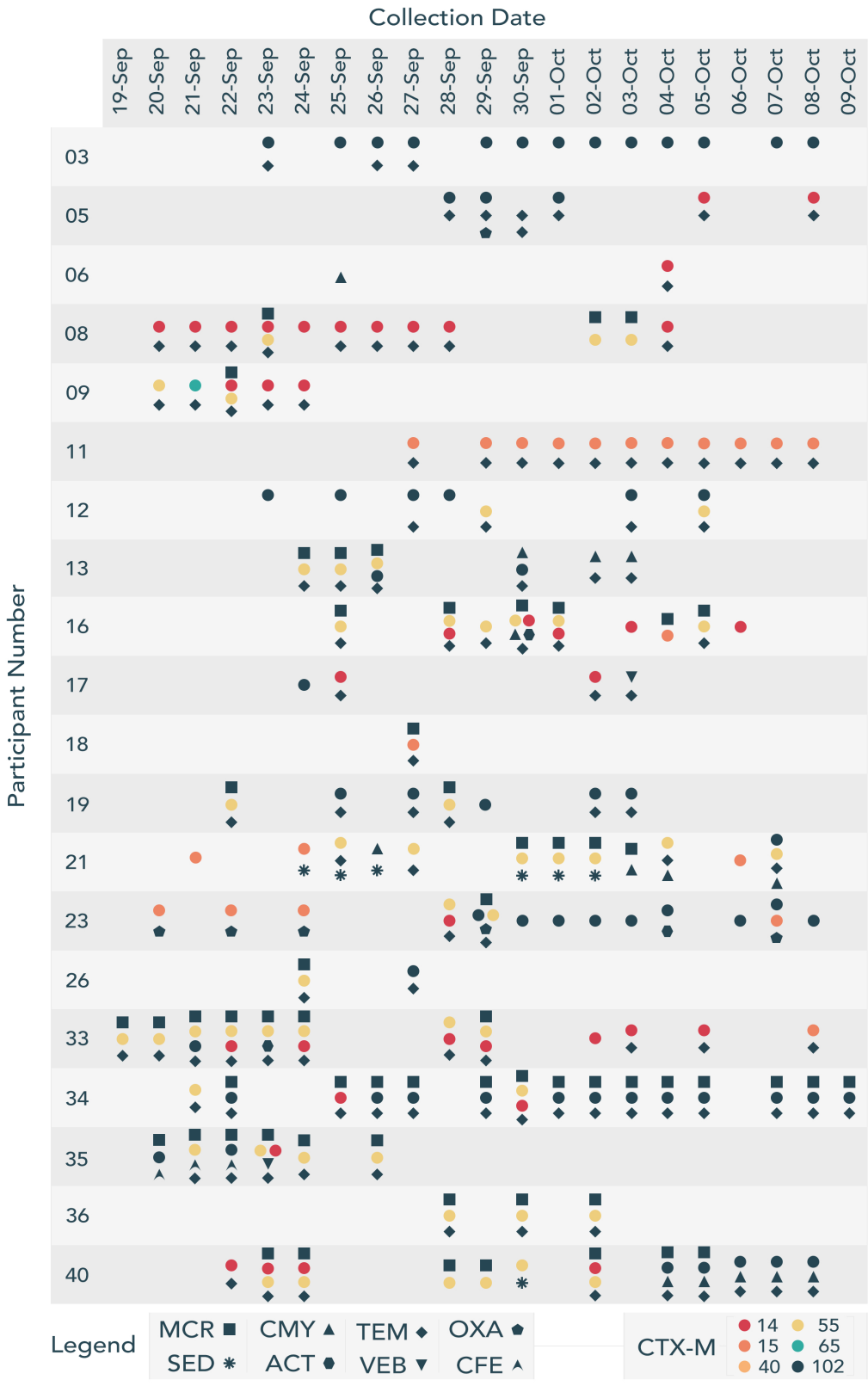
23-Sep
24-Sep
25-Sep
26-Sep
27-Sep
28-Sep
29-Sep
30-Sep
01-Oct
02-Oct
03-Oct
04-Oct
05-Oct
06-Oct
07-Oct
08-Oct
09-Oct
Participant Number
03
05
06
08
09
11
12
13
16
17
18
19
21
23
26
33
34
35
36
40
Legend
Strains
E. coli
Klebsiella
Other
Isolate Links*
No Growth

543

544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Participant Number
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26
33
34
35
36
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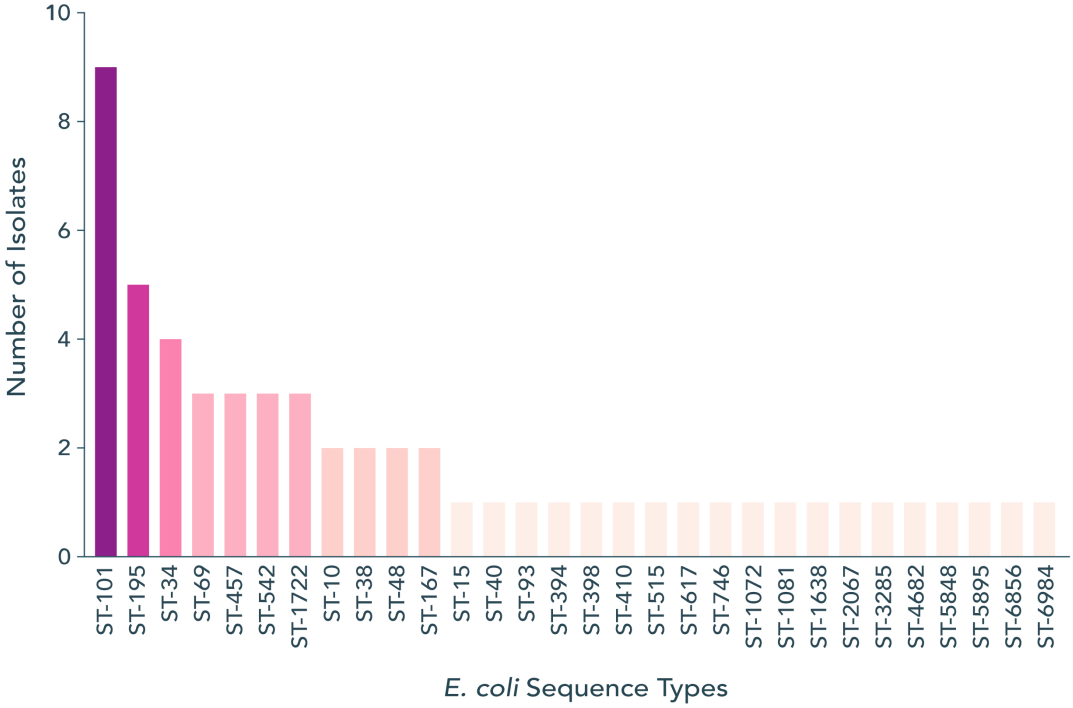
Legend
MCR
CMY
TEM
OXA
SED
ACT
VEB
CFE
CTX-M
14
55
15
65
40
102

545


Number of Isolates
10
8
6
4
2
0
ST-101
ST-195
ST-34
ST-69
ST-457
ST-542
ST-1722
ST-10
ST-38
ST-48
ST-167
ST-15
ST-40
ST-93
ST-394
ST-398
ST-410
ST-515
ST-617
ST-746
ST-1072
ST-1081
ST-1638
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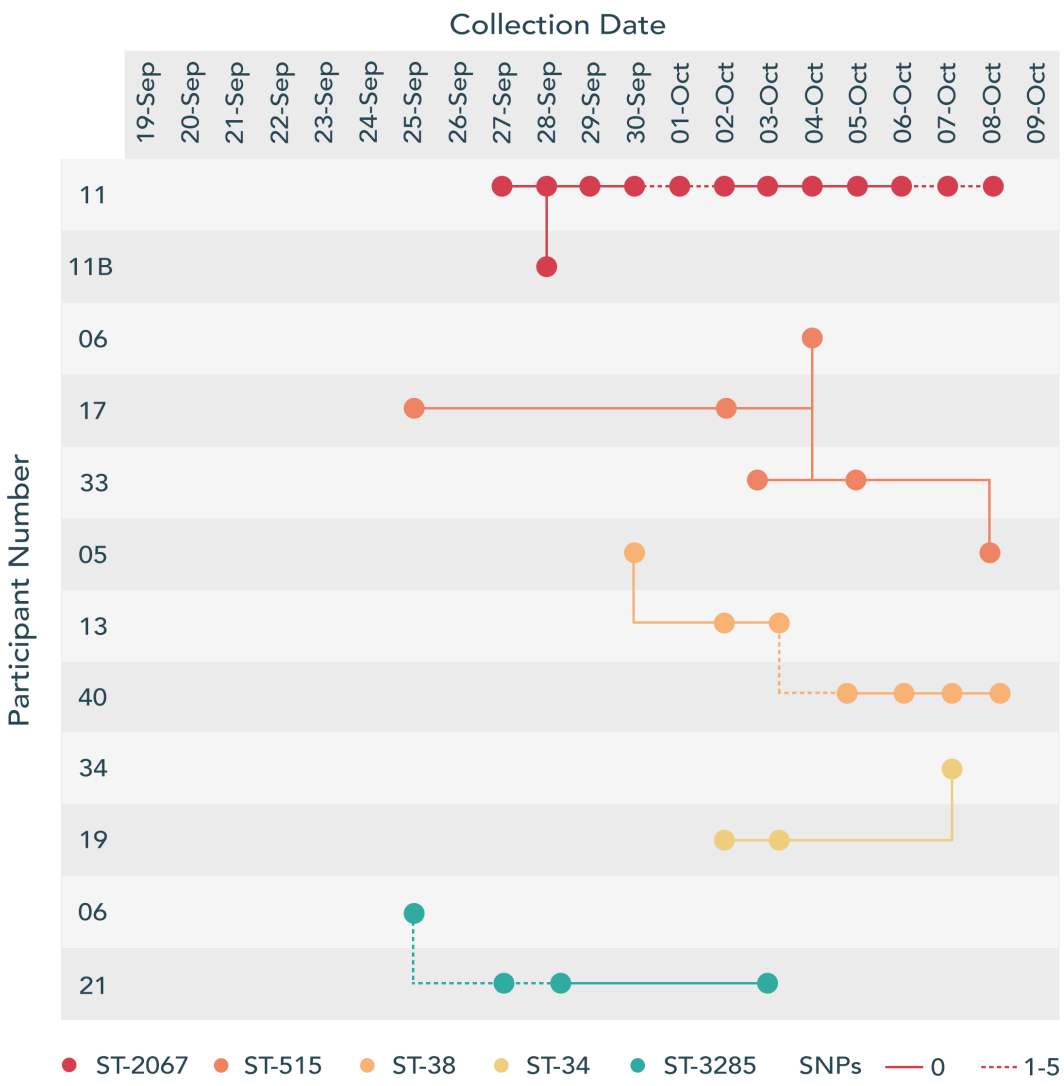
ST-4682
ST-5848
ST-5895
ST-6856
ST-6984
E. coli Sequence Types

546

547


Collection Date
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28-Sep
29-Sep
30-Sep
01-Oct
02-Oct
03-Oct
04-Oct
05-Oct
06-Oct
07-Oct
08-Oct
09-Oct
Participant Number
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11B
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33
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ST-2067
ST-515
ST-38
ST-34
ST-3285
SNPs
0
1-5

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