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

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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 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 than 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 (1), while also providing critical insights into biology and evolution (2). 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 (3-5). 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" (6). 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 (7-13).

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) (14-17). 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 (18, 19).

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* (20-22), with one individual carrying 24 distinct clones (22-24). More recently, whole-genome sequencing has illuminated molecular epidemiological investigations (9), adaptation during and after infection (25, 26), as well as the intra-clonal diversity in healthy hosts (27).

There are two plausible sources of within-host genomic diversity. Although a predominant strain usually colonises the host for extended periods (28), successful immigration events mean that incoming strains can replace the dominant strain or co-exist alongside it as minority populations (29). 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 (20).

Most relevant studies have been limited to Western countries, except for a recent report from Tanzania (21), so little is known about the genomic diversity of *E. coli* in sub-Saharan Africa. The Global Enteric Multicenter Study (GEMS) (30, 31) has documented a high burden of diarrhoea attributable to *E. coli* (including *Shigella*) 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. 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-year-old asymptomatic Gambian children, who had been recruited from Basse, Upper River Region, the Gambia, into the GEMS study (30) as healthy controls from December 1, 2007, to March 3, 2011. Samples had been collected according to a previously described sampling protocol (32). 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). Ten of the original 76 samples proved unavailable for processing in this study.

Bacterial growth and isolation

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

Genomic DNA extraction and genome sequencing

Broth cultures were prepared from pure, fresh cultures of each colony-pick in 1 ml Luria-Bertani broth and incubated overnight to attain between $10^9 – 10^{10}$ cfu per ml. Genomic DNA was then extracted from the overnight broth cultures using the lysate method described in (33). The eluted DNA was quantified by the Qubit high sensitivity DNA assay kit (Invitrogen, MA, USA) and sequenced on the Illumina NextSeq 500 instrument (Illumina, San Diego, CA) as described previously (34).

Following Dixit et al. (20), we sequenced a random selection of isolates twice, using DNA obtained from independent cultures, to help in the determination of clones and the analysis of within-host variants (Supplementary File 5). Bioinformatic analyses of the genome sequences were carried out on the Cloud Infrastructure for Microbial Bioinformatics (CLIMB) platform (35).

Phylogenetic analysis

The paired 150bp reads were quality checked and assembled, as previously described (34). Snippy v4.3.2 (https://github.com/tseemann/snippy) was used for variant calling, using the complete genome sequence of commensal *E. coli* str. K12 substr. MG1655 as a reference strain (NCBI accession: NC_000913.3) and to generate a core-genome alignment, from which a maximum-likelihood phylogeny with 1000 bootstrap replicates was reconstructed using RAxML v8.2.4 (36), 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). 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.

Multi-locus sequence typing, Clermont typing and SNPs

The merged reads were uploaded to EnteroBase (37), where *de novo* assembly and genome annotation were carried out, and *in-silico* multi-locus sequence types (STs) assigned based on the Achtman scheme, allocating new sequence types (ST) if necessary. EnteroBase assigns phylogroups using ClermontTyper and EzClermont (38, 39) and unique core-genome MLST types based on 2, 513 core loci in *E. coli*. Publicly available *E. coli* sequences in EnteroBase (http://enterobase.warwick.ac.uk/species/index/ecoli) (37) were included for comparative analysis, including 23 previously sequenced isolates obtained from diarrhoeal cases recruited in the GEMS study in the Gambia (Supplementary File 1).

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). For the duplicate sequence reads of the same strains, we used SPAdes v3.13.2 (40) to assemble each set of reads and map the raw sequences from one sequencing run to the assembly of the other run and vice versa, as described previously (20). SNPs were detected using the CSIPhylogeny tool (https://cge.cbs.dtu.dk/services/CSIPhylogeny/) and compared between the two steps, counting only those SNPs that were detected in both sets of reads as accurate.

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 and visualised this as a heat map using RStudio v 3.5.1.

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 complexes, the equivalent of clonal complexes achieved with the legacy MLST clustering approaches (37). We reconstructed neighbour-joining phylogenetic trees using NINJA (41), 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 included both pathogenic and commensal *E. coli* strains recovered from the GEMS study. For the clusters that encompassed commensal and pathogenic strains belonging to the same ST, 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).

Ethical statement

The study was approved by the joint Medical Research Council Unit The Gambia-Gambian Government ethical review board.

Results

Population structure

The study population included 27 females and 39 males (Table 1). All but one reported the presence of a domestic animal within the household. Twenty-one samples proved positive for the growth of *E. coli*, yielding 88 isolates. We detected 37 seven-allele sequence types (STs) among the isolates, with a fairly even distribution (Figure 2). Five STs were completely novel (ST9274, ST9277, ST9278, ST9279 and ST9281). These study strains were scattered over all the eight main phylogroups of *E. coli* (Table 2). Hierarchical clustering of core genomic STs revealed twenty-seven cgST clonal complexes (Supplementary File 2).

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 4, yellow highlight), suggesting recent transfer from one child to another or recent acquisition from a common source.

We observed thirteen cases where a single host harboured two or more variants within the same SNP cloud (Table 2). Such within-host evolution accounted for around a quarter of the observed variation, with immigration explaining the remaining three quarters. 22% 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). The average number of SNPs among variants within such SNP clouds was 5 (range 0-18) (Table 3). 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 4, 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 (Table 4). 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 5).

We detected 130 genes encoding putative virulence factors across the 88 study isolates (Figure 2; Supplementary File 3). More than half of the isolates encoded resistance to three or more clinically relevant classes of antibiotics (Figure 3; Supplementary Figure 1). The most common resistance gene network was -*aph(6)-Id_1-sul2* (41% of the isolates), followed by *aph(3'')-Ib_5-sul2* (27%) and *bla-TEM-aph(3'')-Ib_5* (24%). Most isolates (67%) harboured two or more plasmid types (Figure 4). Of the 24 plasmid types detected, IncFIB was the most common (41%), followed by col156 (19%) and IncI_1-Alpha (15%). Nearly three-quarters of the multi-drug resistant isolates carried IncFIB (AP001918) plasmids, suggesting that these large plasmids disseminate resistance genes within our study population.

Discussion

This study provides an overview of the within-host genomic diversity of *E. coli* in healthy children from a rural setting in the Gambia, West Africa. Surprisingly, we recovered a low rate of colonisation than reported elsewhere among children of similar age groups (42), with only a third of our study samples yielding growth of *E. coli*. This may reflect geographical variation but might also be some hard-to-identify effect of the way the samples were handled, even though they were kept frozen and thawed only just before culture.

Several studies have shown that sampling a single colony is insufficient to capture *E. coli* strain diversity in stools (20, 21, 23). Lidin-Janson *et al.* (43) claim that sampling five colonies provides a >99% chance of recovering dominant genotypes from single stool specimens, while Schlager *et al.* (24) calculate that sampling twenty-eight colonies provides a >90% chance of recovering minor genotypes. Our results confirm the importance of multiple-colony picks in faecal surveillance studies, as over half (57%) of our strains would have been missed by picking a single colony.

Although our strains encompassed all eight major phylotypes of *E. coli*, the majority fell into the A and B1 phylogenetic groups, in line with previous reports that these phylogroups dominate in stools from people in low- and middle-income countries (44, 45). The prevalence of putative virulence genes in most of our isolates highlights the pathogenic potential of commensal intestinal strains—regardless of their phylogroup—should they gain access to the appropriate tissues, for example, the urinary tract. Our results complement previous studies reporting genomic similarities between faecal *E. coli* isolates and those recovered from urinary tract infection (25, 46).

We found that within-host evolution plays a minor role in the generation of diversity, in line with Dixit et al. (20), who reported that 83% of diversity originates from immigration events, and with epidemiological data suggesting that the recurrent immigration events account for the high faecal diversity of *E. coli* in the tropics (47). 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 estimated mutation rate for *E. coli* lineages is around one SNP per genome per year (48), 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. 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 (49). However, a limitation of our study is that we did not perform phenotypic antimicrobial resistance testing, although Doyle et al. (50) 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 (51).

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. Besides, this work has added significantly to the number of commensal *E. coli* genomes, which are underrepresented in public repositories. 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.

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Data summary

All genomic assemblies for the strains included in this study are freely available from EnteroBase (http://enterobase.warwick.ac.uk/species/index/ecoli). The EnteroBase genome assembly barcodes are provided in Supplementary Files 1 and 2.

The raw genomic sequences have been deposited in the NCBI SRA, under the BioProject ID PRJNA658685 and accession numbers SAMN15880286 to SAMN15880281.

Conflicts of interest

We declare no conflicts of interest.

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.

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Tables and figure legends

Table 1: Characteristics of the study population

Sample ID	**Lab ID**	**Age (months)**	**Gender**	**Bristol stool index**	**Domestic animal within household**	**Enrolment date**
102135	H1	43	Female	Thick liquid	Goat, sheep	18-Feb-09
102650	H2	45	Female	Soft	Goat, sheep, donkey	27-Jul-09
103296	H3	44	Male	Soft	Goat, horse, donkey, rodent	27-Apr-10
103298	H4	44	Male	Formed	Sheep, fowl, horse, donkey, rodent	27-Apr-10
103621	H5	37	Female	Soft	Sheep, fowl, rodent	01-Sep-10
103650	H6	48	Female	Soft	Fowl, donkey, rodent	29-Sep-10
103649	H7	45	Female	Soft	Goat, sheep, fowl, horse, rodent	29-Sep-10
103071	H8	53	Male	Formed	Goat, sheep, fowl	15-Jan-10
103622	H9	39	Female	Soft	Goat, sheep	01-Sep-10
100167	H10	40	Female	Soft	Goat, sheep, fowl	01-Feb-08
100217	H11	57	Male	Formed	Cat, fowl, horse, rodent	21-Feb-08
100230	H12	51	Male	Soft	Goat, sheep, cat, fowl, rodent	28-Feb-08
100612	H13	55	Female	Formed	Goat, sheep, dog, fowl, horse, donkey, rodent	16-Aug-08
100162	H14	47	Female	Thick liquid	Sheep, horse, donkey, rodent	30-Jan-08
102255	H15	42	Male	Formed	Goat, sheep, fowl, horse, donkey, rodent	26-Mar-09
102250	H16	39	Male	Formed	Fowl	25-Mar-09
102114	H17	54	Male	Formed	Rodent	12-Feb-09
102123	H18	37	Female	Soft	Goat, sheep, fowl, rodent	14-Feb-09
103282	H19	43	Male	Formed	Goat, sheep, dog, cat, cow, fowl,	22-Apr-10
100817	H20	44	Male	Soft	Dog, fowl	03-Dec-08
100816	H21	40	Male	Soft	Goat, sheep, cow, fowl, horse, donkey, rodent	03-Dec-08
102836	H22	47	Male	Thick liquid	Fowl, rodent	12-Oct-09
102837	H23	41	Male	Thick liquid	Sheep, fowl, rodent	12-Oct-09
102843	H24	44	Male	Soft	Fowl, rodent	13-Oct-09
102907	H25	36	Male	Soft	Goat, sheep, fowl	05-Nov-09
102905	H26	37	Male	Soft	Goat, sheep, fowl	05-Nov-09
102262	H27	38	Male	Formed	Goat, sheep, rodent	01-Apr-09
102728	H28	41	Male	Soft	Goat, fowl	24-Aug-09
102729	H29	41	Male	Soft	Goat, dog, cat, fowl, donkey	24-Aug-09
100806	H30	55	Male	Soft	Goat, sheep, dog, fowl	21-Nov-08
102053	H31	37	Female	Formed	Cow, fowl, donkey, rodent	29-Jan-09
102052	H32	38	Female	Formed	Goat, sheep, cow, fowl, donkey, rodent	29-Jan-09
102511	H33	37	Male	Soft	Fowl, horse, donkey, rodent	19-Jun-09

102649	H34	37	Male	Soft	Fowl, horse, donkey, rodent	27-Jul-09
102454	H35	52	Male	Soft	Sheep, fowl, donkey, rodent	02-Jun-09
102459	H36	51	Male	Formed	Goat, sheep, dog, cat, cow, horse, donkey, rodent	04-Jun-09
100303	H37	58	Male	Formed	Sheep, fowl	08-Apr-08
100320	H38	42	Female	Formed	Sheep, fowl, rodent	19-Apr-08
100319	H39	45	Female	Formed	Goat, sheep, fowl, rodent	17-Apr-08
103081	H40	39	Female	Thick liquid	Goat, sheep, fowl, horse, donkey, rodent	20-Jan-10
103082	H41	39	Female	Thick liquid	Goat, sheep, fowl, horse, donkey, rodent	20-Jan-10
100663	H42	36	Male	Thick liquid	Goat, sheep, fowl, donkey	10-Sep-08
100072	H43	51	Female	Formed	Goat, cow, fowl, rodent	03-Jan-08
103171	H44	36	Female	Soft	Goat, sheep, rodent, fowl, rodent	18-Feb-10
103172	H45	36	Female	Soft	Goat, sheep, fowl, rodent	18-Feb-10
103292	H46	39	Male	Soft	Goat, sheep, fowl	23-Apr-10
102952	H47	36	Male	Soft	Goat, sheep, fowl, rodent	20-Nov-09
102953	H48	37	Male	Soft	Goat, sheep, fowl, rodent	20-Nov-09
102964	H49	40	Female	Formed	Goat, fowl, rodent	26-Nov-09
102966	H50	37	Female	Formed	Goat, sheep, fowl, horse, donkey, rodent	22-Apr-10
103281	H51	44	Male	Formed	Goat, sheep, dog, cat, fowl	22-Apr-10
100540	H52	43	Male	Soft	Goat, sheep, fowl, rodent	22-Jul-08
103123	H53	38	Male	Soft	Sheep	03-Feb-10
103124	H54	36	Male	Soft	Fowl	03-Feb-10
102089	H55	38	Female	Soft	Goat, cow, fowl, horse, donkey, rodent	05-Feb-09
103297	H56	38	Male	Soft	Goat, sheep, fowl, horse, donkey, rodent	27-Apr-10
102251	H57	39	Male	Formed	Fowl	25-Mar-09
103602	H58	38	Female	Formed	Goat, sheep, cow, fowl	26-Aug-10
103600	H59	39	Female	Formed	Goat, sheep, fowl	26-Aug-10
100026	H60	49	Female	Soft	Goat, sheep, cow, fowl	14-Dec-07
102102	H61	47	Female	Opaque watery	None	11-Feb-09
102263	H62	38	Male	Formed	Horse, donkey, rodent	01-Apr-09
103070	H63	58	Male	Soft	Goat, sheep, fowl	15-Jan-10
103130	H64	40	Male	Soft	Sheep, fowl	03-Feb-10
102051	H65	36	Female	Formed	Goat, sheep, dog, cat, cow, fowl, donkey, rodent	29-Jan-09
102524	H66	36	Male	Soft	Goat, sheep, fowl, horse, donkey, rodent	24-Jun-09

Table 2: Phylogroup and sequence types of the distinct clones isolated in each patient

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

Table 3: 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

Table 4: Closest relatives to the study isolates

Sample ID	**7-gene ST**	**Neighbour host**	**Neighbour status**	**Neighbour's country of isolation**	**Allelic distance**
H-32_5	10	Human	Unknown	UK	18
H-36_1	59	Human	Unknown	UK	18
H-39_1	452	Human	Commensal	UK	26
H-9_1	2705	Livestock		China	29
H-18_3	9274	Human	Commensal	Unknown	34
H-2_1	9274	Human	Commensal	Unknown	34
H-22_1	316	Human	Commensal	UK	35
H-38_1	394	Human	Pathogen (cystitis)	US	39
H-25_4	337	Human	Unknown	Mali	43
H-37_1	5148	Human	Pathogen (diarrhoea)	Ecuador	43
H-26_1	641	Livestock		US	46
H-26_5	398	Poultry		Kenya	47
H-48_2	485	Human	Commensal	Tanzania	57
H-15_1	9277	Human	Commensal	Zambia	68
H-15_2	747	Human	Commensal	Egypt	72
H-28_1	469	Human	Commensal	Kenya	77
H-21_2	1204	Avian		Kenya	81
H-34_2	10	Livestock		UK	83
H-38_2	58	Human	Pathogen (bloodstream infection)	Australia	87
H-34_4	1727	Unknown	Unknown	Unknown	89
H-35_1	226	Human	Commensal	China	93
H-21_1	58	Unknown	Unknown	Unknown	98
H-21_4	540	Human	Unknown	Belgium	100
H-32_2	2175	Livestock		UK	100
H-26_2	10	Livestock		US	111
H-32_1	101	Unknown	Unknown	Unknown	111
H-50_2	515	Environment		Canada	117
H-41_1	43	Unknown	Unknown	Unknown	120
H-26_4	2741	Human	Commensal	Germany	126
H-50_1	410	Livestock		US	140
H-18_1	38	Poultry		US	144
H-21_5	223	Unknown	Unknown	Unknown	145
H-40_1	155	Unknown	Unknown	US	146
H-41_2	9283	Environment	Commensal	US	191
H-36_4	9278	Avian		Kenya	208
H-9_3	2914	Canine		UK	272
H-9_5	29	Unknown	Unknown	Unknown	288
H-34_1	603	Laboratory		UK	325
H-55_1	9279	Environment		Unknown	333
H-18_2	9281	Unknown	Unknown	France	430
H-25_1	181	Human	Commensal	Tanzania	607

Legends to figures

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.

Figure 3

A: The prevalence of antimicrobial-associated genes detected in the isolates. The y-axis shows the detected AMR-associated genes in the genomes, grouped by antimicrobial class.

B: A histogram depicting the number of antimicrobial classes to which resistance genes were detected in the corresponding strains.

Figure 4

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

Figure 5

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. B: The closest neighbour to a pathogenic strain reported in GEMS [4] is shown to be a commensal isolate recovered from a healthy individual. 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. 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 geographical locations where isolates were recovered are displayed in Figures 4A-C; the genome counts shown in square brackets.

Supplementary material

Supplementary Figure 1

A co-occurrence matrix of acquired antimicrobial resistance genes detected in the study isolates. The diagonal values show how many isolates each individual gene was found in, while the intersections between the columns represent the number of isolates in which the corresponding antimicrobial resistance genes co-occurred.

Supplementary Figure 2

A Neighbour-joining phylogenetic tree depicting the genetic relationships among twenty-four strains isolated from diarrhoeal cases in the GEMS study [4]. The Sequence types identified in these isolates are shown in the legend, with the genome count displayed in square brackets next to the respective sequence types. Three STs (ST38, ST58 and ST10) overlapped with what was found among commensal strains from this study (see Figure 2).

Supplementary File 1

Sequencing statistics and characteristics of twenty-four previously sequenced GEMS cases included in this study.

Supplementary File 2

A summary of the sequencing statistics of the study isolates reported in this study.

Supplementary File 3

A summary of the virulence factors detected among the study isolates and their known functions.

Supplementary File 4

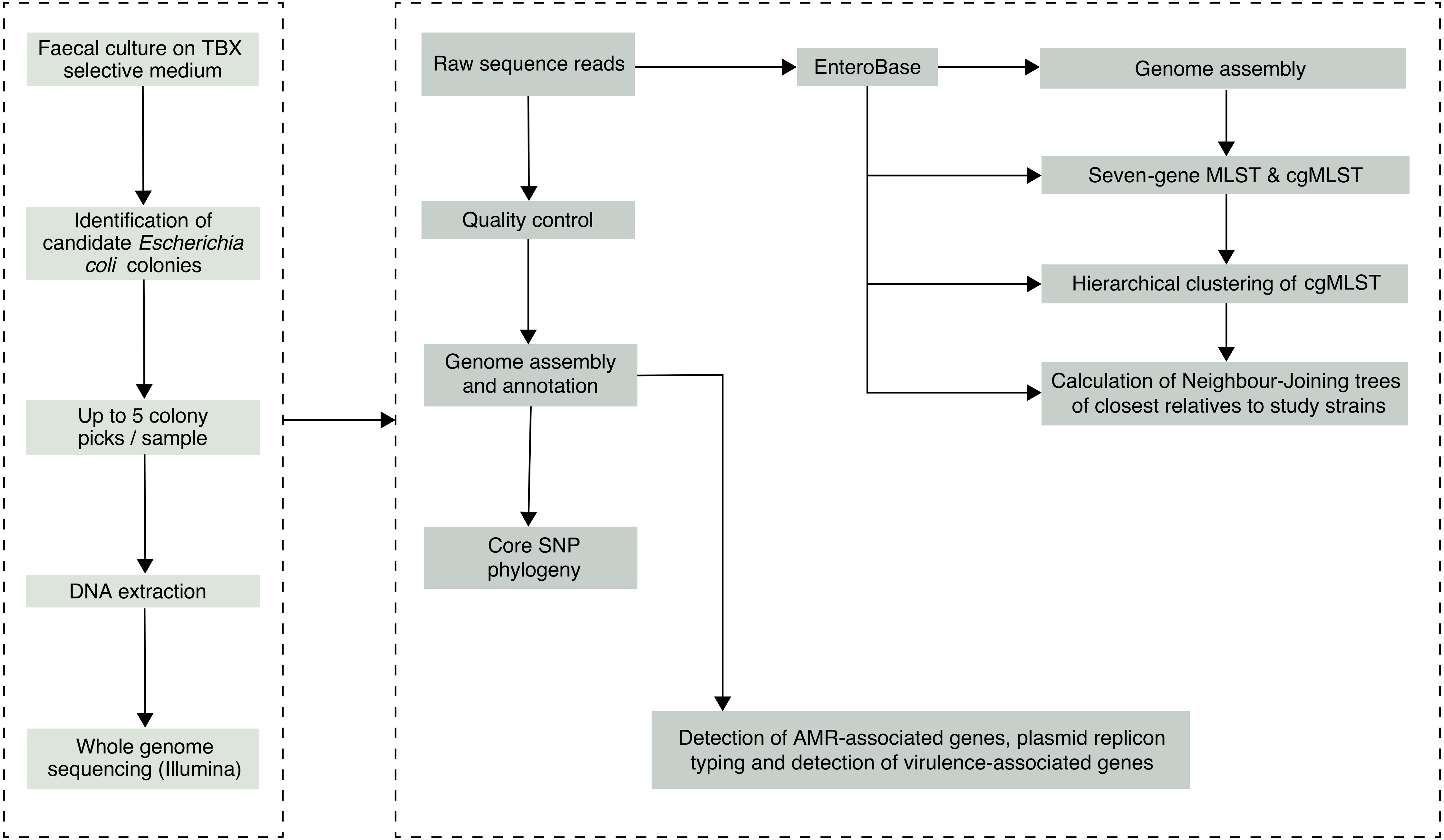
A pairwise single nucleotide polymorphism matrix showing the SNP distances between the study genomes.

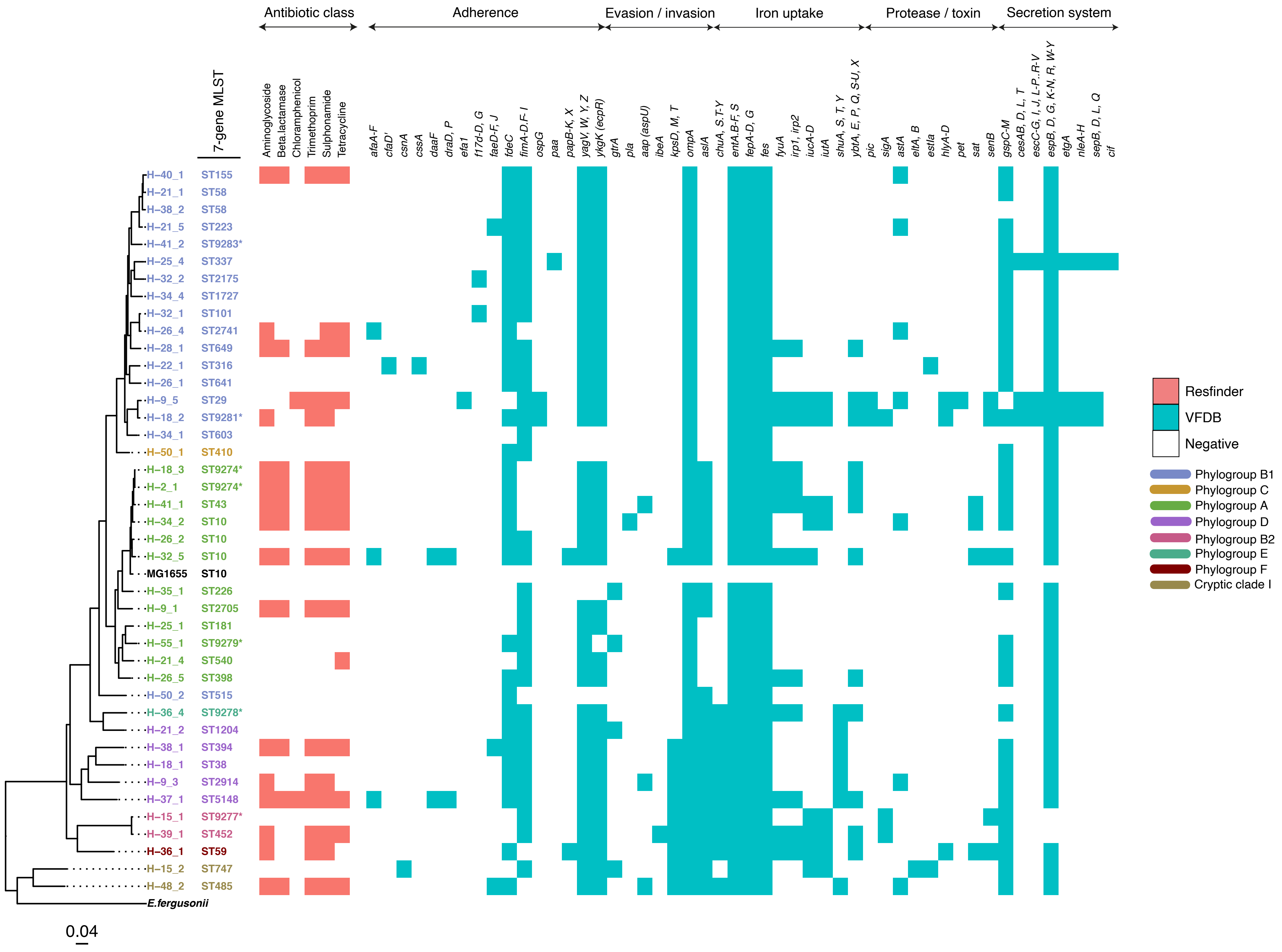
Supplementary File 5

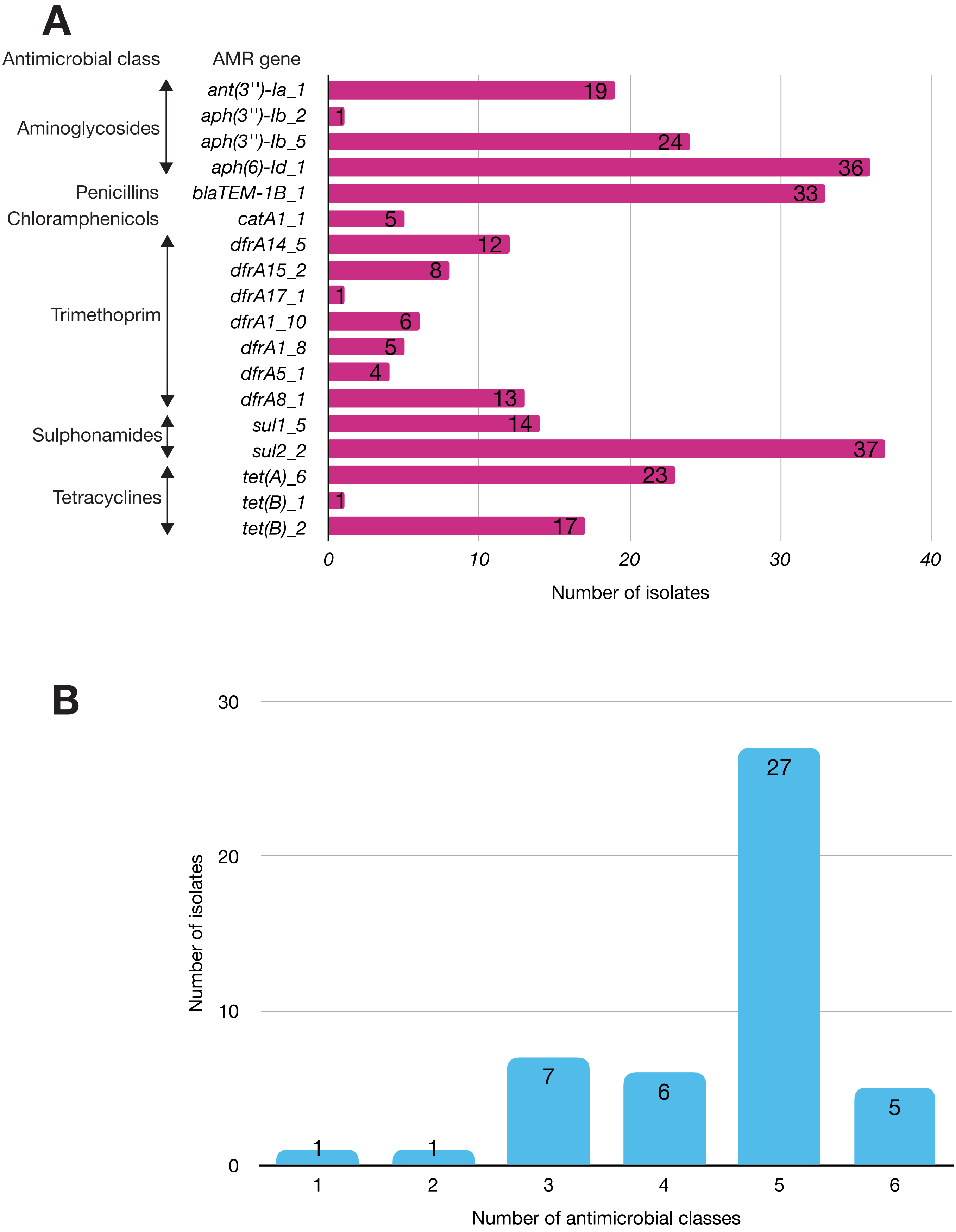
List of the sample clones for which two independent cultures were obtained and sequenced, to find the SNPs between the same clones.

Supplementary File 6

Mutations in variants inferred to have been derived from within-host evolution.


Faecal culture on TBX selective medium
Identification of candidate *Escherichia coli* colonies
Up to 5 colony picks / sample
DNA extraction
Whole genome sequencing (Illumina)
Raw sequence reads
Quality control
Genome assembly and annotation
Core SNP phylogeny
EnteroBase
Genome assembly
Seven-gene MLST & cgMLST
Hierarchical clustering of cgMLST
Calculation of Neighbour-Joining trees of closest relatives to study strains
Detection of AMR-associated genes, plasmid replicon typing and detection of virulence-associated genes


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,T-Y
entA,B-F, S
fepA-D, G
fes
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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AMR gene
Aminoglycosides
Penicillins
Chloramphenicols
Trimethoprim
Sulphonamides
Tetracyclines
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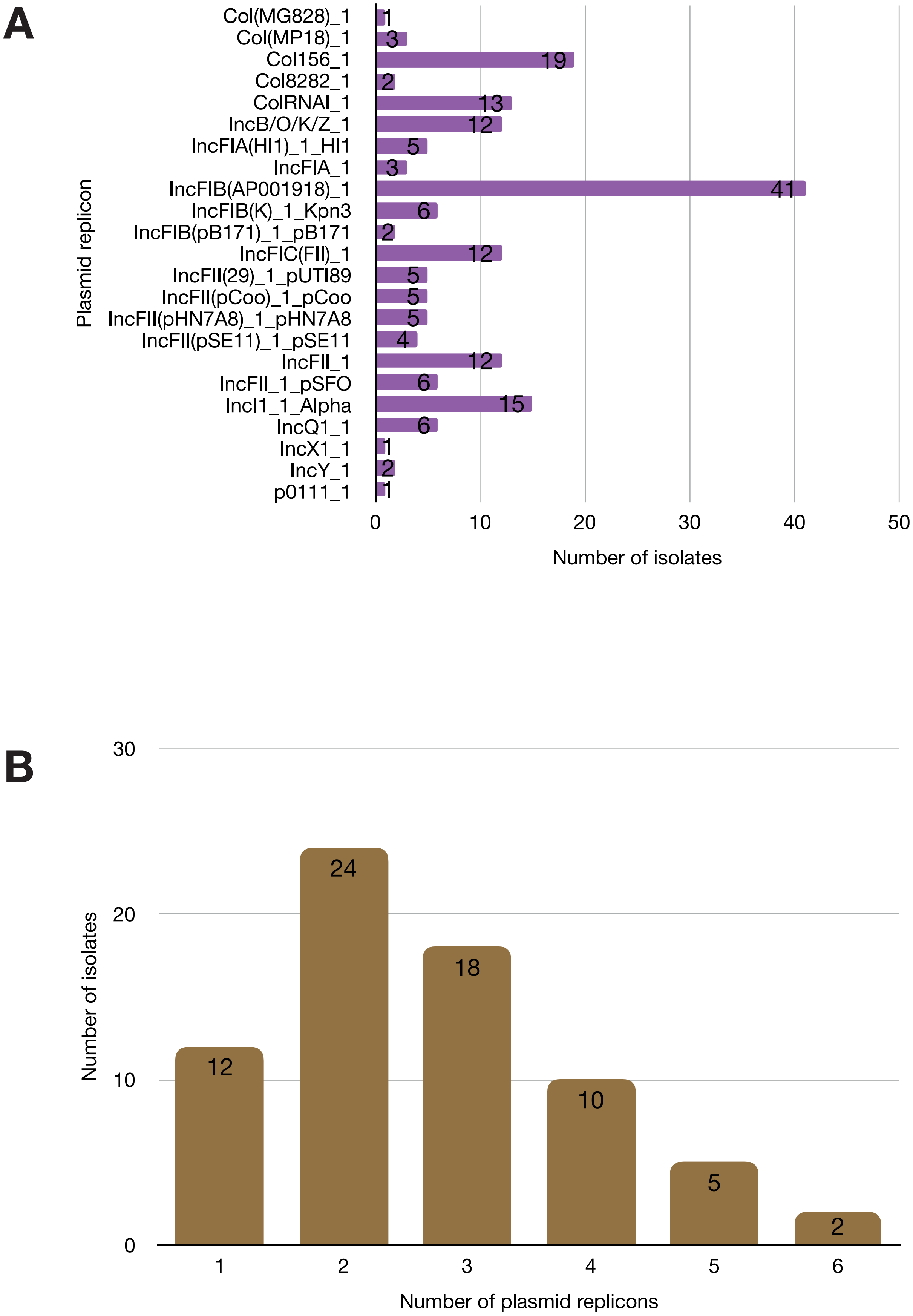
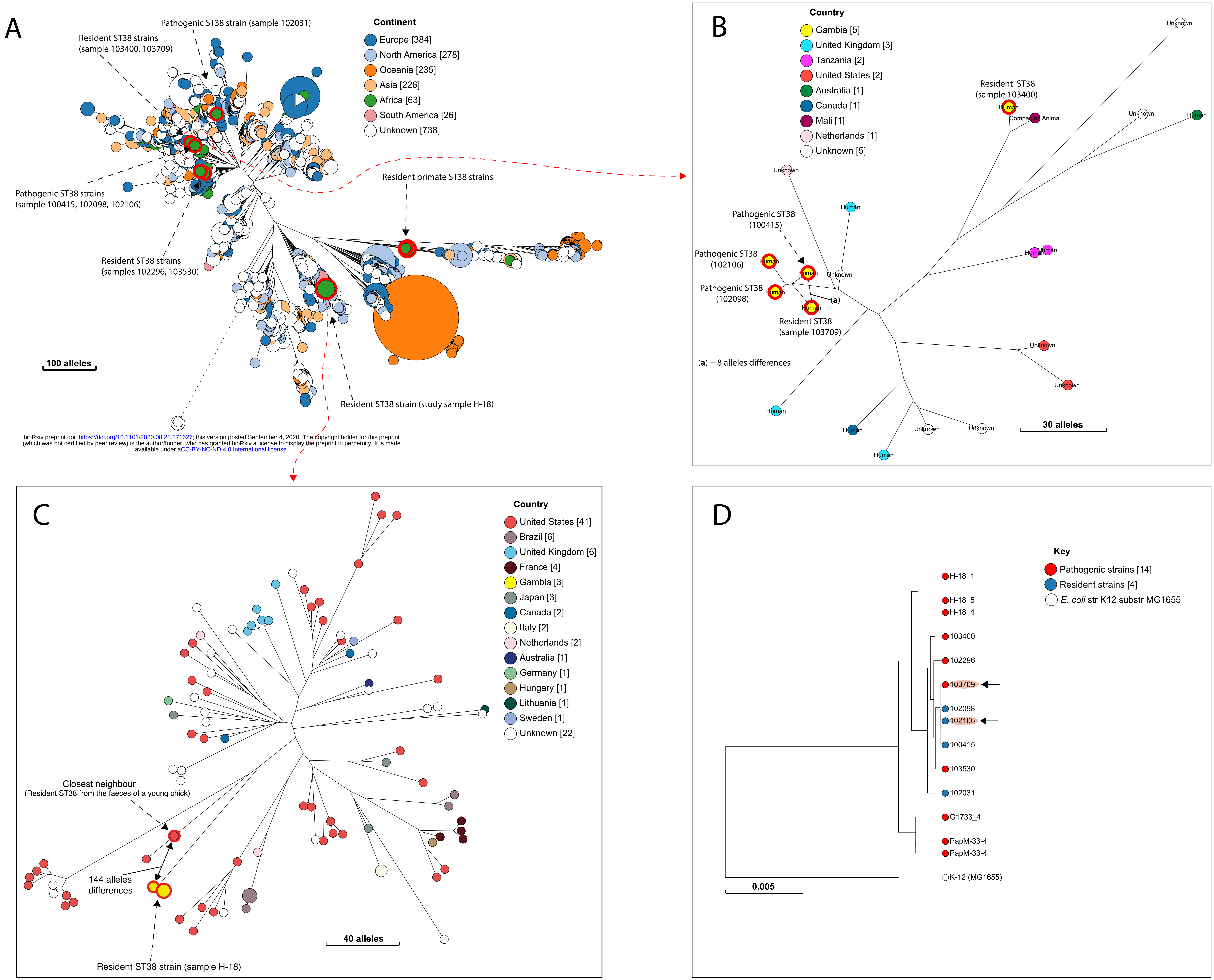
aph(3'')-Ib_2 1
aph(3'')-Ib_5 24
aph(6)-Id_1 36
blaTEM-1B_1 33
catA1_1 5
dfrA14_5 12
dfrA15_2 8
dfrA17_1 1
dfrA1_10 6
dfrA1_8 5
dfrA5_1 4
dfrA8_1 13
sul1_5 14
sul2_2 37
tet(A)_6 23
tet(B)_1 1
tet(B)_2 17
0
10
20
30
40
Number of isolates
B
30
20
10
0
Number of isolates
1 1
2 1
3 7
4 6
5 27
6 5
Number of antimicrobial classes


A
Plasmid replicon
Col(MG828)_1 1
Col(MP18)_1 3
Col156_1 19
Col8282_1 2
ColRNAI_1 13
IncB/O/K/Z_1 12
IncFIA(HI1)_1_HI1 5
IncFIA_1 3
IncFIB(AP001918)_1 41
IncFIB(K)_1_Kpn3 6
IncFIB(pB171)_1_pB171 2
IncFIC(FII)_1 12
IncFII(29)_1_pUTI89 5
IncFII(pCoo)_1_pCoo 5
IncFII(pHN7A8)_1_pHN7A8 5
IncFII(pSE11)_1_pSE11 4
IncFII_1 12
IncFII_1_pSFO 6
IncI1_1_Alpha 15
IncQ1_1 6
IncX1_1 1
IncY_1 2
p0111_1 1
0
10
20
30
40
50
Number of isolates
B
30
20
10
0
Number of isolates
12
24
18
10
5
2
1
2
3
4
5
6
Number of plasmid replicons


A
Pathogenic ST38 strain (sample 102031)
Resident ST38 strains (sample 103400, 103709)
Pathogenic ST38 strains (sample 100415, 102098, 102106)
Resident ST38 strains (samples 102296, 103530)
Resident primate ST38 strains
Resident ST38 strain (study sample H-18)
100 alleles
Continent
Europe [384]
North America [278]
Oceania [235]
Asia [226]
Africa [63]
South America [26]
Unknown [738]
B
Country
Gambia [5]
United Kingdom [3]
Tanzania [2]
United States [2]
Australia [1]
Canada [1]
Mali [1]
Netherlands [1]
Unknown [5]
Resident ST38 (sample 103400)
Companion Animal
Pathogenic ST38 (100415)
Pathogenic ST38 (102106)
Pathogenic ST38 (102098)
Resident ST38 (sample 103709)
(a)
(a) = 8 alleles differences
Human
Unknown
30 alleles
C
Country
United States [41]
Brazil [6]
United Kingdom [6]
France [4]
Gambia [3]
Japan [3]
Canada [2]
Italy [2]
Netherlands [2]
Australia [1]
Germany [1]
Hungary [1]
Lithuania [1]
Sweden [1]
Unknown [22]
Closest neighbour
(Resident ST38 from the faeces of a young chick)
144 alleles differences
Resident ST38 strain (sample H-18)
40 alleles
D
Key
Pathogenic strains [14]
Resident strains [4]
E. coli str K12 substr MG1655
H-18_1
H-18_5
H-18_4
103400
102296
103709
102098
102106
100415
103530
102031
G1733_4
PapM-33-4
PapM-33-4
K-12 (MG1655)
0.005