Genomic diversity of *Escherichia coli* from healthy children in rural Gambia

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Little is known about the genomic diversity of *Escherichia coli* in healthy children from sub-Saharan Africa, even though this is pertinent to understanding bacterial evolution and ecology and their role in infection. We isolated and whole-genome sequenced up to five colonies of faecal *E. coli* from 66 asymptomatic children aged three-to-five years in rural Gambia (n=88 isolates from 21 positive stools). We identified 56 genotypes, with an average of 2.7 genotypes per host. These were spread over 37 seven-allele sequence types and the *E. coli* phylogroups A, B1, B2, C, D, E, F and *Escherichia* cryptic clade I. Immigration events accounted for three-quarters of the diversity within our study population, while one-quarter of variants appeared to have arisen from within-host evolution. Several isolates encode putative virulence factors commonly found in Enteropathogenic and Enteroaggregative *E. coli,* and 53% of the isolates encode resistance to three or more classes of antimicrobials. Thus, resident *E. coli* in these children may constitute reservoirs of virulence- and resistance-associated genes. Moreover, several study strains were closely related to isolates that caused disease in humans or originated from livestock. Our results suggest that within-host evolution plays a minor role in the generation of diversity compared to independent immigration and the establishment of strains among our study population. Also, this study adds significantly to the number of commensal *E. coli* genomes, a group that has been traditionally underrepresented in the sequencing of this species.

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Abstract

Little is known about the genomic diversity of *Escherichia coli* in healthy children from sub-Saharan Africa, even though this is pertinent to understanding bacterial evolution and ecology and their role in infection. We isolated and whole-genome sequenced up to five colonies of faecal *E. coli* from 66 asymptomatic children aged three-to-five years in rural Gambia (n=88 isolates from 21 positive stools). We identified 56 genotypes, with an average of 2.7 genotypes per host. These were spread over 37 seven-allele sequence types and the *E. coli* phylogroups A, B1, B2, C, D, E, F and *Escherichia* cryptic clade I. Immigration events accounted for three-quarters of the diversity within our study population, while one-quarter of variants appeared to have arisen from within-host evolution. Several isolates encode putative virulence factors commonly found in Enteropathogenic and Enteroaggregative *E. coli*, and 53% of the isolates encode resistance to three or more classes of antimicrobials. Thus, resident *E. coli* in these children may constitute reservoirs of virulence- and resistance-associated genes. Moreover, several study strains were closely related to isolates that caused disease in humans or originated from livestock. Our results suggest that within-host evolution plays a minor role in the generation of diversity compared to independent immigration and the establishment of strains among our study population. Also, this study adds significantly to the number of commensal *E. coli* genomes, a group that has been traditionally underrepresented in the sequencing of this species.

Keywords: *Escherichia coli*, genomic diversity, within-host evolution.

Introduction

Ease of culture and genetic tractability account for the unparalleled status of *Escherichia coli* as "the biological rock star", driving advances in biotechnology (Blount 2015), while also providing critical insights into biology and evolution (Good et al. 2017). However, *E. coli* is also a widespread commensal, as well as a versatile pathogen, linked to diarrhoea (particularly in the under-fives), urinary tract infection, neonatal sepsis, bacteraemia and multi-drug resistant infection in hospitals (Camins et al. 2011; Rodríguez-Baño et al. 2010; Russo & Johnson 2003). Yet, most of what we know about *E. coli* stems from the investigation of laboratory strains, which fail to capture the ecology and evolution of this key organism "in the wild" (Hobman et al. 2007). What is more, most studies of non-lab strains have focused on pathogenic strains or have been hampered by low-resolution PCR methods, so we have relatively few genomic sequences from commensal isolates, particularly from low- to middle-income countries (Ahmed et al. 2014; Ferjani et al. 2017; Moremi et al. 2017; Oshima et al. 2008; Rasko et al. 2008; Stoesser et al. 2015; Touchon et al. 2009).

We have a broad understanding of the population structure of *E. coli,* with eight significant phylogroups loosely linked to ecological niche and pathogenic potential (B2, D and F linked to extraintestinal infection; A and B1 linked to severe intestinal infections such as haemolytic-uraemic syndrome) (Alm et al. 2011; Escobar-Paramo et al. 2004; Mellata 2013; Walk et al. 2009). All phylogroups can colonise the human gut, but it remains unclear how far commensals and pathogenic strains compete or collaborate—or engage in horizontal gene transfer—within this important niche (Laxminarayan et al. 2013; Stoppe et al. 2017).

Although clinical microbiology typically relies on single-colony picks (which has the potential to underestimate species diversity and transmission events), within-host diversity of *E.*

coli in the gut is crucial to our understanding of inter-strain competition and co-operation and also for accurate diagnosis and epidemiological analyses. Pioneering efforts using serotyping and molecular typing have shown that normal individuals typically harbour more than one strain of *E. coli* (Chen et al. 2013; Dixit et al. 2018; Richter et al. 2018), with one individual carrying 24 distinct clones (Chen et al. 2013; Schlager et al. 2002; Shooter et al. 1977). More recently, whole-genome sequencing has illuminated molecular epidemiological investigations (Stoesser et al. 2015), for example, studies of the transmission of extended-spectrum beta-lactamase-encoding *E. coli*, multidrug-resistant *Acinetobacter baumannii*, and the genomic surveillance of multidrug-resistant *E. coli* carriage. Whole-genome data has also been applied to studies of *E. coli* adaptation during and after infection (McNally et al. 2013; Nielsen et al. 2016), as well as the intra-clonal diversity in healthy hosts (Stegger et al. 2020).

There are two plausible sources of within-host genomic diversity. Although a predominant strain usually colonises the host for extended periods (Hartl & Dykhuizen 1984), successful immigration events mean that incoming strains can replace the dominant strain or co-exist alongside it as minority populations (Bettelheim et al. 1972). Strains originating from serial immigration events are likely to differ by hundreds or thousands of single-nucleotide polymorphisms (SNPs). Alternatively, within-host evolution can generate clouds of intra-clonal diversity, where genotypes differ by just a handful of SNPs (Dixit et al. 2018).

Most relevant studies have been limited to Western countries, except for a recent report from Tanzania (Richter et al. 2018), so little is known about the genomic diversity of *E. coli* in sub-Saharan Africa. The Global Enteric Multicenter Study (GEMS) (Kotloff et al. 2013; Liu et al. 2016) has documented a high burden of diarrhoea attributable to *E. coli* (including *Shigell*a) among children from the Gambia, probably as a result of increased exposure to this organism

through poor hygiene and frequent contact with animals and the environment. GEMS was a prospective case-control study which investigated the aetiology of moderate-to-severe diarrhoea in children aged less than five years residing in sub-Saharan Africa and South Asia. In the Gambia, children with moderate-to-severe diarrhoea seeking care at the Basse Health centre in the Upper River Division of the country were recruited, with one to three matched control children randomly selected from the community along with each case. In also facilitating access to stool samples from healthy Gambian children, the GEMS study has given us a unique opportunity to study within-host genomic diversity of commensal *E. coli* in this setting.

Methods

Study population

We initially selected 76 faecal samples from three- to five-four-old (36-59 months) asymptomatic Gambian children, who had been recruited into the GEMS study (Kotloff et al. 2013) as healthy controls from December 1, 2007, to March 3, 2011. Samples had been collected according to a previously described sampling protocol (Kotloff et al. 2012) and the results of the original study are publicly available at ClinEpiDB.org. Ten of the original 76 samples were depleted and were therefore unavailable for processing in this study. Of the remaining 66 stools, 62 had previously tested positive for *E. coli*. GEMS isolated three *E. coli* colonies per stool sample but pooled these into a single tube for frozen storage. Thus, we needed to re-culture the stools with multiple colony picks, as the original isolate collection was unsuitable for the investigation of within-host diversity. Archived stool samples were retrieved from -80^{o}C storage and allowed to thaw on ice. A 100-200 mg aliquot from each sample was transferred aseptically into 1.8ml Nunc tubes for microbiological processing below (Figure 1).

110

111 **Bacterial growth and isolation**

112 1 ml of physiological saline (0.85%) was added to each sample tube and vigorously vortexed at
113 4200 rpm for at least 2 minutes. Next, the homogenised sample suspensions were taken through
114 four ten-fold dilution series. A100 µl aliquot from each dilution was then spread evenly on a
115 plate of tryptone-bile-X-glucuronide differential and selective agar. The inoculated plates were
116 incubated overnight at 37°C under aerobic conditions. Colony counts were performed on the
117 overnight cultures for each serial dilution for translucent colonies with entire margins and blue-
118 green pigmentation indicative of *E. coli*. Up to five representative colonies were selected from
119 each sample and sub-cultured on MacConkey agar overnight at 37°C before storing in 20%
120 glycerol broth at -80°C. Individual isolates were assigned a designation comprised of the subject
121 ID followed by the colony number ("1-5").

122

123 **Genomic DNA extraction and genome sequencing**

124 Broth cultures were prepared from pure, fresh cultures of each colony-pick in 1 ml Luria-Bertani
125 broth and incubated overnight to attain between $10^9 – 10^{10}$ cfu per ml. Genomic DNA was then
126 extracted from the overnight broth cultures using the lysate method described in (Foster-Nyarko
127 et al. 2020). The eluted DNA was quantified by the Qubit high sensitivity DNA assay kit
128 (Invitrogen, MA, USA) and sequenced on the Illumina NextSeq 500 instrument (Illumina, San
129 Diego, CA), using a modified Nextera XT DNA protocol for the library preparation as described
130 previously (Foster-Nyarko et al. 2020). The pooled library was loaded on a mid-output flow cell
131 (NSQ 500 Mid Output KT v2 300 cycles; Illumina Catalogue No. FC-404–2003) at a final

concentration of 1.8 pM, following the Illumina recommended denaturation and loading parameters—including a 1% PhiX spike (PhiX Control v3; Illumina Catalogue FC-110–3001).

Following Dixit et al. (Dixit et al. 2018), we sequenced a random selection of ten isolates twice, using DNA obtained from independent cultures, to help in the determination of clones and the analysis of within-host variants (Supplementary File 1). Bioinformatic analyses of the genome sequences were carried out on the Cloud Infrastructure for Microbial Bioinformatics (CLIMB) platform (Connor et al. 2016).

Genome assembly and phylogenetic analysis

The paired 150bp reads were quality checked and concatenated, then quality checked using the FastQC tool v0.11.7 (Wingett & Andrews 2018) and assembled using SPAdes genome assembler v3.12.0 (Bankevich et al. 2012), under default parameters. The quality of the assemblies was assessed using QUAST v5.0.0, de6973bb (Gurevich et al. 2013). We used Snippy v4.3.2 (https://github.com/tseemann/snippy)—a rapid command line tool that finds SNPs (substitutions and insertions/deletions) between a haploid reference genome and input sequence reads and generates a core SNP alignment which can be used to reconstruct a high-resolution phylogeny—to generate a core-genome alignment based on core SNPs under default parameters. The complete genome sequence of commensal *E. coli* str. K12 substr. MG1655 as a reference strain (NCBI accession: NC_000913.3). From the core-genome alignment, we then reconstructed a maximum-likelihood phylogeny with 1000 bootstrap replicates using RAxML v8.2.4 (Stamatakis 2006), based on a general time-reversible nucleotide substitution model. The phylogenetic tree was rooted using the genomic sequence of *E. fergusonii* as an outgroup (NCBI accession: GCA_000026225.1). The phylogenetic tree was visualised in FigTree v1.4.3

(https://github.com/rambaut/figtree/) and annotated in RStudio v3.5.1 and Adobe Illustrator v 23.0.3 (Adobe Inc., San Jose, California). As recombination is known to be widespread in *E. coli* and can blur phylogenetic signals (Wirth et al. 2006), we detected and masked any recombinant regions of the core-genome alignment using Gubbins (Genealogies Unbiased By recomBinations In Nucleotide Sequences) (Croucher et al. 2015) before the phylogenetic reconstruction. For visualisation, a single colony was chosen to represent replicate colonies of the same strain (ST) with identical virulence, plasmid and antimicrobial resistance profiles and a de-replicated phylogenetic tree reconstructed using the representative isolates. We computed pairwise single nucleotide polymorphism (SNP) distances between genomes from the core-genome alignment using snp-dists v0.6 (https://github.com/tseemann/snp-dists).

Multi-locus sequence typing, Clermont typing

The merged reads were uploaded to EnteroBase (Zhou et al. 2020), where *de novo* assembly and genome annotation were carried out, and *in-silico* multi-locus sequence types (MLST) assigned based on the Achtman scheme, allocating new sequence types (ST) if necessary. EnteroBase assigns phylogroups using ClermontTyper and EzClermont (Clermont et al. 2013; Clermont et al. 2015) and unique core-genome MLST types (cgMLST) based on 2, 513 core loci in *E. coli*. Publicly available *E. coli* sequences in EnteroBase (http://enterobase.warwick.ac.uk/species/index/ecoli) (Zhou et al. 2020) were included for comparative analysis, including 23 previously sequenced isolates obtained from diarrhoeal cases recruited in the GEMS study in the Gambia (Supplementary File 2). The isolates can be searched in EnteroBase using the 'Search Strains' parameter and under 'Strain Metadata',

177 selecting the 'Name' option and entering the study sample name (column 1 of Supplementary
178 File 2) in the 'Value' box.
179
180 **Determination of immigration events and within-host variants**
181 For the whole genome sequences of the strains sequenced twice, we used SPAdes v3.13.2
182 (Bankevich et al. 2012) to assemble each set of reads and map the raw sequences from one
183 sequencing run to the assembly of the other run and vice versa, as described previously (Dixit et
184 al. 2018). Briefly, mapping was done using the BWA-MEM algorithm v0.7.17-r1188 under
185 default parameters to generate a SAM alignment. This was then converted to BAM files using
186 Samtools view v1.9 (Li et al. 2009), sorted and indexed. Next, variants were called and written to
187 a VCF file using Samtools mpileup and the "view" module of BCFtools (which is part of the
188 Samtools v1.9 package) and visualised in Tablet v1.19.09.13 (Milne et al. 2013). The number of
189 SNPs, and their positions were determined and compared between the two steps, counting only
190 those SNPs that were detected in both sets of reads as accurate.
191 In line with (Dixit et al. 2018), isolates belonging to different STs recovered from the same
192 host were considered to be separate strains derived from independent exposures and immigration
193 events. As described in (Dixit et al. 2018), we determined the number of SNP differences that
194 existed between assemblies of the same isolate that were sequenced on two separate occasions,
195 to determine if multiple isolates of the same ST from a single host were distinct variants (clones).
196 If the SNP difference between two isolates belonging to the same ST recovered from the same
197 host was less than the SNP difference between the sequences of the same isolate sequenced on
198 two separate occasions, then the two isolates were taken to represent replicate copies of the same
199 clone. Otherwise, they were considered as within-host variants (separate, distinct clones of the

same strain)—provided the SNP differences between such distinct clones were no more than eleven SNPs. This cut-off was chosen based on an estimated mutation rate of 1.1 SNP per genome per year (Reeves et al. 2011), assuming equal rates of mutation in both genomes being compared. Based on these data, we inferred replicate clones with SNP differences of greater than 11 SNPs to represent a divergence of more than five years. Thus, it seems implausible that such replicate clones would have emerged from within-host evolution, considering the age of the study participants (< 5 years old).

We produced a contingency table to summarise the distribution of variants derived from migration events and within-host evolution and visualised this using a clustered bar graph. We then performed Fisher's exact test to investigate the association between phylogroup and the distribution of variants (migration versus within-host evolution). Our calculations were based on the assumption of independence among the observed phylogroups—that is, the finding of one phylogroup does not preclude or predict the co-occurrence of another.

Accessory gene content

We used ABRicate v0.9.8 (https://github.com/tseemann/abricate) to predict virulence factors, acquired antimicrobial resistance (AMR) genes and plasmid replicons by scanning the contigs against the VFDB, ResFinder and PlasmidFinder databases respectively, using an identity threshold of ≥ 90% and a coverage of ≥ 70%. Virulence factors and AMR genes were plotted next to the phylogenetic tree using the ggtree, ggplot2 and phangorn packages in RStudio v3.5.1. We calculated co-occurrence of AMR genes among study isolates by transforming the binary AMR gene content matrix and visualising this as a heat map using the pheatmap package v 1.0.12 (https://CRAN.R-project.org/package=pheatmap) in RStudio v3.5.1. We computed

Fisher's exact tests between the detected virulence factors and the observed phylogroups in RStudio v3.5.1.

Population structure and comparison of commensal and pathogenic strains

We assessed the population structure using the hierarchical clustering algorithm in EnteroBase. Briefly, the isolates were assigned stable population clusters at eleven levels (from HC0 to HC 2350) based on pairwise cgMLST allelic differences. Hierarchical clustering at 1100 alleles differences (HC1100) resolves populations into cgST (core-genome MLST type) complexes, the equivalent of clonal complexes achieved with the legacy MLST clustering approaches (Zhou et al. 2020). We reconstructed neighbour-joining phylogenetic trees using NINJA (Wheeler 2009), based on clustering at HC1100 to display the population sub-clusters at this level as an indicator of the genomic diversity within our study population and to infer the evolutionary relationship among our strains and others in the public domain.

Next, we interrogated the HC1100 clusters that encompassed our study isolates and Gambian pathogenic isolates recovered from diarrhoeal cases and commensal *E. coli* strains recovered from the GEMS study. For the clusters that encompassed commensal and pathogenic strains belonging to the same ST (HC1100_200 cluster, comprising pathogenic isolates from GEMS cases 100415, 102106 and 102098 and the resident ST38 strain recovered from our study subject 18), we reconstructed both neighbour-joining and SNP phylogenetic trees to display the genetic relationships among these strains. We visualised the accessory genomes for the overlapping STs mentioned above to determine genes associated with phages, virulence factors and AMR. The resulting phylogenetic trees were annotated in Adobe Illustrator v 23.0.3 (Adobe Inc., San Jose, California).

246

247 **Ethical statement**

248 The parent study was approved by the joint Medical Research Council Unit The Gambia-
249 Gambian Government ethical review board (SCC 1331). Written informed consents were
250 obtained from all the study participants as previously reported in (Kotloff et al. 2013). The joint
251 Medical Research Council Unit The Gambia-Gambian Government ethical review board gave
252 approval for the use of the stool samples analysed in this study.

253

254 **Results**

255 **Population structure**

256 The study population included 27 females and 39 males (Supplementary File 3). All but one
257 reported the presence of a domestic animal within the household. Twenty-one samples proved
258 positive for the growth of *E. coli*, yielding 88 isolates (Supplementary File 4). We detected 37
259 seven-allele sequence types (STs) among the isolates, with a fairly even distribution (Figure 2).
260 Five STs were completely novel (ST9274, ST9277, ST9278, ST9279 and ST9281). These study
261 strains were scattered over all the eight main phylogroups of *E. coli* : A (27%), B1 (32%), B2
262 (9%), D (15%), C and F (5% each), E (1%), and the cryptic Clade I (7%), although the majority
263 belonged to phylogroups A and B1 (Table 1). Hierarchical clustering of core genomic STs
264 revealed twenty-seven cgST clonal complexes (Supplementary File 4). The raw genomic
265 sequences of the study isolates have been deposited in the NCBI SRA under the BioProject ID
266 PRJNA658685 (accession numbers SAMN15880274 to SAMN15880361).

267

268 **Within-host diversity**

Just a single ST colonised nine individuals, six carried two STs, four carried four STs, and two carried six STs. We found 56 distinct genotypes, which equates to an average of 2.7 genotypes per host. Two individuals (H-18 and H-2) shared an identical strain belonging to ST9274 (zero SNP difference) (Supplementary File 5, yellow highlight), suggesting recent transfer from one child to another or recent acquisition from a common source.

We observed thirteen within-host variants in ten hosts (intra-clonal diversity) (subjects H-15, H-18, H-22, H-25, H-28, H-34, H36, H37, H-38 and H-39), compared to forty-one immigration events (Tables 1 and 2). Overall, immigration events accounted for the majority (76%) of variants (Supplementary Figure 1). The proportion of migration versus within-host evolution events did not appear to be affected by phylogroup ($p=0.42$). Twenty-two percent of within-host mutations represented synonymous changes, 43% were non-synonymous mutations, while 31% occurred in non-coding regions, and 4% represented stop-gained mutations (Supplementary File 6). On an average, Ka/Ks ratios were greater than 1, which seems to suggest that these mutations were under positive Darwinian selection—indicating that most of the mutations were likely to have little effect on fitness. However, these remain to be investigated further. Also, the observed non-synonymous mutations were spread across genes with a variety of functions, including metabolism, transmembrane transport, pathogenesis and iron import into the cell. However, the bulk (42%) occurred in genes involved in metabolism. The average number of SNPs among within-host variants was 5 (range 0-18) (Table 2). However, in two subjects (H36 and H37), pairwise distances between genomes from the same ST (ST59 and ST5148) were as large as 14 and 18 SNPs respectively (Supplementary File 5, grey highlight).

Accessory gene content and relationships with other strains

A quarter of our isolates were most closely related to commensal strains from humans, with smaller numbers most closely related to human pathogenic strains or strains from livestock, poultry or the environment (Supplementary File 7). One isolate was most closely related to a canine isolate from the UK. Three STs (ST38, ST10 and ST58) were shared by our study isolates and diarrhoeal isolate from the GEMS study (Supplementary Figure 2), with just eight alleles separating our commensal ST38 strain from a diarrhoeal isolate from the GEMS study (Figure 3). For ST10 and ST58, hierarchical clustering placed the commensal strains from this study into separate clusters from the pathogenic isolates from diarrhoeal cases, indicating that they were genetically distinct to each other. Yet, the closest relative of our study ST58 strain was an extraintestinal strain isolated from the blood of a 69-year-old male (87 alleles differences, Figure 4). Also, the resident ST10 isolates recovered from this study (H-26_2, H-34_2, and H-32_5) had their closest neighbours in isolates from livestock (83 and 111 alleles each), and an isolate of an unspecified source (18 alleles differences) respectively (Supplementary File 7).

We detected 130 genes encoding putative virulence factors across the 88 study isolates (Figure 2; Supplementary File 8). Notable among these were genes associated with pathogenesis in Enteroaggregative *E. coli* and *Salmonella* referred to as the Serine Protease Autotransporters of *Enterobacteriaceae* (SPATEs) (Pokharel et al. 2019), such as *sat* (13%), *sigA* (11%) and *pic* (1%). Besides, eight isolates harboured known markers of Enteropathogenic *E. coli* (*eltAB* or *estA*). Several strains (across all phylogroups) also harboured virulence genes associated with intestinal or extraintestinal disease in humans, including adhesins, invasins, toxins and iron-acquisition genes such as *fyuA*, several *fim* and *pap genes*, *iroN*, *irp1,*2*, ibeA* and *aslA.* We did not detect any of the well-known markers of EPEC (*eae, bfpA, stx1*, or *stx2*) (Figure 2, Supplementary File 8).

315 The prevalence of some virulence factors involved in invasion/evasion, iron uptake,
316 adherence, and secretion systems appeared to be more or less likely to occur in one or a few
317 phylotypes ($p \leq 0.05$) as follows (Supplementary File 9). The iron acquisition genes *chuA, S-Y* and
318 *shuA, S, T, Y* were found to be present in all cases for phylogroup D (n=5), and absent in
319 virtually all cases for phylogroups A (n=13) and B1 (n=16). On the other hand, *iutA* and *iucA-D*
320 were observed in the two cases from phylogroup B2, and absent from all samples from
321 phylogroup D (n=5). The invasion/evasion genes *kpsD, M, T* and *aslA* were found to be present
322 in almost all cases for phylogroups D (n=5), B2 (n=2), and Clade I (n=2), and absent in B1
323 (n=16). The secretion system gene cluster *espB, D, G, K-N, R, W-Y* was observed in all cases
324 except the two belonging to phylogenetic group B2. The protease gene *sigA* was absent from
325 most samples, except two samples from phylotype B2. The adherence gene *fdeC* was observed in
326 all cases for phylotype D (n=5) and most for B1 (n=16).
327 More than half of the isolates encoded resistance to three or more clinically relevant classes
328 of antibiotics such as aminoglycosides, penicillins, trimethoprim, sulphonamides and
329 tetracyclines (Figure 5; Supplementary Figure 3). The most common resistance gene network
330 was *-aph(6)-Id_1-sul2* (41% of the isolates), followed by *aph(3'')-Ib_5-sul2* (27%) and *bla-TEM-*
331 *aph(3'')-Ib_5* (24%). Most isolates (67%) harboured two or more plasmid types (Figure 6). Of
332 the 24 plasmid types detected, IncFIB was the most common (41%), followed by col156 (19%)
333 and IncI_1-Alpha (15%). Nearly three-quarters of the multi-drug resistant isolates carried IncFIB
334 (AP001918) plasmids (~50kb), suggesting that these large plasmids disseminate resistance genes
335 within our study population.
336
337 **Discussion**

338 This study provides an overview of the within-host genomic diversity of *E. coli* in healthy
339 children from a rural setting in the Gambia, West Africa. Surprisingly, we were able to recover
340 *E. coli* from only 34% of stools which had previously tested positive for *E. coli* in the original
341 study. This low rate of recovery may reflect some hard-to-identify effect of long-term storage
342 (nine to thirteen years) or the way the samples were handled, even though they were kept frozen
343 and thawed only just before culture.

344 Several studies have shown that sampling a single colony is insufficient to capture *E. coli*
345 strain diversity in stools (Dixit et al. 2018; Richter et al. 2018; Shooter et al. 1977). Lidin-Janson
346 *et al.* (Lidin-Janson et al. 1978) claim that sampling five colonies provides a >99% chance of
347 recovering dominant genotypes from single stool specimens, while Schlager *et al.* (Schlager et
348 al. 2002) calculate that sampling twenty-eight colonies provides a >90% chance of recovering
349 minor genotypes. Our results confirm the importance of multiple-colony picks in faecal
350 surveillance studies, as over half (57%) of our strains would have been missed by picking a
351 single colony.

352 Our strains encompassed all eight major phylotypes of *E. coli,* however, the majority fell into
353 the A and B1 phylogenetic groups, in line with previous reports that these phylogroups dominate
354 in stools from people in low- and middle-income countries (Duriez et al. 2001; Escobar-Páramo
355 et al. 2004). Although not fully understood, there appear to be host-related factors that influence
356 the composition of *E. coli* phylogroups in human hosts. For example, the establishment of strains
357 belonging to phylogroups E or F seems to favour subsequent colonisation by other phylotypes,
358 compared to the establishment of phylogroup B2 strains, which tend to limit the heterogeneity
359 within individual hosts (Gordon et al. 2015). Geographical differences have also been reported,
360 with phylogroups A and B1 frequently dominating the stools of people living in developing

361 countries (Duriez et al. 2001; Escobar-Páramo et al. 2004). Conversely, phylogroup B2 and D
362 strains appear to be pervasive among people living in developed countries (Massot et al. 2016;
363 Skurnik et al. 2008). These locale-specific patterns in the distribution of *E. coli* phylotypes have
364 been attributed to differences in diet and climate (Duriez et al. 2001; Escobar-Páramo et al.
365 2004).

366 The prevalence of putative virulence genes in most of our isolates highlights the pathogenic
367 potential of commensal intestinal strains—regardless of their phylogroup—should they gain
368 access to the appropriate tissues, for example, the urinary tract. Our results complement previous
369 studies reporting genomic similarities between faecal *E. coli* isolates and those recovered from
370 urinary tract infection (McNally et al. 2013; Wold et al. 1992).

371 We found that within-host evolution plays a minor role in the generation of diversity in our
372 study population. This might be due to the low prevalence of B2 strains, which are thought to
373 inhibit the establishment of strains from other phylogroups, as discussed above (Gordon et al.
374 2015); or it may indicate that members of phylogroups A and B1 might favour a more
375 heterogeneous composition of *E. coli* phylotypes in stools of healthy individuals. However, this
376 remains to be properly investigated, as we did not find statistical evidence that the distribution of
377 variants (independent migration versus within-host evolution) was influenced by phylogroup.
378 Our findings are similar to that reported, in line with Dixit et al. (Dixit et al. 2018), who reported
379 that 83% of diversity originates from immigration events, and with epidemiological data
380 suggesting that the recurrent immigration events account for the high faecal diversity of *E. coli* in
381 the tropics (Tenaillon et al. 2010).

382 The estimated mutation rate for *E. coli* lineages is around one SNP per genome per year
383 (Reeves et al. 2011), so that two genomes with a most recent common ancestor in the last five

years would be expected to be around ten SNPs apart. However, in two subjects, pairwise distances between genomes from the same ST (ST59 and ST5148) were large enough (14 and 18 respectively) to suggest that they might have arisen from independent immigration events, as insufficient time had elapsed in the child's life for such divergence to occur within the host. However, it remains possible that the mutation rate was higher than expected in these lineages, although we found no evidence of damage to DNA repair genes. Co-colonising variants belonging to the same ST tended to share an identical virulence, AMR and plasmid profile, signalling similarities in their accessory gene content.

The sources of novel variation that account for within-host diversity include point mutation and small insertions or deletions (indels), indels and the loss or acquisition of mobile genetic elements. Among the variants inferred to have been derived from within-host evolution, we observed dominance of mutations that were predicted to result in changes in protein function, in the form of missense mutations and non-sense mutations (leading to a premature stop codon). Although the mutations appeared to be heterogeneously distributed, a higher number was observed in genes associated with metabolism. These appeared to be under positive selection, although it remains to be seen if these changes confer any effects on fitness. It will be desirable to investigate this in future studies. Due to the cross-sectional nature of our sampling, we were unable to analyse the dynamics of strain gain or loss and variation in gene content over time. Homologous recombination has also been noted to contribute to the generation of diversity (Golubchik et al. 2013; González-González et al. 2013), however, we detected and remove recombinant regions prior to phylogenetic reconstruction and thus focused on our analysis on SNPs.

More than half of our isolates encode resistance to three or more classes of antimicrobials echoing the high rate of MDR (65%; confirmed by phenotypic testing) in the GEMS study. IncFIB (AP001918) was the most common plasmid Inc type from our study, in line with the observation that IncF plasmids are frequently associated with the dissemination of resistance (Carattoli 2009). However, a limitation of our study is that we did not perform phenotypic antimicrobial resistance testing, although Doyle et al. (Doyle et al. 2020) reported that only a small proportion of genotypic AMR predictions are discordant with phenotypic results.

Comparative analyses confirm the heterogeneous origins of the strains reported here, documenting links to other human commensal strains or isolates sourced from livestock or the environment. This is not surprising, as almost all study participants reported that animals are kept in their homes and children in rural Gambia are often left to play on the ground, close to domestic animals such as pets and poultry (Dione et al. 2011).

Conclusions

Our results show that the commensal *E. coli* population in the gut of healthy children in rural Gambia is richly diverse, with the independent immigration and establishment of strains contributing to the bulk of the observed diversity. An obvious limitation to our study is the low recovery of *E. coli* from frozen stools—which potentially implies we may have underestimated the extent of genetic diversity present within our study population. Although solely observational, our study paves the way for future studies aimed at a mechanistic understanding of the factors driving the diversification of *E. coli* in the human gut and what it takes to make a strain of *E. coli* successful in this habitat. Besides, this work has added significantly to the number of commensal *E. coli* genomes, which are underrepresented in public repositories.

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Author contributions

Conceptualization: MA, MP; data curation, MP, NFA; formal analysis: EFN; analytical support: MD; funding: MA and MP; sample collection and storage: MJH, UNI, PET, CO; data management: SG; laboratory experiments, EFN, supervision, NFA, MP, JO, MA; manuscript preparation – original draft, EFN; review and editing, NFA, MP; review of the final manuscript, all authors.

References

Ahmed SF, Ali MM, Mohamed ZK, Moussa TA, and Klena JD. 2014. Fecal carriage of extended-spectrum β-lactamases and AmpC-producing *Escherichia coli* in a Libyan community. *Annals of Clinical Microbiology and Antimicrobials* 13:22. 10.1186/1476-0711-13-22

Alm EW, Walk ST, and Gordon DM. 2011. The niche of *Escherichia coli*. *Population Genetics of Bacteria*: American Society of Microbiology

Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev

452 MA, and Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its
453 applications to single-cell sequencing. *Journal of Computational Biology* 19:455-477.
454 10.1089/cmb.2012.0021
455 Bettelheim KA, Faiers M, and Shooter RA. 1972. Serotypes of *Escherichia coli* in normal stools.
456 *The Lancet* 2:1223-1224.
457 Blount ZD. 2015. The unexhausted potential of *E. coli*. *Elife* 4. 10.7554/eLife.05826
458 Camins BC, Marschall J, DeVader SR, Maker DE, Hoffman MW, and Fraser VJ. 2011. The
459 clinical impact of fluoroquinolone resistance in patients with *E. coli* bacteremia. *Journal*
460 *of Hospital Medicine* 6:344-349. 10.1002/jhm.877
461 Carattoli A. 2009. Resistance plasmid families in *Enterobacteriaceae*. *Antimicrobial Agents and*
462 *Chemotherapy* 53:2227-2238. 10.1128/AAC.01707-08
463 Chen SL, Wu M, Henderson JP, Hooton TM, Hibbing ME, Hultgren SJ, and Gordon JI. 2013.
464 Genomic diversity and fitness of *E. coli* strains recovered from the intestinal and urinary
465 tracts of women with recurrent urinary tract infection. *Science Translational Medicine*
466 5:184ra160. 10.1126/scitranslmed.3005497
467 Clermont O, Christenson JK, Denamur E, and Gordon DM. 2013. The Clermont *Escherichia coli*
468 phylo-typing method revisited: improvement of specificity and detection of new phylo-
469 groups. *Environmental Microbiology Reports* 5:58-65. 10.1111/1758-2229.12019
470 Clermont O, Gordon D, and Denamur E. 2015. Guide to the various phylogenetic classification
471 schemes for *Escherichia coli* and the correspondence among schemes. *Microbiology*
472 161:980-988. 10.1099/mic.0.000063
473 Connor TR, Loman NJ, Thompson S, Smith A, Southgate J, Poplawski R, Bull MJ, Richardson
474 E, Ismail M, Thompson SE, Kitchen C, Guest M, Bakke M, Sheppard SK, and Pallen MJ.

2016. CLIMB (the Cloud Infrastructure for Microbial Bioinformatics): an online resource for the medical microbiology community. *Microbial Genomics* 2:e000086. 10.1099/mgen.0.000086

Croucher NJ, Page AJ, Connor TR, Delaney AJ, Keane JA, Bentley SD, Parkhill J, and Harris SR. 2015. Rapid phylogenetic analysis of large samples of recombinant bacterial whole genome sequences using Gubbins. *Nucleic Acids Research* 43:e15-e15. 10.1093/nar/gku1196

Dione MM, Ikumapayi UN, Saha D, Mohammed NI, Geerts S, Ieven M, Adegbola RA, and Antonio M. 2011. Clonal differences between Non-Typhoidal *Salmonella* (NTS) recovered from children and animals living in close contact in the Gambia. *PLoS Neglected Tropical Diseases* 5:e1148. 10.1371/journal.pntd.0001148

Dixit OVA, O'Brien CL, Pavli P, and Gordon DM. 2018. Within-host evolution versus immigration as a determinant of *Escherichia coli* diversity in the human gastrointestinal tract. *Environmental Microbiology* 20:993-1001. 10.1111/1462-2920.14028

Doyle RM, O'Sullivan DM, Aller SD, Bruchmann S, Clark T, Coello Pelegrin A, Cormican M, Diez Benavente E, Ellington MJ, McGrath E, Motro Y, Phuong Thuy Nguyen T, Phelan J, Shaw LP, Stabler RA, van Belkum A, van Dorp L, Woodford N, Moran-Gilad J, Huggett JF, and Harris KA. 2020. Discordant bioinformatic predictions of antimicrobial resistance from whole-genome sequencing data of bacterial isolates: an inter-laboratory study. *Microbial Genomics* 6. 10.1099/mgen.0.000335

Duriez P, Clermont O, Bonacorsi S, Bingen E, Chaventré A, Elion J, Picard B, and Denamur E. 2001. Commensal *Escherichia coli* isolates are phylogenetically distributed among

497 geographically distinct human populations. *Microbiology* 147:1671-1676.
498 10.1099/00221287-147-6-1671
499 Escobar-Paramo P, Clermont O, Blanc-Potard AB, Bui H, Le Bouguenec C, and Denamur E.
500 2004. A specific genetic background is required for acquisition and expression of
501 virulence factors in *Escherichia coli*. *Molecular Biology and Evolution* 21:1085-1094.
502 10.1093/molbev/msh118
503 Escobar-Páramo P, Grenet K, Le Menac'h A, Rode L, Salgado E, Amorin C, Gouriou S, Picard
504 B, Rahimy MC, Andremont A, Denamur E, and Ruimy R. 2004. Large-scale population
505 structure of human commensal *Escherichia coli* isolates. *Applied and Environmental*
506 *Microbiology* 70:5698-5700. 10.1128/AEM.70.9.5698-5700.2004
507 Ferjani S, Saidani M, Hamzaoui Z, Alonso CA, Torres C, Maamar E, Slim AF, and Boutiba BB.
508 2017. Community fecal carriage of broad-spectrum cephalosporin-resistant *Escherichia*
509 *coli* in Tunisian children. *Diagnostic Microbiology and Infectious Disease* 87:188-192.
510 10.1016/j.diagmicrobio.2016.03.008
511 Foster-Nyarko E, Alikhan NF, Ravi A, Thilliez G, Thomson NM, Baker D, Kay G, Cramer JD,
512 O'Grady J, Antonio M, and Pallen MJ. 2020. Genomic diversity of *Escherichia coli*
513 isolates from non-human primates in the Gambia. *Microbial Genomics* 6.
514 10.1099/mgen.0.000428
515 Golubchik T, Batty EM, Miller RR, Farr H, Young BC, Larner-Svensson H, Fung R, Godwin H,
516 Knox K, Votintseva A, Everitt RG, Street T, Cule M, Ip CLC, Didelot X, Peto TEA,
517 Harding RM, Wilson DJ, Crook DW, and Bowden R. 2013. Within-host evolution of
518 *Staphylococcus aureus* during asymptomatic carriage. *PLoS One* 8:e61319.
519 10.1371/journal.pone.0061319

González-González A, Sánchez-Reyes LL, Delgado Sapien G, Eguiarte LE, and Souza V. 2013. Hierarchical clustering of genetic diversity associated to different levels of mutation and recombination in *Escherichia coli*: a study based on Mexican isolates. *Infections, Genetics and Evolution* 13:187-197. 10.1016/j.meegid.2012.09.003

Good BH, McDonald MJ, Barrick JE, Lenski RE, and Desai MM. 2017. The dynamics of molecular evolution over 60,000 generations. *Nature* 551:45-50. 10.1038/nature24287

Gordon DM, O'Brien CL, and Pavli P. 2015. *Escherichia coli* diversity in the lower intestinal tract of humans. *Environmental Microbiology Reports* 7:642-648. 10.1111/1758-2229.12300

Gurevich A, Saveliev V, Vyahhi N, and Tesler G. 2013. QUAST: quality assessment tool for genome assemblies. *Bioinformatics* 29:1072-1075. 10.1093/bioinformatics/btt086

Hartl DL, and Dykhuizen DE. 1984. The population genetics of *Escherichia coli*. *Annual Reviews of Genetics* 18:31-68. 10.1146/annurev.ge.18.120184.000335

Hobman JL, Penn CW, and Pallen MJ. 2007. Laboratory strains of *Escherichia coli*: model citizens or deceitful delinquents growing old disgracefully? *Molecular Microbiology* 64:881-885. 10.1111/j.1365-2958.2007.05710.x

Kotloff KL, Blackwelder WC, Nasrin D, Nataro JP, Farag TH, van Eijk A, Adegbola RA, Alonso PL, Breiman RF, Faruque AS, Saha D, Sow SO, Sur D, Zaidi AK, Biswas K, Panchalingam S, Clemens JD, Cohen D, Glass RI, Mintz ED, Sommerfelt H, and Levine MM. 2012. The Global Enteric Multicenter Study (GEMS) of diarrheal disease in infants and young children in developing countries: epidemiologic and clinical methods of the case/control study. *Clinical Infectious Diseases* 55 Suppl 4:S232-245. 10.1093/cid/cis753

542 Kotloff KL, Nataro JP, Blackwelder WC, Nasrin D, Farag TH, Panchalingam S, Wu Y, Sow SO,
543 Sur D, Breiman RF, Faruque ASG, Zaidi AKM, Saha D, Alonso PL, Tamboura B,
544 Sanogo D, Onwuchekwa U, Manna B, Ramamurthy T, Kanungo S, Ochieng JB, Omore
545 R, Oundo JO, Hossain A, Das SK, Ahmed S, Qureshi S, Quadri F, Adegbola RA,
546 Antonio M, Hossain MJ, Akinsola A, Mandomando I, Nhampossa T, Acácio S, Biswas
547 K, O'Reilly CE, Mintz ED, Berkeley LY, Muhsen K, Sommerfelt H, Robins-Browne
548 RM, and Levine MM. 2013. Burden and aetiology of diarrhoeal disease in infants and
549 young children in developing countries (the Global Enteric Multicenter Study, GEMS): a
550 prospective, case-control study. *The Lancet* 382:209-222. 10.1016/S0140-
551 6736(13)60844-2

552 Laxminarayan R, Duse A, Wattal C, Zaidi AK, Wertheim HF, Sumpradit N, Vlieghe E, Hara
553 GL, Gould IM, Goossens H, Greko C, So AD, Bigdeli M, Tomson G, Woodhouse W,
554 Ombaka E, Peralta AQ, Qamar FN, Mir F, Kariuki S, Bhutta ZA, Coates A, Bergstrom R,
555 Wright GD, Brown ED, and Cars O. 2013. Antibiotic resistance-the need for global
556 solutions. *The Lancet Infectious Diseases* 13:1057-1098. 10.1016/s1473-3099(13)70318-
557 9

558 Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R,
559 and Subgroup GPDP. 2009. The Sequence Alignment/Map format and SAMtools.
560 *Bioinformatics* 25:2078-2079. 10.1093/bioinformatics/btp352

561 Lidin-Janson G, Kaijser B, Lincoln K, Olling S, and Wedel H. 1978. The homogeneity of the
562 faecal coliform flora of normal school-girls, characterized by serological and biochemical
563 properties. *Medical Microbiology and Immunology* 164:247-253. 10.1007/BF02125493

564 Liu J, Platts-Mills JA, Juma J, Kabir F, Nkeze J, Okoi C, Operario DJ, Uddin J, Ahmed S,
565 Alonso PL, Antonio M, Becker SM, Blackwelder WC, Breiman RF, Faruque AS, Fields
566 B, Gratz J, Haque R, Hossain A, Hossain MJ, Jarju S, Qamar F, Iqbal NT, Kwambana B,
567 Mandomando I, McMurry TL, Ochieng C, Ochieng JB, Ochieng M, Onyango C,
568 Panchalingam S, Kalam A, Aziz F, Qureshi S, Ramamurthy T, Roberts JH, Saha D, Sow
569 SO, Stroup SE, Sur D, Tamboura B, Taniuchi M, Tennant SM, Toema D, Wu Y, Zaidi A,
570 Nataro JP, Kotloff KL, Levine MM, and Houpt ER. 2016. Use of quantitative molecular
571 diagnostic methods to identify causes of diarrhoea in children: a reanalysis of the GEMS
572 case-control study. *Lancet* 388:1291-1301. 10.1016/S0140-6736(16)31529-X
573 Massot M, Daubié AS, Clermont O, Jauréguy F, Couffignal C, Dahbi G, Mora A, Blanco J,
574 Branger C, Mentré F, Eddi A, Picard B, Denamur E, and The Coliville Group. 2016.
575 Phylogenetic, virulence and antibiotic resistance characteristics of commensal strain
576 populations of *Escherichia coli* from community subjects in the Paris area in 2010 and
577 evolution over 30 years. *Microbiology* 162:642-650. 10.1099/mic.0.000242
578 McNally A, Alhashash F, Collins M, Alqasim A, Paszckiewicz K, Weston V, and Diggle M.
579 2013. Genomic analysis of extra-intestinal pathogenic *Escherichia coli* urosepsis.
580 *Clinical Microbiology and Infection* 19:E328-334. 10.1111/1469-0691.12202
581 Mellata M. 2013. Human and avian extraintestinal pathogenic *Escherichia coli*: infections,
582 zoonotic risks, and antibiotic resistance trends. *Foodborne Pathogens and Disease*
583 10:916-932. 10.1089/fpd.2013.1533
584 Milne I, Stephen G, Bayer M, Cock PJ, Pritchard L, Cardle L, Shaw PD, and Marshall D. 2013.
585 Using Tablet for visual exploration of second-generation sequencing data. *Briefings in*
586 *Bioinformatics* 14:193-202. 10.1093/bib/bbs012

587 Moremi N, Claus H, Vogel U, and Mshana SE. 2017. Faecal carriage of CTX-M extended-
588 spectrum beta-lactamase-producing *Enterobacteriaceae* among street children dwelling
589 in Mwanza city, Tanzania. *PLoS One* 12:e0184592. 10.1371/journal.pone.0184592
590 Nielsen KL, Stegger M, Godfrey PA, Feldgarden M, Andersen PS, and Frimodt-Møller N. 2016.
591 Adaptation of *Escherichia coli* traversing from the faecal environment to the urinary
592 tract. *International Journal of Medical Microbiology* 306:595-603.
593 10.1016/j.ijmm.2016.10.005
594 Oshima K, Toh H, Ogura Y, Sasamoto H, Morita H, Park SH, Ooka T, Iyoda S, Taylor TD,
595 Hayashi T, Itoh K, and Hattori M. 2008. Complete genome sequence and comparative
596 analysis of the wild-type commensal *Escherichia coli* strain SE11 isolated from a healthy
597 adult. *DNA Research* 15:375-386. 10.1093/dnares/dsn026
598 Pokharel P, Habouria H, Bessaiah H, and Dozois CM. 2019. Serine Protease Autotransporters of
599 the *Enterobacteriaceae* (SPATEs): Out and about and chopping it up. *Microorganisms* 7.
600 10.3390/microorganisms7120594
601 Rasko DA, Rosovitz MJ, Myers GS, Mongodin EF, Fricke WF, Gajer P, Crabtree J, Sebaihia M,
602 Thomson NR, Chaudhuri R, Henderson IR, Sperandio V, and Ravel J. 2008. The
603 pangenome structure of *Escherichia coli*: comparative genomic analysis of *E. coli*
604 commensal and pathogenic isolates. *Journal of Bacteriology* 190:6881-6893.
605 10.1128/JB.00619-08
606 Reeves PR, Liu B, Zhou Z, Li D, Guo D, Ren Y, Clabots C, Lan R, Johnson JR, and Wang L.
607 2011. Rates of mutation and host transmission for an *Escherichia coli* clone over 3 years.
608 *PloS One* 6:e26907-e26907. 10.1371/journal.pone.0026907

609 Richter TKS, Hazen TH, Lam D, Coles CL, Seidman JC, You Y, Silbergeld EK, Fraser CM, and
610 Rasko DA. 2018. Temporal variability of *Escherichia coli* diversity in the gastrointestinal
611 tracts of Tanzanian children with and without exposure to antibiotics. *mSphere* 3.
612 10.1128/mSphere.00558-18
613 Rodríguez-Baño J, Picón E, Gijón P, Hernández JR, Cisneros JM, Peña C, Almela M, Almirante
614 B, Grill F, Colomina J, Molinos S, Oliver A, Fernández-Mazarrasa C, Navarro G,
615 Coloma A, López-Cerero L, and Pascual A. 2010. Risk factors and prognosis of
616 nosocomial bloodstream infections caused by extended-spectrum-beta-lactamase-
617 producing *Escherichia coli*. *Journal of Clinical Microbiology* 48:1726-1731.
618 10.1128/JCM.02353-09
619 Russo TA, and Johnson JR. 2003. Medical and economic impact of extraintestinal infections due
620 to *Escherichia coli*: focus on an increasingly important endemic problem. *Microbes and*
621 *Infection* 5:449-456. 10.1016/s1286-4579(03)00049-2
622 Schlager TA, Hendley JO, Bell AL, and Whittam TS. 2002. Clonal diversity of *Escherichia coli*
623 colonizing stools and urinary tracts of young girls. *Infection and Immunity* 70:1225-1229.
624 10.1128/iai.70.3.1225-1229.2002
625 Shooter RA, Bettleheim KA, Lennox-King SM, and O'Farrell S. 1977. *Escherichia coli*
626 serotypes in the faeces of healthy adults over a period of several months. *Journal of*
627 *Hygiene (Lond)* 78:95-98. 10.1017/s0022172400055972
628 Skurnik D, Bonnet D, Bernède-Bauduin C, Michel R, Guette C, Becker JM, Balaire C, Chau F,
629 Mohler J, Jarlier V, Boutin JP, Moreau B, Guillemot D, Denamur E, Andremont A, and
630 Ruimy R. 2008. Characteristics of human intestinal *Escherichia coli* with changing

environments. *Environmental Microbiology* 10:2132-2137. 10.1111/j.1462-2920.2008.01636.x

Stamatakis A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22:2688-2690. 10.1093/bioinformatics/btl446

Stegger M, Leihof RF, Baig S, Sieber RN, Thingholm KR, Marvig RL, Frimodt-Møller N, and Nielsen KL. 2020. A snapshot of diversity: Intraclonal variation of *Escherichia coli* clones as commensals and pathogens. *International Journal of Medical Microbiology* 310:151401. 10.1016/j.ijmm.2020.151401

Stoesser N, Sheppard AE, Moore CE, Golubchik T, Parry CM, Nget P, Saroeun M, Day NP, Giess A, Johnson JR, Peto TE, Crook DW, Walker AS, and Group MMMI. 2015. Extensive within-host diversity in fecally carried extended-spectrum-beta-lactamase-producing *Escherichia coli* isolates: Implications for transmission analyses. *Journal of Clinical Microbiology* 53:2122-2131. 10.1128/JCM.00378-15

Stoppe NC, Silva JS, Carlos C, Sato MIZ, Saraiva AM, Ottoboni LMM, and Torres TT. 2017. Worldwide phylogenetic group patterns of *Escherichia coli* from commensal human and wastewater treatment plant isolates. *Frontiers in Microbiology* 8:2512. 10.3389/fmicb.2017.02512

Tenaillon O, Skurnik D, Picard B, and Denamur E. 2010. The population genetics of commensal *Escherichia coli*. *Nature Reviews Microbiology* 8:207-217. 10.1038/nrmicro2298

Touchon M, Hoede C, Tenaillon O, Barbe V, Baeriswyl S, Bidet P, Bingen E, Bonacorsi S, Bouchier C, Bouvet O, Calteau A, Chiapello H, Clermont O, Cruveiller S, Danchin A, Diard M, Dossat C, Karoui ME, Frapy E, Garry L, Ghigo JM, Gilles AM, Johnson J, Le

654 Bouguénec C, Lescat M, Mangenot S, Martinez-Jéhanne V, Matic I, Nassif X, Oztas S,
655 Petit MA, Pichon C, Rouy Z, Ruf CS, Schneider D, Tourret J, Vacherie B, Vallenet D,
656 Médigue C, Rocha EP, and Denamur E. 2009. Organised genome dynamics in the
657 *Escherichia coli* species results in highly diverse adaptive paths. *PLoS Genetics*
658 5:e1000344. 10.1371/journal.pgen.1000344
659 Walk ST, Alm EW, Gordon DM, Ram JL, Toranzos GA, Tiedje JM, and Whittam TS. 2009.
660 Cryptic lineages of the genus *Escherichia*. *Applied and Environmental Microbioogy*
661 75:6534-6544. 10.1128/aem.01262-09
662 Wheeler TJ. 2009. Large-Scale Neighbor-Joining with NINJA. in A*lgorithms in Bioinformatics*.
663 *Berlin, Heidelberg: Springer Berlin Heidelberg*.
664 Wingett SW, and Andrews S. 2018. FastQ Screen: A tool for multi-genome mapping and quality
665 control. *F1000Res* 7:1338. 10.12688/f1000research.15931.2
666 Wirth T, Falush D, Lan R, Colles F, Mensa P, Wieler LH, Karch H, Reeves PR, Maiden MCJ,
667 Ochman H, and Achtman M. 2006. Sex and virulence in *Escherichia coli*: an
668 evolutionary perspective. *Molecular Microbiology* 60:1136-1151. 10.1111/j.1365-
669 2958.2006.05172.x
670 Wold AE, Caugant DA, Lidin-Janson G, de Man P, and Svanborg C. 1992. Resident colonic
671 *Escherichia coli* strains frequently display uropathogenic characteristics. *Journal of*
672 *Infectious Diseases* 165:46-52. 10.1093/infdis/165.1.46
673 Zhou Z, Alikhan NF, Mohamed K, Fan Y, Achtman M, and Group AS. 2020. The EnteroBase
674 user's guide, with case studies on *Salmonella* transmissions, *Yersinia pestis* phylogeny,
675 and *Escherichia* core genomic diversity. *Genome Research* 30:138-152.
676 10.1101/gr.251678.119

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Table 1(on next page)

Phylogroup and sequence types of the distinct clones isolated in each study subject

Table 1: Phylogroup and sequence types of the distinct clones isolated in each study subject

	Colony or isolate number					Number of distinct genotypes (clones)	Migration events	Within-host evolution events
Host	**1**	**2**	**3**	**4**	**5**		Phylotype (number of events)	Phylotype (number of events)
H-2	A (9274)	A (9274)	A (9274)	A (9274)	A (9274)	1	A (1)	0
H-9	A (2705)	A (2705)	A (2705)	D (2914)	B1 (29)	3	A (1), D (1), B1 (1)	0
H-15	B2 (9277)	B2 (9277)	B2 (9277)	Clade I (747)	Clade I (747)	3	B2 (1), Clade I (1)	Clade I (1)
H-18	D (38)	D (38)	B1 (9281)	A (9274)		4	D (1), B1 (1), A (1)	D (1)
H-21	B1 (58)	B1 (58)	B1 (223)	A (540)	D (1204)	4	B1(2) A (1), D (1)	0
H-22	B1 (316)	B1 (316)	B1 (316)	B1 (316)		2	B (1)	B1(1)
H-25	A (181)	A (181)	A (181)	A (181)	B1 (337)	4	A (1), B1 (1)	A (2)
H-26	B1 (641)	B1 (2741)	A (10)	A (398)		4	B1(2), A (1), D (1)	0
H-28	B1 (469)	B1 (469)	B1 (469)	B1 (469)		2	B1(1)	B1(1)
H-32	B1 (101)	B1 (101)	B1 (101)	B1 (2175)	A (10)	3	B1(2), A (1)	0
H-34	B1 (603)	B1 (603)	B1 (603)	B1 (1727)	A (10)	4	B1(2), A (1)	B1(1)
H-35	A (226)					1	A (1)	0
H-36	F (59)	F (59)	F (59)	F (59)	E (9278)	4	F (1), E (1)	F (1)
H-37	D (5148)	D (5148)	D (5148)	D (5148)	D (5148)	3	D (1)	D (2)
H-38	D (394)	D (394)	D (394)	D (394)	B1 (58)	4	D (1), B1(1)	D (2)
H-39	B2 (452)	B2 (452)	B2 (452)	B2 (452)	B2 (452)	2	B2(1)	B2 (1)
H-40	B1 (155)					1	B1(1)	0
H-41	A (43)	A (43)	A (43)	A (43)	B1 (9283)	2	A (1), B1(1)	0
H-48	Clade I (485)	Clade I (485)	Clade I (485)	Clade I (485)		3	Clade I (1)	0
H-50	C (410)	C (410)	C (410)	C (410)	B1 (515)	2	C (1), B1(1)	0
H-55	A (9279)					1	A(1)	0

Table 2(on next page)

Pairwise SNP distances between variants arising from within-host evolution

Table 2: Pairwise SNP distances between variants arising from within-host evolution

Host	Sequence type (ST)	Colonies per ST	Pairwise SNP distances between multiple colonies of the same ST
H2	9274	5	0-9
H9	2705	3	0-1
H15	9277	3	0-1
H15	747	2	3
H18	38	2	3
H21	58	2	0
H22	316	4	0-3
H25	181	4	1-5
H28	469	4	0-3
H32	101	3	1-9
H34	603	3	2-8
H36	59	4	0-14
H37	5148	5	2-18
H38	394	4	1-3
H39	452	5	0-2
H41	43	4	0-1
H48	485	4	1-9
H50	410	4	0

Figure 1

The study sample-processing flow diagram.

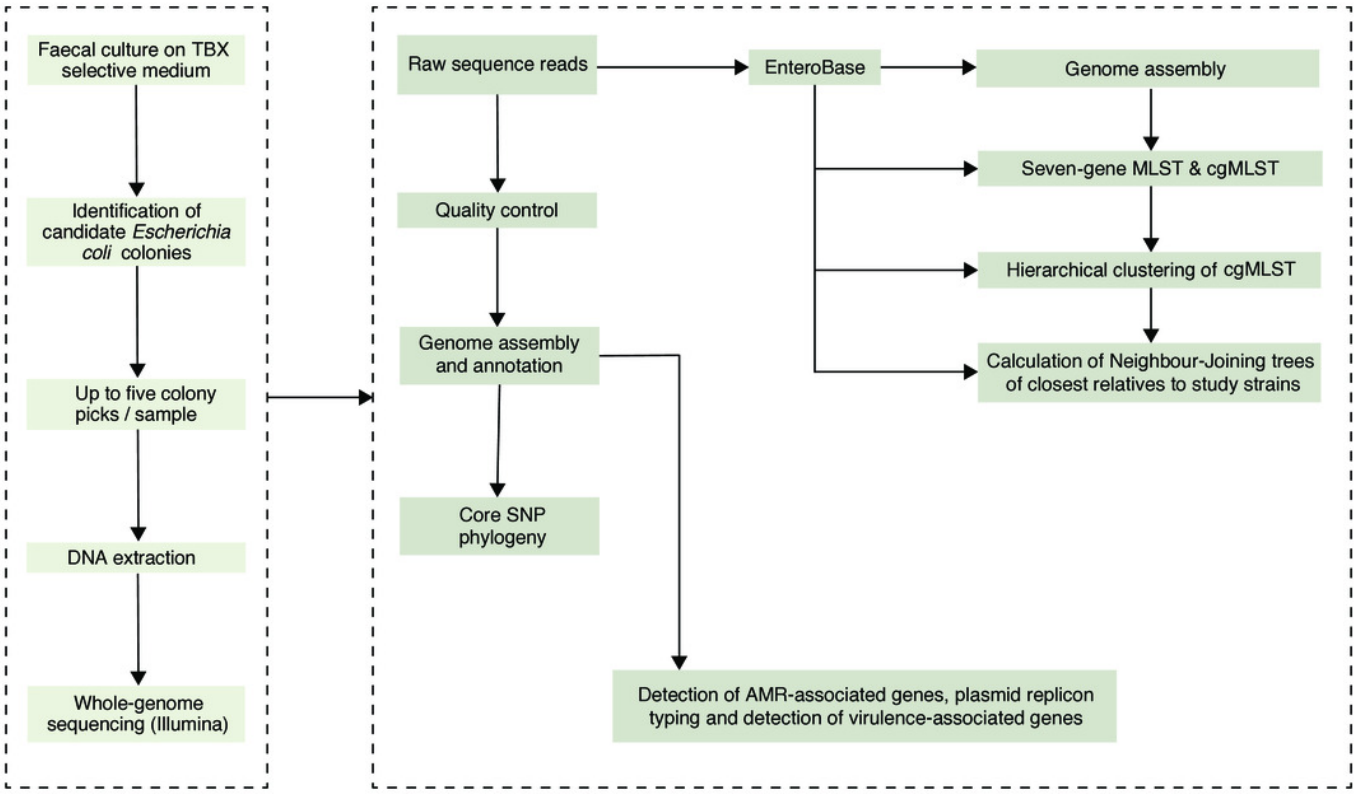


Figure 2

A maximum-likelihood tree depicting the phylogenetic relationships among the study isolates.

The tree was reconstructed with RAxML, using a general time-reversible nucleotide substitution model and 1,000 bootstrap replicates. The genome assembly of *E. coli* str. K12 substr. MG1655 was used s as the reference, and the tree rooted using the genomic assembly of *E. fergusonii* as an outgroup. The sample names are indicated at the tip, with the respective Achtman sequence types (ST) indicated beside the sample names. The respective phylogroups the isolates belong to are indicated with colour codes as displayed in the legend. *E. coli* reference genome is denoted in black. Asterisks (*) are used to indicate novel STs. The predicted antimicrobial resistance genes and putative virulence factors for each isolate are displayed next to the tree, with the virulence genes clustered according to their function. Multiple copies of the same strain (ST) isolated from a single host are not shown. Instead, we have shown only one representative isolate from each strain. Virulence and resistance factors were not detected in the reference strain either. A summary of the identified virulence factors and their known functions are provided in Supplementary File 3.

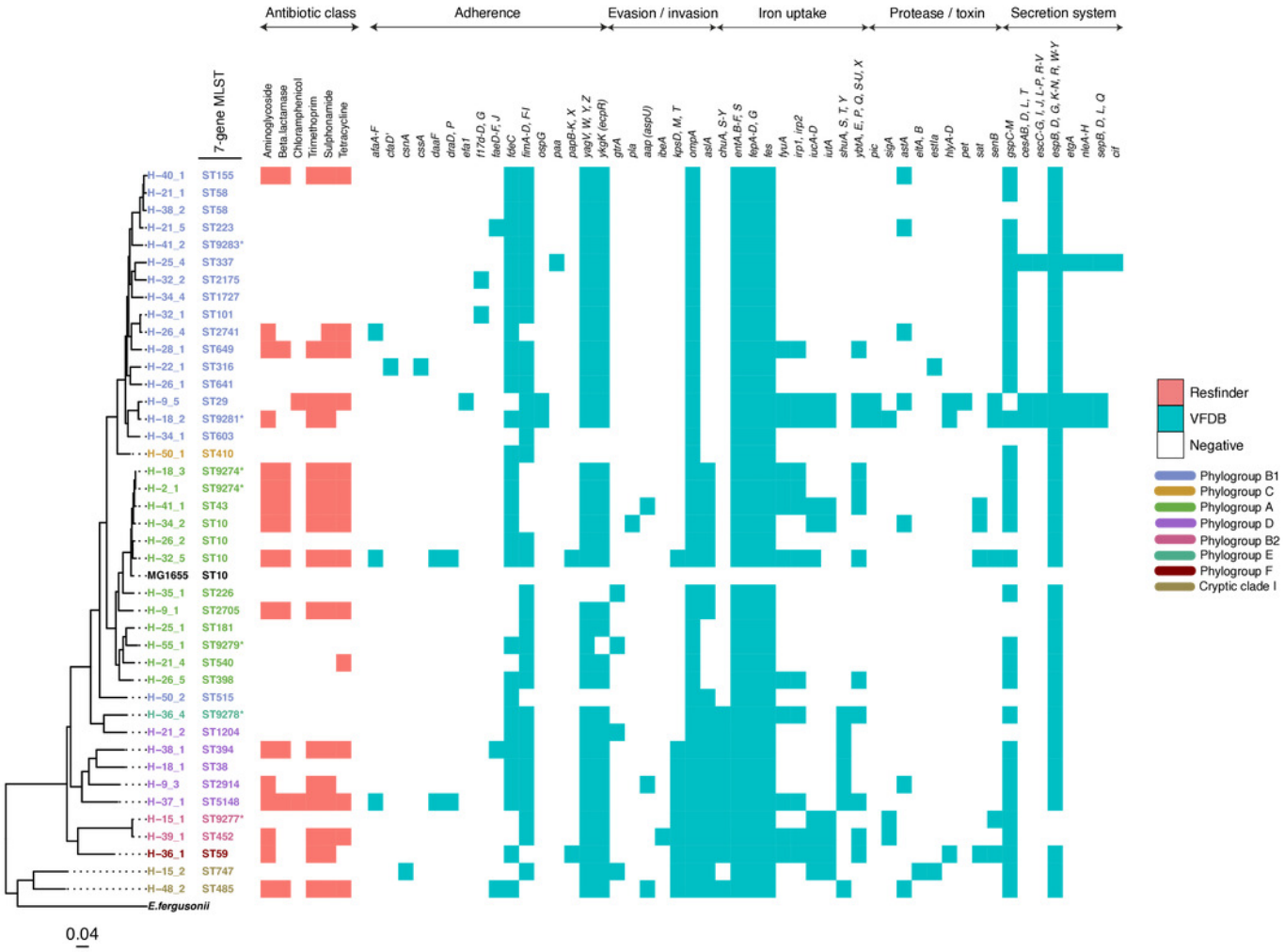

Antibiotic class
Adherence
Evasion / invasion
Iron uptake
Protease / toxin
Secretion system
7-gene MLST
Aminoglycoside
Beta-lactamase
Chloramphenicol
Trimethoprim
Sulphonamide
Tetracycline
afaA-F
cfaD'
csnA
cssA
daaF
draD, P
efa1
f17d-D, G
faeD-F, J
fdeC
fimA-D, F-I
ospG
paa
papB-K, X
yagV, W, Y, Z
ykgK (ecpR)
gtrA
pla
aap (aspU)
ibeA
kpsD, M, T
ompA
aslA
chuA, S-Y
entA, B-F, S
fepA-D, G
tes
fyuA
irp1, irp2
iucA-D
iutA
shuA, S, T, Y
ybtA, E, P, Q, S-U, X
pic
sigA
astA
eltA, B
estIa
hlyA-D
pet
sat
senB
gspC-M
cesAB, D, L, T
escC-G, I, J, L-P, R-V
espB, D, G, K-N, R, W-Y
etgA
nleA-H
sepB, D, L, Q
cif
H−40_1 ST155
H−21_1 ST58
H−38_2 ST58
H−21_5 ST223
H−41_2 ST9283*
H−25_4 ST337
H−32_2 ST2175
H−34_4 ST1727
H−32_1 ST101
H−26_4 ST2741
H−28_1 ST649
H−22_1 ST316
H−26_1 ST641
H−9_5 ST29
H−18_2 ST9281*
H−34_1 ST603
H−50_1 ST410
H−18_3 ST9274*
H−2_1 ST9274*
H−41_1 ST43
H−34_2 ST10
H−26_2 ST10
H−32_5 ST10
MG1655 ST10
H−35_1 ST226
H−9_1 ST2705
H−25_1 ST181
H−55_1 ST9279*
H−21_4 ST540
H−26_5 ST398
H−50_2 ST515
H−36_4 ST9278*
H−21_2 ST1204
H−38_1 ST394
H−18_1 ST38
H−9_3 ST2914
H−37_1 ST5148
H−15_1 ST9277*
H−39_1 ST452
H−36_1 ST59
H−15_2 ST747
H−48_2 ST485
E.fergusonii
0.04
Resfinder
VFDB
Negative
Phylogroup B1
Phylogroup C
Phylogroup A
Phylogroup D
Phylogroup B2
Phylogroup E
Phylogroup F
Cryptic clade I

Figure 3

The population structure of ST38.

A: A NINJA neighbour-joining tree showing the population structure of *E. coli* ST38, drawn using the genomes found in the core-genome MLST hierarchical cluster at HC1100, which corresponds to ST38 clonal complex. The size of the nodes represents the number of isolates per clade. The geographical locations where isolates were recovered are displayed in the legend; with the genome counts shown in square brackets. The study resident ST38 strains and the pathogenic ST38 strains recovered from GEMS cases are highlighted with red circles around the nodes. B: The closest neighbour to a pathogenic strain reported in GEMS [reference 30] is shown to be a commensal isolate recovered from a healthy individual. The size of the nodes represents the number of isolates per clade. The geographical locations where isolates were recovered are displayed in the legend; with the genome counts shown in square brackets. Red circles around the nodes are used to highlight the study resident ST38 strains and the pathogenic ST38 strains recovered from GEMS cases within this cluster. C: The closest relatives to the commensal ST38 strain recovered from this study is shown (red highlights), with the number of core-genome MLST alleles separating the two genomes displayed. The geographical locations where isolates were recovered are displayed in the legend; with the genome counts shown in square brackets, with the size of the nodes depicting the number of isolates per clade. D: A maximum-likelihood phylogenetic tree reconstructed using the genomes found in the cluster in C above, comprising both pathogenic and commensal ST38 strains is presented, depicting the genetic relationship between strain 100415 (pathogenic) and 103709 (commensal) (red highlights). The nodes are coloured to depict the status of the strains as pathogenic (red) or commensal (blue). The size of the nodes represents the number of isolates per clade. The geographical locations where isolates were recovered are displayed in the legend; with the genome counts shown in

square brackets.

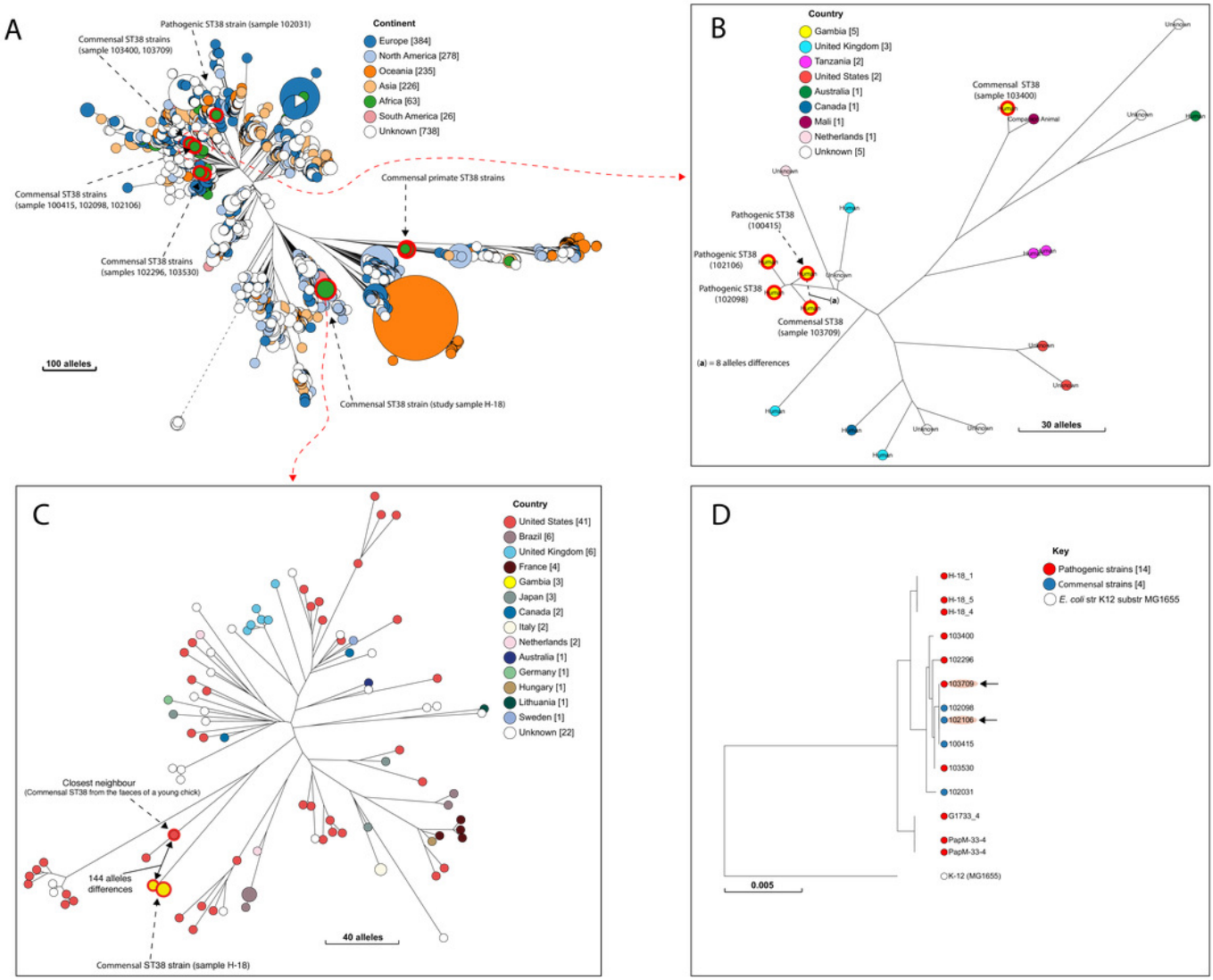


Figure 4

The population structure of ST58.

A: A NINJA neighbour-joining tree depicting the population structure of *E. coli* ST58, drawn using the genomes found that clustered together in the same in HC1100 hierarchical cluster in the core-genome MLST scheme. Commensal ST58 strains from this study and Gambian pathogenic ST58 isolates from GEMS are highlighted in red. The geographical locations where isolates were recovered are displayed in the legend; with the genome counts shown in square brackets. The size of the nodes represents the number of isolates per clade. B and C: The closest relatives to the study ST58 strains are shown. Geographical locations where isolates were recovered are displayed in the legend, with the genome counts displayed in square brackets. The red highlights around the nodes depict the study commensal ST58 strains and their closest neighbours. The size of the nodes represents the number of isolates per clade, and the geographical locations where isolates were recovered are displayed in the legend; with the genome counts shown in square brackets.

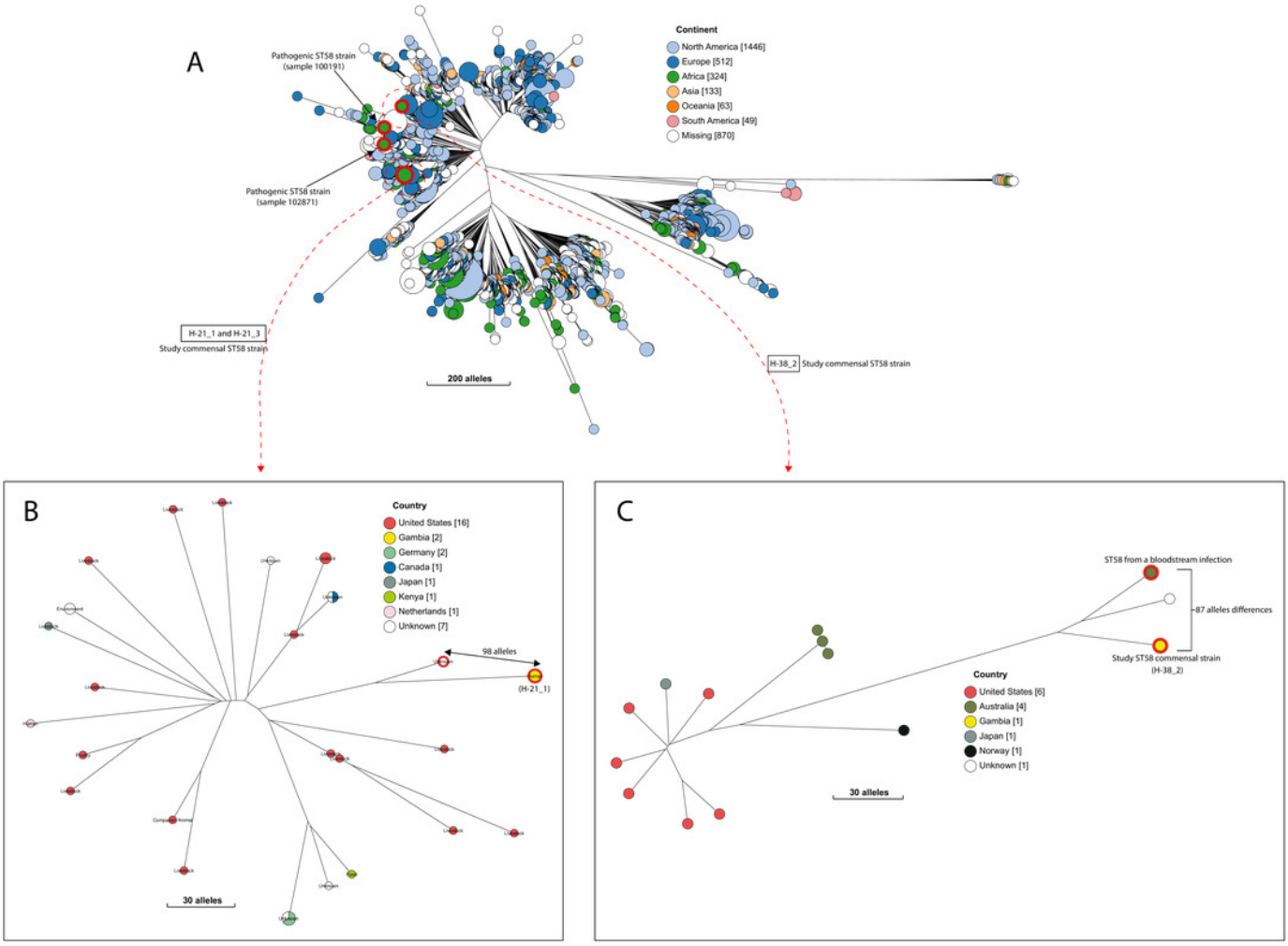
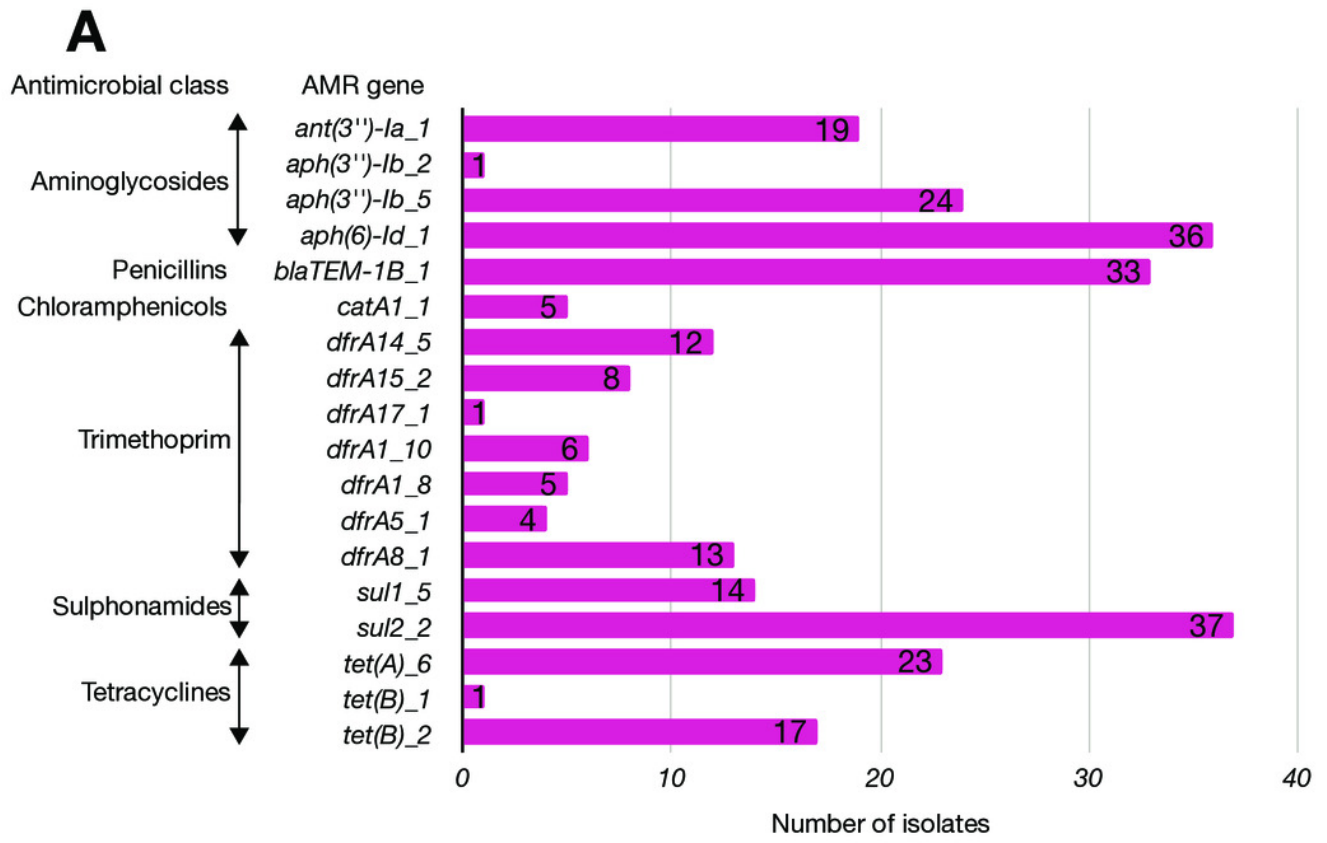

A
Pathogenic ST58 strain (sample 100191)
Pathogenic ST58 strain (sample 102871)
Continent
North America [1446]
Europe [512]
Africa [324]
Asia [133]
Oceania [63]
South America [49]
Missing [870]
H-21_1 and H-21_3
Study commensal ST58 strain
200 alleles
H-38_2 Study commensal ST58 strain
B
Country
United States [16]
Gambia [2]
Germany [2]
Canada [1]
Japan [1]
Kenya [1]
Netherlands [1]
Unknown [7]
98 alleles
(H-21_1)
30 alleles
C
ST58 from a bloodstream infection
87 alleles differences
Study ST58 commensal strain (H-38_2)
Country
United States [6]
Australia [4]
Gambia [1]
Japan [1]
Norway [1]
Unknown [1]
30 alleles

Figure 5

The prevalence of antimicrobial-associated genes detected in the isolates.

(A) The y-axis shows the prevalence of the detected AMR-associated genes in the study isolates, grouped by antimicrobial class. (B) A histogram depicting the number of antimicrobial classes to which resistance genes were detected in the corresponding strains.


A
Antimicrobial class
AMR gene
Aminoglycosides
Penicillins
Chloramphenicols
Trimethoprim
Sulphonamides
Tetracyclines
ant(3'')-Ia_1
aph(3'')-Ib_2
aph(3'')-Ib_5
aph(6)-Id_1
blaTEM-1B_1
catA1_1
dfrA14_5
dfrA15_2
dfrA17_1
dfrA1_10
dfrA1_8
dfrA5_1
dfrA8_1
sul1_5
sul2_2
tet(A)_6
tet(B)_1
tet(B)_2
19
1
24
36
33
5
12
8
1
6
5
4
13
14
37
23
1
17
0
10
20
30
40
Number of isolates

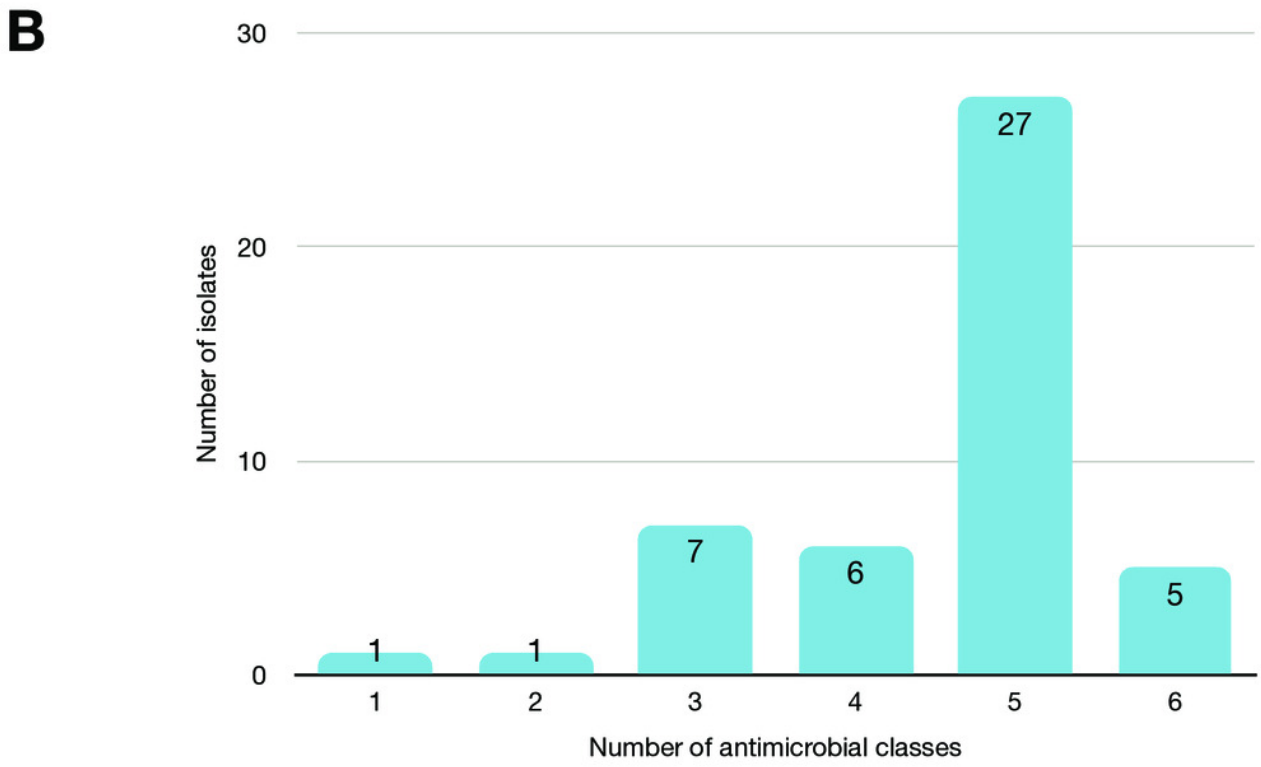
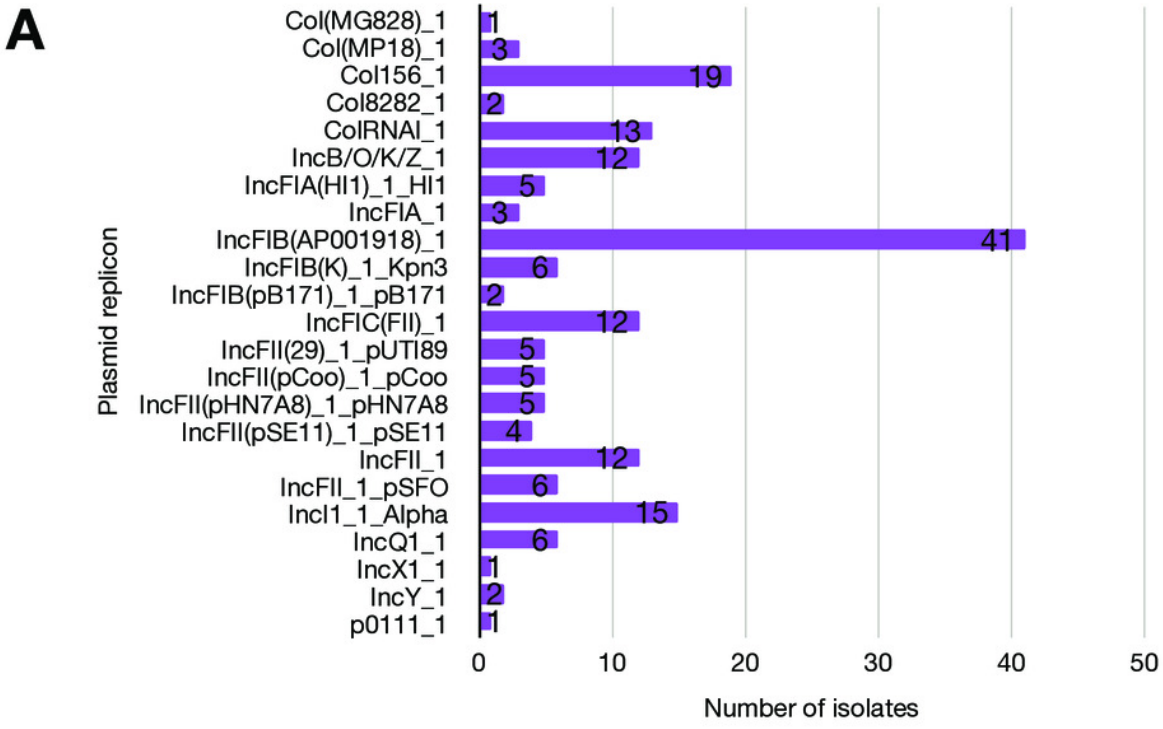
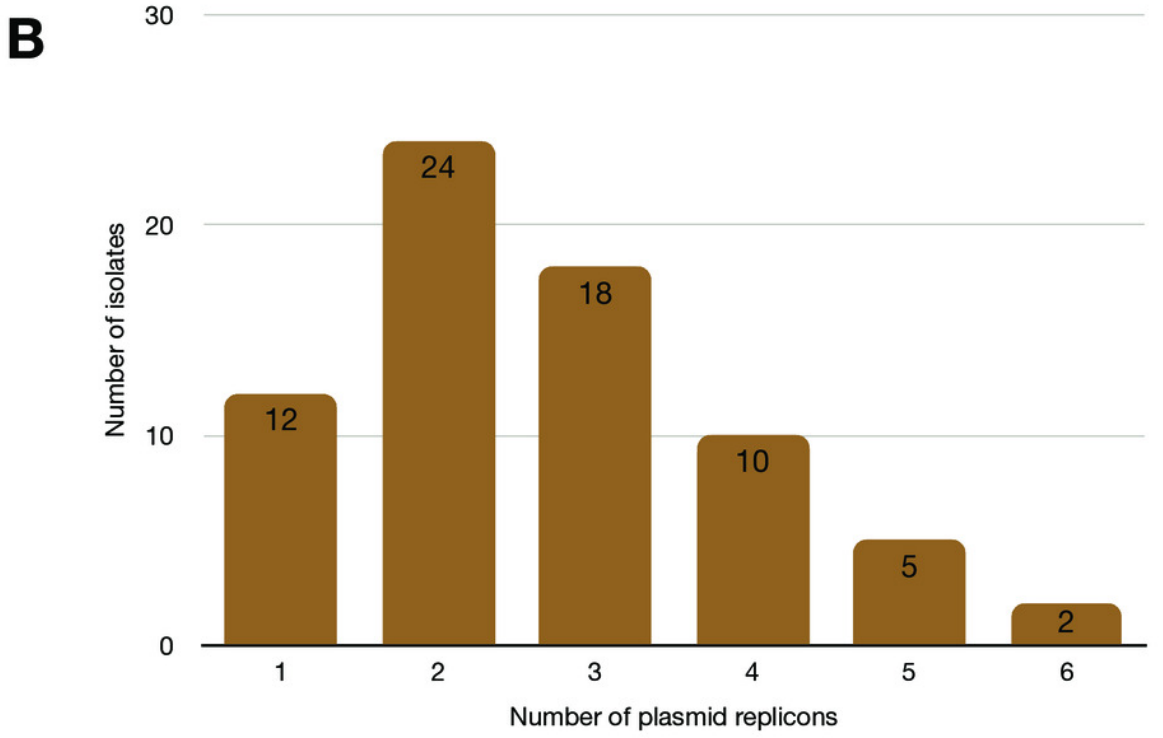

B
30
20
10
0
Number of isolates
1
1
7
6
27
5
1
2
3
4
5
6
Number of antimicrobial classes

Figure 6

Prevalence of plasmid replicons among the study isolates.

(A) Plasmid replicons detected in the study isolates. (B) A histogram depicting the number of plasmids co-harboured in a single strain.


A
Col(MG828)_1
Col(MP18)_1
Col156_1
Col8282_1
ColRNAI_1
IncB/O/K/Z_1
IncFIA(HI1)_1_HI1
IncFIA_1
IncFIB(AP001918)_1
IncFIB(K)_1_Kpn3
IncFIB(pB171)_1_pB171
IncFIC(FII)_1
IncFII(29)_1_pUTI89
IncFII(pCoo)_1_pCoo
IncFII(pHN7A8)_1_pHN7A8
IncFII(pSE11)_1_pSE11
IncFII_1
IncFII_1_pSFO
IncI1_1_Alpha
IncQ1_1
IncX1_1
IncY_1
p0111_1
1
3
19
2
13
12
5
3
41
6
2
12
5
5
5
4
12
6
15
6
1
2
1
Plasmid replicon
0
10
20
30
40
50
Number of isolates


B
30
20
10
0
24
18
12
10
5
2
Number of isolates
1
2
3
4
5
6
Number of plasmid replicons