Plasmids shape the diverse accessory resistomes of *Escherichia coli* ST131

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Abstract

The human gut microbiome includes beneficial, commensal and pathogenic bacteria that possess antimicrobial resistance (AMR) genes and exchange these predominantly through conjugative plasmids. *Escherichia coli* is a significant component of the gastrointestinal microbiome and is typically non-pathogenic in this niche. In contrast, extra-intestinal pathogenic *E. coli* (ExPEC) including ST131 may occupy other environments like the urinary tract or bloodstream where they express genes enabling AMR and host adhesion like type 1 fimbriae. The extent to which non-pathogenic *E. coli* and uropathogenic ExPEC ST131 share AMR genes remains understudied at a genomic level, and here we examined this using a preterm infant resistome. We found that individual ST131 isolates had extensive AMR gene diversity even though there was a large core resistome across all *E. coli* examined here. Comparisons with a range of plasmids common in ST131 showed this AMR gene composition was driven by conjugation, recombination and mobile genetic elements, highlighting that analyses restricted to the core genome may be limiting and could miss AMR gene transfer patterns. We found that the segments of plasmid pEK499 were ancestral to most ST131 Clade C isolates, and along with pEK204 had evidence of a co-evolutionary signal with chromosomal genes. We also demonstrate that ST131 possessed extensive genetic diversity at key type 1, type IV, P and F17-like fimbriae genes, some of which are plasmid-encoded, and that this variability in fimbrial operons was highest in subclade C2. The type, structure and composition of AMR genes, plasmids and fimbriae vary widely in ST131 Clade C and this may mediate pathogenicity and infection outcomes.

Keywords: *Genome, fimbrial, plasmid, ST131, Escherichia coli, evolution, infection.*

Introduction

Extra-intestinal pathogenic *E. coli* (ExPEC) cause extensive infections outside the gut, from which they can originate. ExPEC have a wide range of virulence factor (VF) [1-4] and antimicrobial resistance (AMR) genes [5], especially sequence type (ST) 131 from serotypes O25b:H4 and O16:H5 in phylogroup B2 [6]. ST131 causes a substantial minority of ExPEC cases, and possibly a majority of extended-spectrum beta-lactamase (ESBL)-producing ones [7]. ST131's acquisition of key AMR genes encoded on plasmids [4,8] has coincided with its adaptation to new environments [9,10].

ExPEC adherence factors that assist with colonising different host niches are mainly produced by the Chaperone-Usher secretion pathway: type 1, P, F1C/S and AFA fimbriae [11-14]. Within ExPEC ST131, fluoroquinolone-resistant Clade C is the main cause of human infection globally [15-16]. Its D-mannose-specific type 1 fimbriae encoded by the *fim* operon bind the host mucosal epithelium to cause urinary tract and kidney infection [18-21]. However, the genetic diversity of fimbrial operons beyond *fim* in Clade C has not been examined in large collections of isolates.

ExPEC infection can be associated with an imbalance in microbiome composition [22] and *E. coli* is the most common initial coloniser of infant intestines [23]. Although most are commensal [24], and others be protective against pathogen invasion [25], AMR is prevalent in neonatal units [26] and within-host gene exchange between commensal and pathogenic bacteria may be common [27]. ST131 spreads between mother-infant pairs [28] likely via an oro-faecal transmission route [29], and such colonisation of infants can last for long periods [30]. Consequently, AMR gene screening can inform on pathogen treatment strategies [31] and the effect of antibiotic treatment on microbiome compositions [32].

The ST131 resistome (the set of AMR genes) includes ESBL genes [33-34] that allow third-generation cephalosporin-resistance [35] and are associated with three main cefotaximase (CTX-M) resistance alleles named $bla_{\text{CTX-M-14/15/27}}$ [36]. Like most AMR genes, these are sandwiched by mobile genetic elements (MGEs) on plasmids and thus are part of the mobile accessory resistome that can be changed by horizontal gene transfer (HGT) [37-39], or lost if no longer beneficial [40-41]. Of nine common bacterial pathogens, *E. coli* has the highest MGE numbers and high levels of transposases and phage-associated MGEs [42]. Diverse pathogenic bacteria can share MGEs [43-45] and HGT may make nosocomial outbreaks worse [46-48]. Most plasmids common to ST131 have IS*Ecp1*, IS*903D* and IS*26* elements [49] that encode transpose (*tnpA*) genes flanked by short inverted repeats and are typically adjacent to ESBL genes. Moreover, ESBL genes can be chromosomally transferred and so can form part of the core resistome [50].

Fluctuating antibiotic exposure, host type, anatomical niche and host immunity mean that the resistome within isolates varies [51]. Conjugation, recombination and MGE-mediated rearrangements help shape ST131 resistome dynamics [1,10,52-55]. ST131's plasmids typically are from incompatibility (Inc) groups F, I1, N and A/C [56-58]. Some plasmids in ST131 encode genes for post-segregation killing and stable inheritance to ensure their propagation, but these genes can be lost or may recombine with other plasmids [58-60]. As a result of this mixing and their extensive array of MGEs, plasmids may rearrange extensively even within a clonal radiation [61-62]. Plasmids may also impair cell reproduction due to the energetic cost of their replication and maintenance, and conjugation and recombination could allow for coevolutionary optimisation of gene dosage and coordinated gene expression.

There is extensive evidence of AMR gene conjugation in the gut between species including *E. coli* [63-66], and also between its ST, such as: transfer of a $bla_{\text{CTX-M-1}}$-positive IncI1 plasmid among ST1640, ST2144 and ST6331 in a single patient's gut [67]; a $bla_{\text{OXA-48}}$ gene on a *K. pneumoniae* IncL/M-type plasmid from ST14 to ST666 [68] and from ST648 to ST866 [69]; a 113.6 kb IncF plasmid with a mercury detoxification operon $bla_{\text{KPC-2}}$, $bla_{\text{OXA-9}}$ and $bla_{\text{TEM-1A}}$ genes from ST69 to ST131 [70]; and transfer of a range of sulphonamide- (*sul2*) and ampicillin-resistance genes ($bla_{\text{TEM-1b}}$) on a pNK29 plasmid within *E. coli* subtypes [71-72]. Moreover, the frequency of conjugation was ten times higher and more stable in $bla_{\text{CTX-M-15}}$-producing ST131 than in *K. pneumoniae* [73].

This study focused on uropathogenic ST131 Clade C (O25b:H4, *fimH30*) because recent work has found high accessory genome, plasmid composition and AMR gene diversity in ST131 [50,61], but has yet to examine in detail the mobile resistome and how plasmids mediate AMR gene composition. We differentiated between a large shared core resistome from a smaller accessory one that varied between closely related isolates. This was caused by distinct events of plasmid turnover and rearrangement, affecting fimbrial adhesion genes as well. We hypothesise that certain plasmids are more compatible with ST131 genomes, and applied topological data analysis (TDA) to understand plasmid-chromosome co-evolution.

Results

A core preterm infant resistome in *E. coli*

We examined the resistome and plasmid composition of seven *E. coli* ST131 genome assemblies in the context of related reference genomes and non-pathogenic isolates. The reference resistome in this study was a set of 794 genes associated with antibiotic resistance in preterm infants [74] to overcome inconsistencies in existing AMR gene databases (see Methods). This reference resistome came from 401 stool samples longitudinally collected from 84 infants undergoing antibiotic treatment, and was assembled as 2,004 redundant contigs experimentally tested *in vitro* for resistance to 16 antibiotics (see Data Access, Table S1) [74].

Seven ST131 Clade C genome assemblies originating from human urinary tract infections (UTIs) together with subclade C2 reference genomes EC958 and NCTC13441 had between 262 and 291 unique AMR contigs from the reference resistome [74] (Table 1). ST131 Clade A reference SE15 (O150:H5, *fimH41*) was a commensal isolate with no plasmid-encoded AMR genes and here had 251 AMR contigs. These ten ST131 had no linear association AMR gene levels with year of sampling (r^2=0.31, p=0.1), highlighting a need to further explore AMR gene gain and loss. For context, an *E. coli* Human Microbiome Project (HMP) assembly representing intestinal *E. coli* from ST95 had comparable numbers (269 for 3_2_53FAA, Table 1). In contrast, well-studied asymptomatic urinary tract HMP sample 83972 from ST73 [75] has just 213 unique AMR contigs. This indicated a distinct subset of AMR genes were associated with ST131 causing symptomatic UTIs.

ID	SRA ID	Clade	bla_{CTX-M} allele(s)	Country & year	Unique AMR contigs
8289_1#91	ERR191724	C1	14	Ireland, 2007	268
8289_1#35	ERR191668	C1	14	Ireland, 2008	270
8289_1#3	ERR191636	C2	15	Ireland, 2005	274
8289_1#34	ERR191667	C2	15	Ireland, 2010	274
8289_1#24	ERR191657	C2	14 & 15	Ireland, 2009	283
8289_1#60	ERR191693	C2	15	Ireland, 2010	262
8289_1#27	ERR191660	C2	15	Ireland, 2010	262
SE15	AP009378	A	none	Japan, 2010	251
NCTC13441	ERS530440	C2	15	UK, 2003	280
EC958	HG941718	C2	15	UK, 2005	291
3_2_53FAA	-	ST95	none	Canada, 2007	269
83972	-	ST73	none	Sweden, 1978	213

Table 1. *E. coli* genome assembly collection and metadata. The table shows the sample ID, SRA accession ID, ST131 Clade, bla_{CTX-M} allele(s), year of isolation, and numbers of unique AMR contigs. All seven ST131 assemblies, EC958 and NCTC13441 were isolated from pathogenic UTIs. EC958 had 18 AMR contigs on plasmid pEC958A (and none on pEC958B). NCTC13441 had 27 AMR contigs on its plasmid. SE15 was a faecal commensal isolate with had no plasmid-encoded AMR genes. The seven ST131 and two HMP samples were assembled from short Illumina reads, and the three references were assembled from long PacBio reads.

Comparing the AMR contigs' gene product functions identified a core (intrinsic) resistome of 210 AMR contigs shared by all these samples (Figure 1), highlighting that these genes may be essential for non-pathogenic isolates. This core resistome differed from the accessory (acquired) resistome of 109 contigs, of which 50 that were in ST131 Clade C only corresponded to 18 unique AMR genes (Figure 1). These accessory AMR genes were initially identified with ARIBA v2.11.1 [76] and similarity searches against the Comprehensive Antibiotic Resistance Database (CARD) [77], and candidate matches were verified by mapping the reads to the reference resistome using GROOT [78].

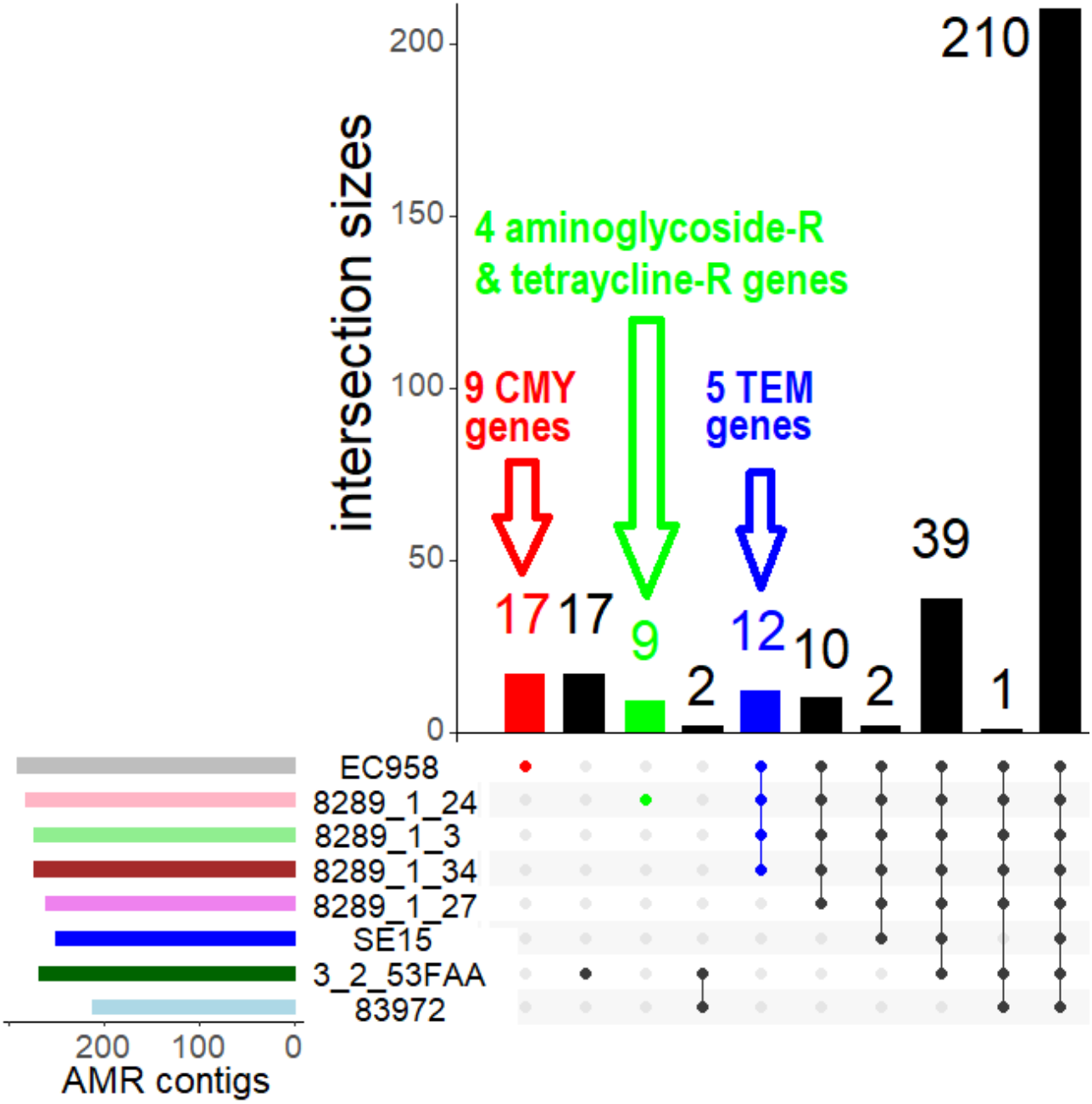

Figure 1. The overlap of preterm infant AMR contigs sharing across four ST131 subclade C2 genome assemblies (8289_1#3, 8289_1#24, 8289_1#27, 8289_1#34), two ST131 reference genomes (EC95 and SE15) and two HMP assemblies (83972 and 3_2_53FAA). Top: The intersection sizes (y-axis) and the numbers of AMR contigs per set showed that most (210) AMR contigs were shared across all isolates. The AMR contigs were redundant and indicated smaller numbers of unique AMR genes, so EC958's 17 AMR contigs (red) corresponded to nine unique bla_{CMY} genes (Table S2), and 8289_1#24's nine contigs (green) represented to four unique aminoglycoside and tetracycline resistance genes (Table S3). All Clade C members bar 8289_1#27 had 12 contigs (blue) denoting to five unique bla_{TEM} genes (Table S4). Bottom: The numbers of AMR contigs per sample with the corresponding sets shown by black circles. 8289_1#60 (not shown) had the same results as 8289_1#27.

A diverse accessory preterm infant resistome among closely related ST131 isolates

Nine *bla*$_{CMY}$ variants encoding ESBL AmpC (alleles CMY-37, 51, 66, 67, 85, 98, 101, 105) resolved from 17 initial AMR contig matches were unique to EC958 (Figure 1). *Bla*$_{CMY}$ is typically associated with cephalosporin-resistance [79]. All Subclade C2 isolates bar 8289_1#27 had five *bla*$_{TEM}$ alleles (TEM-57, 104, 116, 215, 220) encoding an Ambler class A β-lactamase. The sole *bla*$_{CTX-M-14}$- and *bla*$_{CTX-M-15}$-positive isolate (8289_1#24) had unique four genes linked to aminoglycoside and tetracycline resistance (Table S2), a combination that may neutralise the effect of aminoglycoside susceptibility associated with tetracycline efflux pump TetA expression [80-81].

Additional comparisons using the NCTC13441 genome showed that it had five *bla*$_{OXY}$ genes (OXY-1-1, 1-2, 2-3, 6-2, 6-4) encoding an Ambler class A ESBL (Text S1). Ten AMR contigs in all C2 isolates corresponded to a single gene encoding a helix-turn-helix-like transcriptional regulator (Figure 1). The two contigs present in all ST131 encoded *araC* (a transcriptional regulator) and *sugE* (associated with resistance to quaternary ammonium salts). The 39 AMR contigs in all assemblies bar 83972 (Figure 1) included 28 copies of *mdfA* encoding a multi-drug efflux pump linked to broad spectrum AMR [82] (Text S1). 3_2_53FAA and 83972 shared *ftsI* encoding a transpeptidase catalysing peptidoglycan crosslinking [83] also called penicillin-binding protein 3 (PBP3) [84-85] that can be inactivated by ESBLs [86].

For a wider context on ST131 dynamics, comparing the preterm infant resistome with *Klebsiella pneumoniae* showed that the interspecies core resistome had 58 chromosomal genes and that the *K. pneumoniae* core resistome of 308 genes included *bla*$_{SHV}$ (Figure S1, Text S2). Like NCTC13441, nine of ten genes unique to pathogenic *K. pneumoniae* isolate PMK1 but absent from urinary tract microbiome WGLW1 were *bla*$_{OXY}$ alleles. Repeating the comparison with the more divergent *Staphylococcus aureus* and *lugdunensis* showed no interspecies chromosomal core resistome (Figure S2, Text S3) but did find 14 AMR genes on *S. aureus* plasmids.

Extensive plasmid rearrangements in closely related ST131 Clade C genomes

Initial tests of Inc groups in these ST131 Clade C assemblies using PlasmidFinder [94] showed that all Clade C had IncF1A, some C2 had lost IncF1B and generally extensive diversity requiring further scrutiny (Table S3). To evaluate the origin and relationships of the AMR genes in these Clade C samples (Table 1), we aligned their assemblies and mapped their read libraries to plasmids common to ST131 (pEK499, pEK516, pEK204, pCA14, pV130). Plasmids pEK499 (isolated in England), pEK516 (England) and pEK204 (Belfast) were geographically near the other ST131 that were all isolated in Ireland. SE15's pECSF1 (also called pSE15) 122.3 Kb IncF2A/F1B plasmid served as a contrast because it has no AMR genes [87]. 8289_1#35 and 8289_1#91 (both from C1) had identical results, and *vice versa* for 8289_1#60 and 8289_1#27 (both from C2) and so are not shown below.

Alignment and read mapping showed that Clade C had far more regions similar to IncF2/F1A *bla*$_{CTX-M-15}$-positive plasmid pEK499 than SE15 and the two HMP samples, but with extensive variation (Figure 2). This plasmid has 185 genes, lacks *traX* for conjugation, is stably inherited (Table S4), and may have been gained early in the emergence of Clade C where it originated as a fusion of type F2 and F1A replicons [88]. It has ten key AMR genes, nine of which are in a 25 Kb segment demarcated by *bla*$_{TEM}$, *bla*$_{OXA-1}$ and *bla*$_{CTX-M-15}$ at 40, 58 and 63 Kb (respectively). In C2, 8289_1#3 and 8289_1#24 had all three genes, 8289_1#27, 8289_1#60 and 8289_1#34 had *bla*$_{OXA-1}$ and *bla*$_{CTX-M-15}$ but not *bla*$_{TEM}$, whereas the C1 genomes (8289_1#35 and 8289_1#91) were *bla*$_{TEM}$- and *bla*$_{CTX-M-14}$-positive. Conjugation (*tra*) genes were in all ST131 except 8289_1#34.

IncF2A plasmid pEK516 has ~75% similarity to pEK499 but is shorter (64.6 Kb) [59] and is usually non-conjugative [89]. This plasmid has 103 genes including *bla*$_{TEM}$, *bla*$_{OXA-1}$ and *bla*$_{CTX-M-15}$, and a type I partitioning locus absent in pEK499 for stable inheritance (Table S4). Read mapping to pEK516 showed that Clade C had high similarity to it, unlike SE15 and the HMP samples (Figure S3). Regions at pEK516's *bla*$_{OXA-1}$, *bla*$_{CTX-M-15}$ and *bla*$_{TEM}$ genes had variable copy numbers.

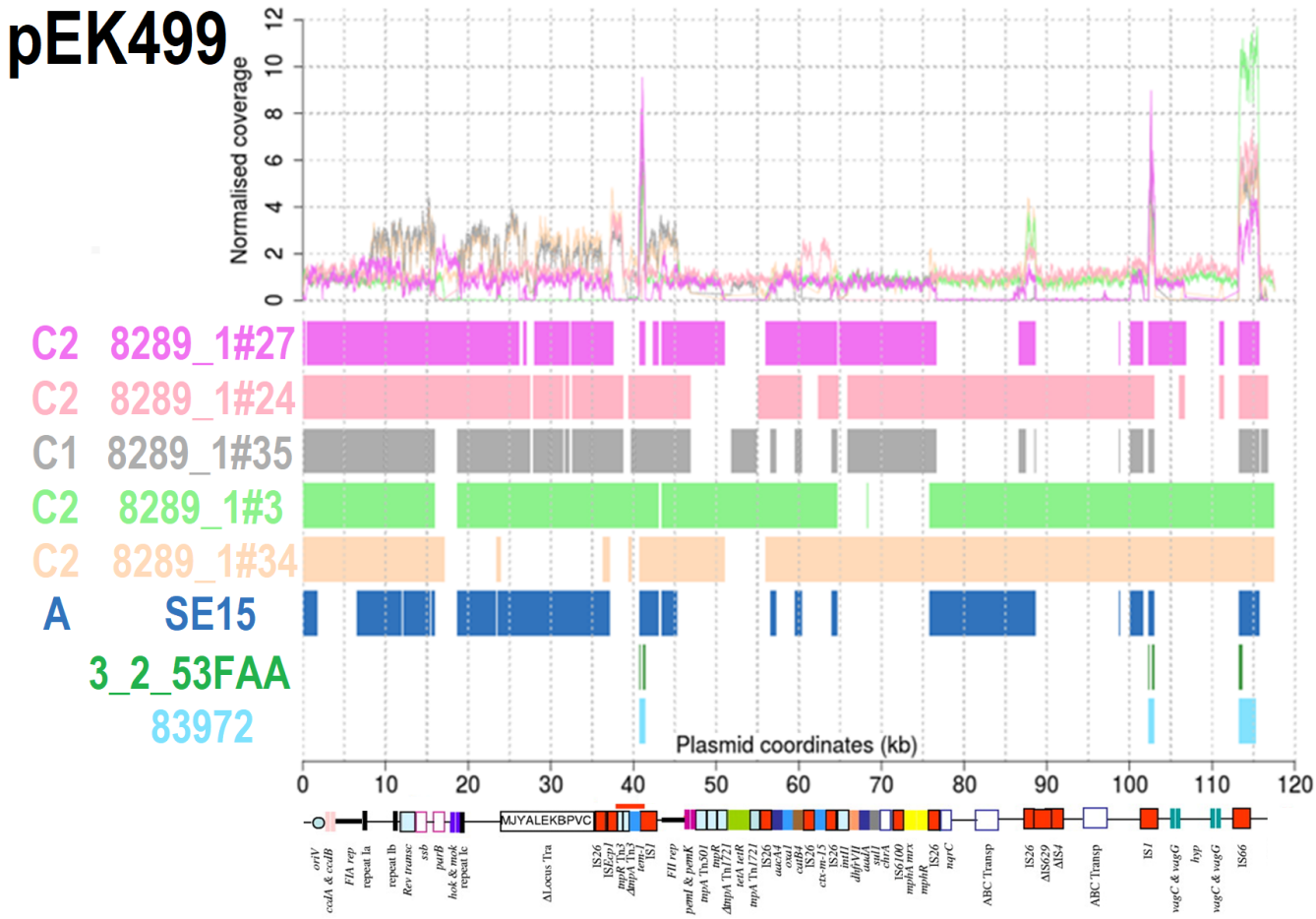

Figure 2. Comparison of pEK499 (117,536 bp) with five ST131 Subclade C1 and C2 isolates, ST131 Clade A reference SE15 (navy) and two HMP assemblies (3_2_53FAA in dark green and 83972 in cyan). Top: Normalised read coverage showed high copy numbers of IS*1* (at 41 and 103 Kb) and IS*66* (at 113 Kb) elements. Bottom: BLAST alignments showed limited similarity for the HMP assemblies, and low matching for SE15 relative to high levels for Clade C assemblies: 8289_1#27 (C2, mauve), 8289_1#24 (C2, pink), 8289_1#35 (C1, grey), 8289_1#3 (C2, light green) and 8289_1#34 (C2, beige). Genes encoding bla_{TEM}, bla_{OXA-1} and $bla_{CTX-M-15}$ were at 40, 58 and 63 Kb (respectively). The annotation was modified from [59]. Matches spanning >300 bp are shown.

Contrasting sharply with pEK499 and pEK516, reads mapped to conjugative IncI1 plasmid pEK204 found 72.8 Kb regions of similarity in 8289_1#27 (C2) where the other isolates had <10 Kb of total matching regions (Figure 3). Plasmid pEK204 is similar to IncI1 plasmid R64, has 112 genes, a complete *tra* region (Table S4), and a type I partitioning locus [59]. 8289_1#27 had a complete I1 replicon, *oriT*, *tra*, shufflons subject to site-specific recombinase activity, and a *pil* cluster encoding a type IV fimbriae associated with enhanced cell adherence and biofilm formation in entero-aggregative and Shiga toxin-producing *E. coli* [90]. A 9.3 Kb region on pEK204 contained bla_{TEM-1b}, along with an inactive transposase Tn*3*-*tnpA*, IS*Ecp1*, bla_{CTX-M} and a 5' orf477-*tnpA*-*tnpR* region. The 14 bp IRL 5' of IS*Ecp1* and IRR at the distal end of the inverted *orf477* element can mobilise bla_{CTX-M}, and an additional IRR at *impB* 3' of the bla_{TEM-1b} gene (7.4 Kb further away) can mobilise the 9.3 Kb unit [91], which has been found on IncFIA, IncFIA-FIB, IncN and IncY plasmids and arose on a pCOL1b-P9-like plasmid [59]. *ImpB* and *impA* encode an error-prone DNA repair protein (like UmuDC) [92].

Further investigation of the ST131 plasmid-matching regions showed variable plasmid similarity within closely related isolates. Read mapping to conjugative plasmid pCA14 showed that all Clade C isolates bar 8289_1#3 had *Mrx* and *mph(A)* genes associated with macrolide resistance (Figure S4). This was also found by comparing with contigs from non-conjugative plasmids pV130a and pV130b from sewage

treatment plant water in India [93], where 8289_1#27 and 8289_1#60 (both C2) had similarity spanning all of the pV130a contigs (Figure S5).

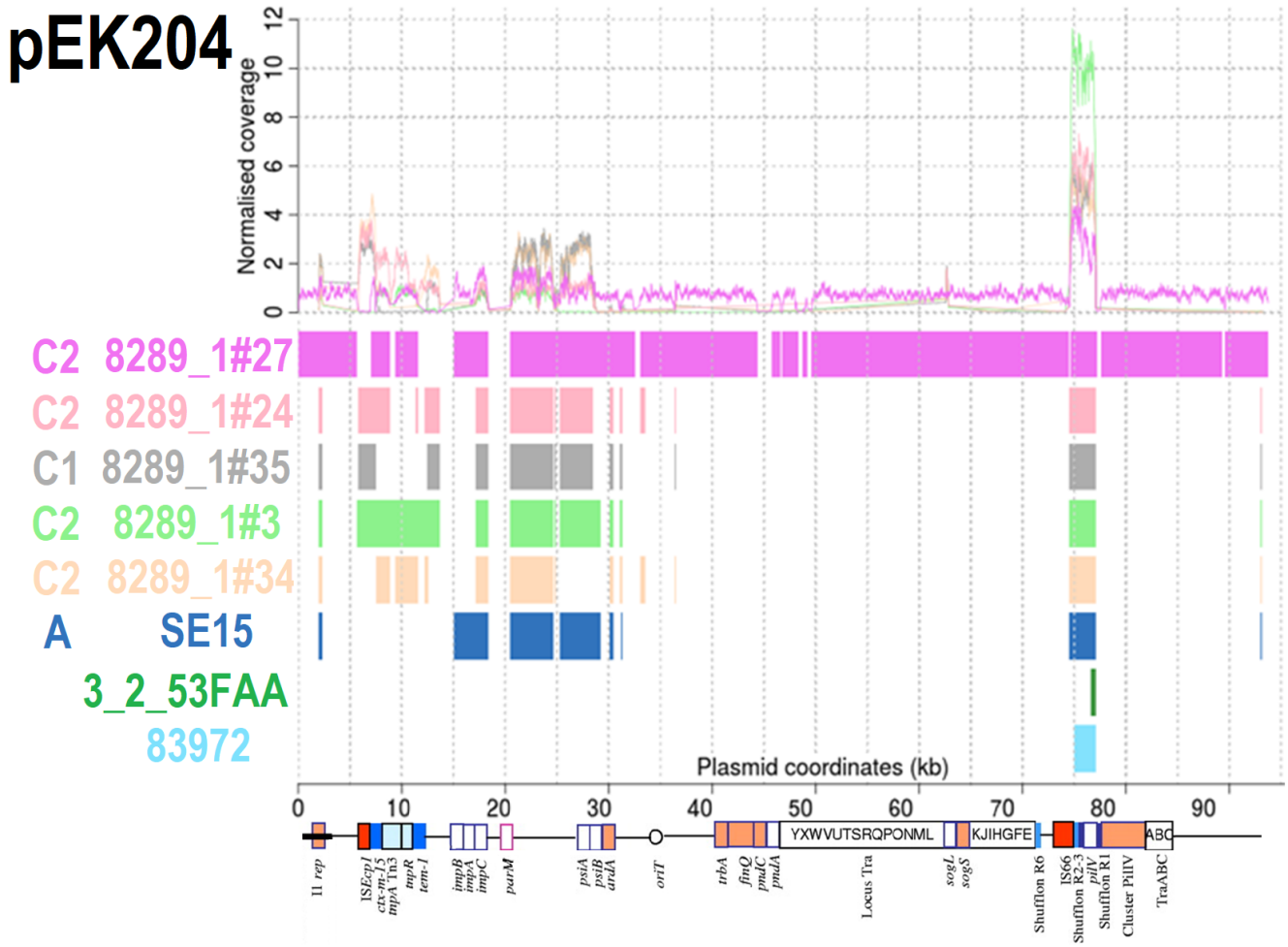

Figure 3. Comparison of pEK204 (93,732 bp) with five ST131 Subclade C1 and C2 isolates, Clade A reference SE15 (navy) and two HMP assemblies (3_2_53FAA in dark green and 83972 in cyan). Top: Normalised read coverage showed high copy numbers at 75-77 Kb encoding an IS*66* and unannotated genes. Bottom: BLAST alignments showed limited similarity for the HMP assemblies, SE15 and Clade C assemblies: 8289_1#24 (C2, pink), 8289_1#35 (C1, grey), 8289_1#3 (C2, light green) and 8289_1#34 (C2, beige). In contrast, 8289_1#27 (C2, mauve) had many regions similar to pEK204. The $bla_{\text{CTX-M}}$ gene was at 8 Kb, bla_{TEM} was at 13 Kb, followed by mixed conjugation and segregation genes at 36-70 Kb. The $bla_{\text{CTX-M-3}}$ gene typically associated with pEK204 differs from $bla_{\text{CTX-M-15}}$ by a R240G substitution, and so the $bla_{\text{CTX-M}}$ genes detected for samples here were $bla_{\text{CTX-M-15}}$ (not $bla_{\text{CTX-M-3}}$). Matches spanning >300 bp are shown. The annotation was modified from [59].

The IncF2A plasmid pEK516 has ~75% similarity to pEK499 but is shorter (64.6 Kb) [59] and is usually non-conjugative [89]. This plasmid has 103 genes including bla_{TEM}, $bla_{\text{OXA-1}}$ and $bla_{\text{CTX-M-15}}$, and a type I partitioning locus (*parM* and *stbB*, absent in pEK499) for stable inheritance (Table S9). Read mapping to pEK516 showed that Clade C had high similarity to this plasmid unlike SE15 and the HMP samples (Figure S3). Regions at pEK516's $bla_{\text{OXA-1}}$, $bla_{\text{CTX-M-15}}$ and bla_{TEM} genes at 12, 20 and 24 Kb (respectively) had variable copy numbers.

Contrasting sharply with pEK499 and pEK516, reads mapped to conjugative IncI1 plasmid pEK204 found 72.8 Kb regions of similarity in 8289_1#27 (C2) where the other isolates had <10 Kb of total matching regions (Figure 3). Plasmid pEK204 is similar to IncI1 plasmid R64, has 112 genes, a complete *tra* region (Table S9), and a type I partitioning locus [59]. 8289_1#27 had a complete I1

replicon, *oriT*, *tra*, shufflons subject to site-specific recombinase activity, and a *pil* cluster encoding a type IV fimbriae associated with enhanced cell adherence and biofilm formation in entero-aggregative and Shiga toxin-producing *E. coli* [90]. Regions absent from 8289_1#27 encoded polymerase and UV protection genes (at 13-15 Kb) and the type I partitioning locus (at 18-20 Kb). A 9.3 Kb region on pEK204 contained $bla_{\text{TEM-1b}}$, along with an inactive transposase Tn*3*-*tnpA*, IS*Ecp1*, $bla_{\text{CTX-M}}$ and a 5' orf477-*tnpA*-*tnpR* region. The 14 bp IRL 5' of IS*Ecp1* and IRR at the distal end of the inverted *orf477* element can mobilise $bla_{\text{CTX-M}}$, and an additional IRR at *impB* 3' of the $bla_{\text{TEM-1b}}$ gene (7.4 Kb further away) can mobilise the 9.3 Kb unit [91], which has been found on IncFIA, IncFIA-FIB, IncN and IncY plasmids and arose on pCOL1b-P9-like plasmid [59]. *ImpB* and *impA* encode an error-prone DNA repair protein (like UmuDC) [92].

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High rates of pEK499 and pEK204 protein-protein interactions with chromosomal *E. coli* proteins

The extensive diversity of Clade C AMR genes and plasmids raised the question of how their plasmids' gene products interact with chromosomally encoded ones. Protein-protein interaction (PPI) networks can examine plasmid-chromosome coevolution based on gene products' topological proximity [95-96], which should be higher for plasmid and chromosomal proteins that have co-existed for longer and so interact more often [97]. We used topological data analysis (TDA) to measure the number of non-trivial (indirect) loops where a loop is a chain of at least four PPIs ending at the same protein where it has started. We focus on a type of loops called "non-trivial" (see Methods). The number of non-trivial loops was scaled by the number of PPIs per dataset (Table 2).

Plasmid	Number of genes	Number of unique genes	Length (Kb)	Number of unique PPIs		Non-trivial loops per PPI
				Within plasmid	With chromosome	
pEK204	112	87	93.7	21	548	0.096
pEK499	185	87	117.5	8	758	0.117
pEK516	103	55	64.6	8	758	0.117
pJIE186-2	138	78	137.7	2	32	0.015
pCA14	181	93	155.4	5	0	0
pEC958A	142	75	135.6	5	0	0
pECSF1	150	70	122.3	0	0	0

Table 2. The numbers of protein-coding genes per plasmid, unique genes per plasmid, length, and numbers of non-redundant PPIs within the plasmid and with the chromosome, and the rate of indirect interactions (non-trivial loops) per PPI. Plasmid pEK516 had the same PPI results as pEK499.

PPI data for 4,146 *E. coli* protein-coding genes with 105,457 PPIs (all combined scores >400) from the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database v10 [98] was processed to get the numbers of non-redundant PPIs and loops per PPI for a plasmid's proteins among themselves, and then between the plasmid's proteins and all 4,146 chromosomal *E. coli* proteins. This included assessing the numbers of PPIs up to a combined score threshold of 900 with a step size of 25. We tested our approach using a published *E. coli* clustering of 60 genes [99] that had a rate of 0.244 non-trivial loops per PPI, which was lower than the rate of 0.518 obtained for all 4,146 chromosomal proteins (Table S5, Figure S6). There were lower rates of non-trivial loops per PPI for pEK499 (0.117) and pEK204 (0.096) compared to the chromosomal rate (0.518), but the plasmids' rates were almost constant across

the range of combined score thresholds used (Figure S7). This held even though the number of PPIs per protein was negatively correlated with the combined score (Figure S8), suggesting that the rate of non-trivial loops per PPI was a robust measure of connectivity. Whereas pEK499 and pEK204 interacted with chromosomally encoded proteins, pCA14, pEC958A and pECSF1 did not (Table S6). Plasmid pJIE186-2 did have a small number of PPIs (Table 2) that may be VFs [100].

ST131 genomes had an ancestral pEK499-like plasmid but some gained a pEK204-like one

The seven ST131 genome assemblies above were from a set of 4,071 assemblies [61]. Of these 4,071, 837 (20.6%) had >10 Kb of regions similar to pEK204 (Figure S9) based on BLAST alignments, but 3,108 (76.3%) with >10 Kb matching pEK499 (Figure S10). 193 assemblies had >40 Kb of pEK204-like segments, all from Clade C: 17 from C0 (out of 52, 33%), 82 from C1 (out of 1,119, 7%) and 94 from C2 (out of 2,051, 5%) (Figure 4). All 17 from C0 had an I1 replicon, *bla*$_{\text{CTX-M}}$ gene, *bla*$_{\text{TEM}}$ gene (bar one isolate) and at least a partial *tra* region, and 13 had the *pil* operon (76%). The 82 from C1 and 94 from C2 had lower rates of I1 replicon presence (C1 77%, C2 81%), partial *tra* regions (C1 94%, C2 86%) and *pil* clusters (C1 n=60 or 73%, C2 n=71 or 76%) but differed in the rates of gene presence for *bla*$_{\text{CTX-M}}$ (C1 28% vs C2 83%, odds ratio = 12.5, 95% CI 6.1-25.8, p=e-11) and *bla*$_{\text{TEM}}$ (C1 59% vs C2 30%, odds ratio = 3.3, 95% CI 1.8-6.2, p=2.2e-5). This implied *bla*$_{\text{CTX-M}}$ (via *orf477*) was common in C2 and *bla*$_{\text{TEM}}$ (via *impB*) in C1 due to different ancestral transposition of the 9.3 Kb region.

To resolve the origin of pEK204-related *pil* HGT, we searched individually for the 14 *pil* operon genes *pilIJKMNOPQRSTUV* in the 4,071 ST131 assemblies [61]. These genes were conserved as a unit in 376 isolates (9%), including 61 from A (out of 414, 15%), 97 from B (out of 433, 22%), 17 from C0 (out of 52, 33%), 95 from C1 (out of 1,121, 8.5%) and 106 from C2 (out of 2,051, 5.2%) (Table 3) (see Data Access). Tallying this with the pEK204 matching patterns indicated potential for non-pEK204 *pil* gain in Clades A and B, the putative Clade C ancestor may have had a pEK204-like plasmid with no *pil* region, which was sometimes subsequently gained by recombination.

Operon			Subclade										
pil	***pap***	***ucl***	**A**	**%**	**B**	**%**	**C0**	**%**	**C1**	**%**	**C2**	**%**	**Totals**
-	*papIBA*	-	279	67%	107	25%	3	6%	772	69%	1,192	58%	2,353
-	all	all	21	5%	13	3%	23	44%	82	7%	481	24%	620
all	*papIBA*	-	57	14%	2	<1%			75	7%	66	3%	200
-	all	-	1	<1%	94	22%			8	<1%	14	<1%	117
all	-	-	3	<1%	71	16%			5	<1%	3	<1%	82
all	all	all			16	4%	1	2%	10	<1%	33	2%	60
all	all	-	1	<1%	6	1%	16	31%			4	<1%	27
all	-	all							1	<1%			1
-	*papIBA*	all									1	<1%	1
all	*papIBA*	all											0
-	-	all											0
Partial matches			52	13%	124	29%	9	17%	168	15%	257	13%	610
Totals			414		433		52		1,121		2,051		4,071

Table 3. *Fim*, *pil*, *pap* and *ucl* operon genes detected in 4,071 ST131 genome assembles. The numbers and percentages of isolates per subclade with complete or absent (-) *pil* (*pilK-pilV*) or *pap* or *ucl* (*uclABCD*) operon genes where a subset also had three *pap* genes (*papIBA*). This focused on isolates allocated to the eleven main allelic options, isolates with ssome level of partial operon presence or absence were too sparse to be informative. *UclBCD* but not *uclA* was in an additional 2% (n=77), including 7 from A (2%), 28 from B (6%), none from C0, 24 from C1 (2%) and 18 from C2 (1%).

Given the *fim* operon's key role in ST131 evolution, we verified that *fim* was present intact in >99% of the 4,071. As expected, the IS*Ec55* insertion at *fimB* [101] that delays *fim* expression in Clade C

isolates like EC958 [20] was nearly fixed in C1 (1,117, 99.6%) and C2 (2,041, 99.5%), but was rarer in A (236, 57%), B (93, 21%) and C0 (18, 35%) (including 32 samples with additional *fimB* rearrangements). Isolates with intact *fimB* genes were common in A (178, 43%), B (321, 74%) and C0 (34, 65%).

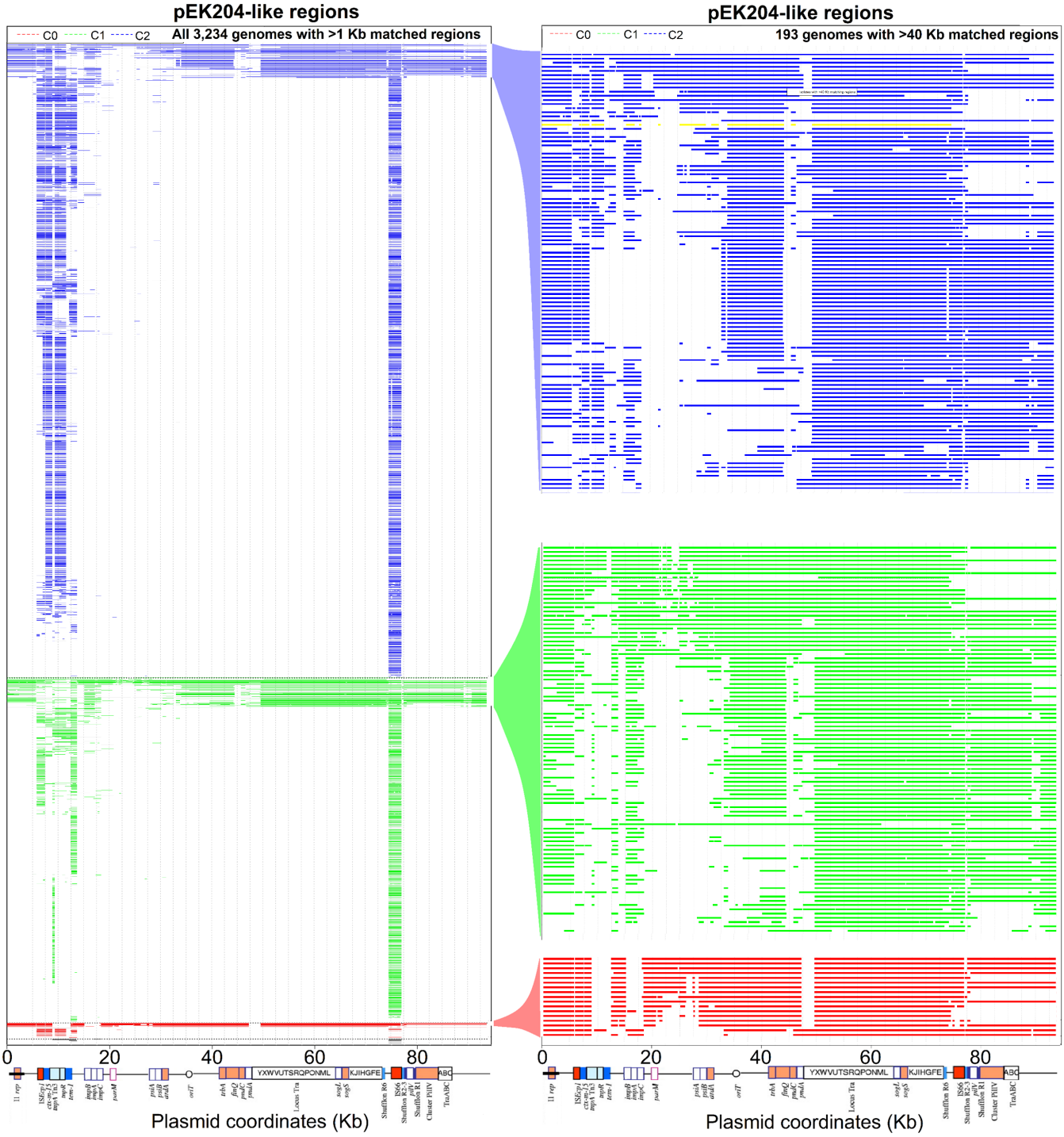

Figure 4. The distributions of pEK204-like regions in ST131 Clade C genome assemblies with >1 Kb (left, n=3,234) and >40 Kb (right, n=193) of matching segments. The regions of similarity were examined in (left) Subclades B0 (n=14 in black), C0 (n=51 in red), C1 (n=1,119 in green) and C2 (n=2,051 in blue), and (right) C0 (n=17 in red), C1 (n=82 in green) and C2 (n=94 in blue). 8289_1#27 (C2) is highlighted in yellow. Similarity across pEK204 (93,732 bp) was based on BLAST alignment. The *bla*$_{CTX-M}$ gene was at 8 Kb, *bla*$_{TEM}$ was at 13 Kb, followed by a mix of conjugation and segregation genes at 36-70 Kb. This suggested independent integrations and rearrangements of pEK204-like plasmids across the subclades. The annotation was modified from [59].

We extended this to two other important fimbrial operons: *pap* (pyelonephritis-associated pili) and *ucl* (uroepithelial cell adhesin-like). All four *ucl* genes were in 17.5% (n=712) of isolates, including 21 from A (5%), 30 from B (7%), 25 from C0 (48%), 94 from C1 (8%) and notably 542 from C2 (26%) (Table 3). All the *ucl*-positive C2 genomes had a complete *pap* operon (with a single exception). The *pap* operon was in a minority (26%, n=1,049) of isolates, including 28 from A (7%), 163 from B (38%), 49 from C0 (94%), 127 from C1 (11%) and 682 from C2 (33%) (Table 3). Most isolates had *papIBA* and not the genes 3' of them (*papHCDJKEF*) (65%, n=2,661): 359 from A (87%), 118 from B (27%), 3 from C0 (6%), 888 from C1 (79%) and 1,293 from C2 (63%). *PapI* (234 bp), which is associated with more P fimbriae expression in *E. coli* CFT073 [102], was amplified to two, three or four copies in 22% of these ST131: 26 in Clade A (6%), 51 in B (12%), 45 in C0 (87%), 119 in C1 (11%) and 658 in C2 (32%). Only 45 isolates across clades had an amplified *papB*, and 265 assemblies had a duplicated or triplicated *papA*. These two genes (*papBA*) share a promoter *pBA*, and *papI* has a separate one (*pI*).

This highlighted that most isolates from A, C1 and C2 and a minority of B had no *pil* nor *ucl* operons and lost the *papH-F* segment (Table 3). Clade B differed from A because some from this heterogeneous clade have either *pil* or *pap*, but not both. 83 isolates with no detected *pap* genes all had a *pil* operon, and most (71) were from Clade B. Clades A and B have *pil* independently of pEK204, so Clade C isolates may have gained *pil* on multiple occasions. During the lengthy period prior to the divergence of the rarer C0 subclade from the fluoroquinolone-resistant C1/C2 ancestor [50], the Clade C ancestor likely had a complete *pap* operon. This C1/C2 ancestor gained the *fimB* IS*Ec55* insertion and a minority of C1 gained either *pil* (80, 7%) or *ucl* (82, 7%) or both (11, 1%), whereas a sizeable minority of C2 gained *ucl* (482, 24%), rather than *pil* alone (73, 4%) or both together (33, 2%).

Unlike *ucl* gene products that are functionally independent [103], regulatory protein PapB reduces *fim* operon expression by inhibiting FimB and activating FimE, both tyrosine site-specific recombinases that invert *fimS*, including in *E. coli* isolates CFT073 and 536 [104-106]. PapI and PapB regulate *pap* expression [105-107] depending on their concentrations and protein binding at the 416 bp regulatory region between *papI* and *papB*. Thus, retention of *papIBA* in >65% of ST131 could be linked to *fim* transcriptional regulation via *pap*B. Using the STRING PPI network data above for *E. coli* samples 536 and CFT073 that have the *fim*, *pap* and *ucl* operons [108-109] (no data for *pil* gene products was found), there were ten *fim-pap* inter-operon PPIs in both 536 and CFT073, including pilus rod subunit PapA with FimD and FimF (as well as FimC), potentially matching the PPIs of FimA [110]. If only *papIBA* was present, the functional effects (if any) of *papI* and *papA* were uncertain: it is not clear that at the inner cell membrane FimC (replacing PapD) could mediate periplasmic transport of PapA (like FimA) subunits via the chaperone FimI (instead of PapH) to usher FimD (rather than PapC) for pilus rod assembly at the outer cell membrane, extended later by FimF (in lieu of PapK) for the base of the tip. Nonetheless, presence of these genes can inform pilicide and coilicide design, such as antibodies targeting VF PapA [110].

Discussion

AMR genes in commensal or environmental bacteria are a major reservoir for pathogens [111]. This gene flow is driven by HGT and recombination in bacteria [112], including in *E. coli* [113-116], and microbiome species [117-118]. Previous work identified contigs from preterm infants that were functionally screened for resistance to 16 antibiotics [74]. We showed that this resistome was shared extensively between ExPEC ST131 and microbiome *E. coli* assembled contigs, indicating likely transfer of these genes across commensal and pathogenic bacteria inhabiting the human gut and urinary tract, as expected [119].

E. coli 83972 does not express functional fimbrial adhesins and is used for therapeutic urinary bladder colonisation in patients in which it protects against super-infection by more virulent strains [120-122]. Although 83972 has lost virulence during its adaptation to commensalism [123], it had key AMR genes like PBP3, which it shared with an *E. coli* microbiome from the gut (3_2_53FAA). This highlighted the

long-term retention of certain AMR genes in asymptomatic specimens, and was a contrast for assessing the VF and AMR gene evolution in ST131 [124-125].

Within ST131, Clade C core genomes are highly conserved but its accessory genomes have extensive differences in AMR gene content [50,52,61]. Here, this was evidenced by NCTC13441's repertoire of bla_{TEM} and bla_{OXY} genes, contrasting with EC958's variety of bla_{CMY} ones, with equivalent accessory genome diversity in the other Clade C genomes examined here. This reinforced the view that ST131's accessory resistome is shaped by the environment mediated by plasmids, rather than population structure or geographic factors [126]. Accessory genome diagnostics tracking plasmids and MGEs in addition to AMR genes [42,127] could assist clinical treatment [128].

By combining the seven Clade C genomes with 4,064 assemblies, we found that most (78%) ST131 had >10 Kb of regions similar to plasmid pEK499 and some (9%) also had regions with high similarity to IncI1 plasmid pEK204, suggesting either discrete gains of pEK204-like plasmids in each C subclade or its presence in the Clade C ancestor. IncI1 plasmids are common in ExPEC and are associated with different ESBL genes [129-131], such as $bla_{CTX\text{-}M\text{-}1}$ that can be transferred between *E. coli* STs in the human gut [67]. Given that the mosaic array of backbone plasmid genes may determine fitness effects more than individual ESBL genes [132], and evidence of long-term IncF plasmid persistence in ST131 [62], plasmids pEK204, (pEK516,) and pEK499 proteins' higher interaction rates with chromosomally-encoded proteins relative to other relevant plasmids could be a co-evolutionary process. Measuring the indirect connectivity as PPI network loops per interaction showed evidence of this long-term retention, and may help identify plasmids compatible with *E. coli* chromosomes.

ST131's fitness advantage is tightly correlated with type 1 fimbriae variant *fimH30* and delayed *fim* operon expression due to an insertion at *fimB* [16,133-134]. Here, a minority of ST131 had type IV pilus biosynthesis (*pil*) genes and most from Clade C with *pil* had >40 Kb of regions similar to a pEK204-like plasmid, whereas Clade A and B isolates with *pil* did not have pEK204-like tracts. *Pil* may allow different epithelial cell adhesion via a thinner pilus and biofilm formation [90]. In parallel, the F17-like *ucl* operon was more common in subclade C2 at 24%: UclD (like FimH) is a two-domain tip adhesin that binds intestinal epithelial cells via O-glycans (FimH uses N-glycans) [103]. The *ucl* operon is associated with uroepithelial cell adhesion, biofilm formation and could be mobilised by its flanking MGEs [135].

The *pap* operon encodes a P fimbriae with high specificity for kidney epithelial cell and erythrocyte receptor glycolipids (α-D-galactopyranosol(1-4)-α-D-galactopyranoside) [136]. Delayed *fim* expression in Clade C isolates [20,101] may be reduced further by PapB: *papIBA* alone was retained in >65% of Clade C isolates here. Delayed *fim* expression [137] and having multiple P fimbrial gene clusters [107] associates more with pyleonephritis than cystitis. Given that individual *E. coli* express single fimbriae at the cell surface [104,138] and isogenic cell populations express distinct fimbrial types [139], type 1 fimbriae may allow initial bladder colonisation followed by reduced expression in favour of P fimbriae to colonise the kidney [138]. This study's results on plasmid-mediated dynamism across the ST131 resistome and extensive diversity in fimbrial gene composition could inform on potential infection [140] and biofilm formation patterns [20].

Methods

E. coli genome isolate collection

Of the 12 *E. coli* genome assemblies examined (Table 1), three were well-studied ST131 reference genomes: SE15, NCTC13441 and EC958. Seven were ST131 isolated in Ireland during 2005-2010 (Table 1) of which two from subclade C1 were $bla_{CTX\text{-}M\text{-}14}$-positive, as was 8289_1#24 from Subclade C2, and all five from C2 were $bla_{CTX\text{-}M\text{-}15}$-positive. All seven were isolated from urine except 8289_1#34, which was a rectal swab. All seven belonged to rST1503 except 8289_1#24 from rST1850 [50]. The two HMP samples were [141] (Table 1): 3_2_53FAA (also called EC3_2_53FAA) was from a colon

biopsy from a 52-year-old male Canadian with Crohn's disease. 83972 (also called EC83972) was from the urine of a Swedish girl with a three-year history of asymptomatic bacteriuria and stable renal function [142]. 83972's shares common ancestry with the virulent pyelonephritis-causing ExPEC CFT073 [143].

ST131 subclade C2 reference genomes NCTC13441 and EC958 were from UTIs in the UK, and were assembled from long PacBio reads that had $bla_{\text{CTX-M-15}}$-positive plasmids [9,59,133]. The NCTC13441 genome has 4,983 predicted protein-coding genes and EC958 has 4,982. EC958 has numerous virulence-associated genes that encode adhesins, autotransporter proteins and siderophore receptors, and can cause impairment of uterine contractility in mice [144]. Plasmid pEC958A has 85% similarity with pEK499 but lacks the latter's second *tra* region due to an IS26-mediated $bla_{\text{TEM-1}}$ insertion [9,60,133].

ST131 Clade A reference genome SE15 was examined as a genetic outgroup to Clade A and as a commensal control because it lacks many virulence-associated genes (like α-hemolysin and cytotoxic necrotizing factor). It has a 4,717,338 bp chromosome with 4,338 protein-coding genes and a plasmid pSE15 (122 Kb) with 150 protein-coding genes [87].

Illumina library quality control and read mapping

The paired-end Illumina HiSeq libraries for each ST131 isolate were screened for low quality (Phred score <30) and short (<50 bp) reads using Trimmomatic v0.36 [145] and were corrected using BayesHammer from SPAdes v3.9 [145] (Table 4). These corrected read libraries were mapped to reference sequences with SMALT v7.6 (www.sanger.ac.uk/resources/software/smalt/), and the resulting SAM files were converted to BAM format, sorted and PCR duplicates removed using SAMtools v1.19 [146].

Library	Clade	Number of reads	Read length			ARG-ANNOT	CARD	ResFinder
			Median	Mean	SD			
8289_1#34_1	C2	2,145,896	101	99.3	6.5	9	17	15
8289_1#34_2			100	97.7	8.0			
8289_1#3_1	C2	1,824,030	101	99.4	6.5	17	39	22
8289_1#3_2			100	97.6	8.2			
8289_1#35_1	C1	2,297,624	101	99.4	6.5	44	52	45
8289_1#35_2			100	97.7	8.0			
8289_1#91_1	C1	2,227,251	101	99.3	6.6	44	57	42
8289_1#91_2			100	97.7	8.1			
8289_1#24_1	C2	2,014,250	101	99.3	6.5	56	71	55
8289_1#24_2			100	97.7	8.1			
8289_1#27_1	C2	5,756,848	101	99.4	6.5	6	9	6
8289_1#27_2			100	97.7	8.1			
8289_1#60_1	C2	1,800,070	101	99.3	6.5	14	26	21
8289_1#60_2			100	97.6	8.1			
NCTC13441		2,857,729	43	42.5	1.4			
SE15		418,045	218	192.2	67.3			
EC958		1,514	1,486	1,401.6	549.2			

Table 4. Read library summary statistics for each main ST131 sample and the three main reference genomes. The read distributions differed for NCTC13441, SE15, and EC958 because they were generated using long read approaches. For GROOT usage, paired-end read library files were mapped individually. SD stands for standard deviation. The numbers of AMR genes from ST131 genome assemblies identified in AMR databases ARG-ANNOT, CARD and ResFinder are shown.

Variable resistome overlaps complicate a clear reference gene set

The reference resistome outlined above was a set of 794 AMR genes assembled as 2,004 redundant AMR contigs tested *in vitro* for resistance to 16 antibiotics (Table S1) [74]. We compared this set with existing AMR databases to test the robustness of resistome comparisons by aligning the 794 contigs with the CARD [77] and mapping the reads for each of the seven ST131 libraries to the CARD, ARG-ANNOT [147] and ResFinder [148] databases using GROOT [78] where the reads for each isolate were indexed using the median read length (Table 4). This showed considerable variation in the numbers and types of AMR genes determined by each database and extensive AMR gene diversity within Clade C (Table 4). As a negative control, the read library for faecal isolate S250 from ST131 Clade B [149] had no AMR genes in ARG-ANNOT and ResFinder, and five in CARD.

Homology-based resistome screening and comparison

Contig annotation and protein domain recognition were implemented using the Pfam v27.0 and ProSite databases using InterProScan v5.22-61 [150]. The protein homolog dataset in CARD (2,239 genes) [77] was aligned with the isolates' genomes to annotate the resistomes using BLAST v2.2.31, where matches with a bit score >500 and >99% homology were considered valid. Alignments were visualised using R's VennDiagram v1.6.1, Seqinr v3.4-5, UpSetR v1.4.0 and WriteXLS v5.0.0 packages. Detected genes and elements were verified and visualised with Artemis and the Artemis Comparison Tool (ACT) [151].

Assessment of plasmids prevalent in ST131

Sequence and annotation files for pEK499 (NC_013122.1, EU935739), pEK516 (NC_013121.1, EU935738), pEK204 (NC_013120.1, EU935740) and all pV130 contigs (LC056314.1 to LC056328.1) [93] were downloaded. The pV130 contigs were aligned to one another to determine their uniqueness with Clustal Omega v1.2.1 [152]. Replicon typing was completed using PlasmidFinder [56]. Each plasmid was compared to the CARD. Each E. coli read library was mapped to each plasmid to verify local genetic features and quantify copy number levels, visualised with Artemis [151] and R v3.4.2's reshape2 v1.4.3, ggridges v0.5.1, ggplot2 v2.3.2.1, readr v1.3.1, dplyr v0.8.3 and ape v5.3 packages. Plasmid and gene sequence similarity was calculated using the Sequence Identity and Similarity (SIAS) tool (http://imed.med.ucm.es/Tools/sias.html). Homology searches for contigs with high similarity to pEK204 and pEK499 in 4,071 assembled ST131 genomes [60] examined matches >300 bp length with >98% sequence identity.

Protein-protein interaction network construction and topological data analysis

TDA has been used to investigate complex and high-dimensional biological datasets, like breast cancer genomes [153], and here we applied it to PPI networks with a focus on direct and indirect connectivity across different network topologies by quantifying rates of absent PPIs (non-trivial loops). The numbers of non-trivial loops per PPI was used as a metric for indirect connectivity because it was consistent across parameters and was independent of network size. We assessed pCA14, pEK204, pEK499, pEK516 and pEC958A given the immediate relevance to ST131 genome composition, along with two plasmids with no known AMR genes as negative controls: pECSF1 (aka pSE15), and non-conjugative plasmid pJIE186-2 that has numerous VF genes that are chromosomal in some *E. coli* [100]. We examined unique genes with PPI network information only: here, results for pEK516 were identical to those for pEK499.

We extracted *E. coli* K12 MG1655 PPI data from the STRING database v10 [98] with R v3.5.2 packages BiocManager v1.30.4, dplyr v0.8.0.1, genbankr v1.10.0, rentrez v1.2.1, STRINGdb v1.22.0, tidyverse v1.2.1 and VennDiagram v1.6.20. STRING's combined scores were used because they integrate multiple types of evidence while controlling for random PPIs [98]. Using a score threshold of 400+, the K12 dataset had 4,146 distinct protein-coding genes, of which 4,121 had interaction information, resulting in 105,457 PPIs (14,555 PPIs were present for a score threshold of 900). For each plasmid or set of genes, we obtained the unique genes and for these determined the number of pairwise PPIs within that set and

with the chromosome. We tested our TDA-based approach using the results of relevant previous work [99]. For *E. coli* 536, FooB was used as an equivalent for PapB, and likewise for protein F7-2 that matched VF PapA in CFT073, and FooG for PapG.

We constructed the Vietoris-Rips complex [154] where the proteins were the vertices with PPIs as edges, so that proteins A and B with an PPI would be joined by a single edge (two proteins in one dimension, 1-D). Similarly, proteins A, B and C that are joined by three PPIs would get their PPI triangle filled in with a solid 2-D triangle. For four proteins A, B, C and D joined by all six possible pairwise PPIs, their four filled 2-D triangles A/B/C, B/C/D, A/C/D and A/B/D constitute a tetrahedral surface which gets filled in with a solid 3-D tetrahedron. This can be extended to $m+1$ pairwise connected proteins, which get a solid m-D polytope filled into their skeleton of edges, triangles, tetrahedra, and so on. A loop connects m proteins, where m is 4, 5, 6 or more, by precisely m PPIs, such that each protein is involved in precisely two of these PPIs. For instance, four proteins A, B, C, D can be joined to a loop by the four PPIs A-B, B-C, C-D, D-A, where A-B is a PPI between the proteins A and B (and so on). Any loop which can be filled with triangles, along existing PPIs, is called trivial. So if there is an interaction between A and C, the loop is filled by the two triangles AB-BC-CA and CD-DA-CA and hence trivial. If there are no interactions between A and C, and none between B and D, then the loop is non-trivial.

Due to the large K12 dataset size (4,146 proteins with 105,457 interactions), instead of using general purpose implementations of the Vietoris-Rips complex (such as SAGE), specialised software [155] to optimise the Betti number computations was used. This software used the sparsity of the boundary matrices to process the rank computations efficiently in LinBox [156], with a hard-coded dimensional truncation of the Vietoris-Rips complex to avoid the large number of high-dimension simplices that could be obtained for the full dataset. For each analysis across STRING combined scores 400 to 900 with a step of 25, we computed the first Betti number of the Vietoris-Rips complex that counted the numbers of non-trivial loops (with missing PPIs inside), which was adjusted for the numbers of PPIs above the score threshold (ie, loops per PPI). The number of PPIs for the complete K12 chromosomal dataset was negatively correlated with the combined score threshold (r^2=0.964), as was the loops per PPI (r^2=0.905), consequently the chromosomal loops per PPI rate was used as a baseline metric across different score thresholds.

Operon gene homology search approach

Homology searches for each *fim* operon gene were implemented using the NCTC13441 annotation coordinates to extract the corresponding sequence with SAMtools v1.9 and align each gene to the 4,071 ST131 genome assemblies [60] with BLAST v2.2.31 as above, processed with R packages tidyverse v1.2.1 and dplyr v0.8.3. For all operons, minor individual gene partial matches or losses were not examined due to the large number of samples and lack of consistent patterns for rare combinations. Results on amplified genes were restricted to those with prevalence >1%. The CDS of *fimB* is normally 600 bp, and with the IS*Ec55* insertion the homologous region typically spanned 2,493 bp. NCTC13441 has only three *pap* genes (*papIBA*) and lacks any *ucl* or *pil* genes, so the *pil* genes were from pEK204. *PilI* and *pilJ* encoding IncI1 conjugal transfer proteins were mainly absent with no clear association with the other *pil* genes, and so were not examined here. *PilV* encoding a pilus tip adhesin had more than one copy in 292 assemblies, as expected for a locus rearranged to change pilus binding specificity [157]. The 5 Kb *ucl* operon has four genes, a major subunit (*uclA*), chaperone (*uclB*), usher (*uclC*) and adhesin (*uclD*), whose reference sequences were determined from *E. coli* 83972 (CP001671). *E. coli* UTI89's chromosome (CP000243) and 143-gene 114 Kb plasmid (CP000244) [112] were used to obtain the *pap* operon: this has regulatory protein gene *papI*, structural protein genes *papA/B* and seven structural subunit genes (*papHCDJKEF*).

<u>Supplementary Data</u>

Table S1 - The 16 antibiotics used to identify 794 AMR genes on contigs originally from preterm infants.

Table S2 - The five AMR genes unique to all ST131 Clade C genomes except 8289_1#27 encoded *bla*$_{TEM}$ genes (an Ambler class A β-lactamase).
Text S1 - The preterm infant resistome in ST131 genomes including NCTC13441.
Text S2 - A small shared infant resistome between ST131 and *Klebsiella pneumoniae*
Figure S1 - Chromosomal AMR genes in *K. pneumoniae* reference isolate PMK1 versus a *K. pneumoniae* microbiome (WGLW1), relative to *E. coli* ST131 EC958 and commensal *E. coli* SE15.
Text S3 - No shared infant resistome between ST131 and *Staphylococcus lugdunensis*
Figure S2 - Out of 794 preterm infant gut AMR genes, there were no overlapping chromosomal genes in *S. lugdenensis* reference HKU versus a *S. lugdenensis* microbiome (M23590) relative to ST131.
Table S3 - PlasmidFinder Inc group alignments against pV130 and seven ST131 isolates.
Table S4 - Key known AMR, plasmid persistence and conjugation genes for pEK204, pEK499, pEK516, pCA14, pV130a and pV130b.
Figure S3 - Comparison of pEK516 (64,471 bp) with ST131 and two HMP assemblies.
Figure S4 - Read mapping distributions for seven ST131 to pV130 contigs.
Figure S5 - Comparison of plasmid pCA14 (155 Kb) with seven ST131 C1/C2 isolates.
Table S5 - The PPIN characteristics of the protein-coding genes from Miryala et al (2018).
Table S6 - The seven plasmids' accessions, numbers of genes with interactions given a combined score of 400+, Inc groups, conjugative ability and AMR genes.
Figure S6 - The ratio of non-trivial loops per PPI (y-axis) versus the STRING combined score for all 60 genes from Miryala et al (2018) Table 1 [99].
Figure S7 - The ratio of non-trivial loops per interaction versus the STRING combined score for all chromosomal genes, all genes on pEK499 and all those on pEK204.
Figure S8 - The ratio of interactions per protein for genes encoded on the chromosome or the pEK499 and pEK204 plasmids across the STRING combined score.
Figure S9 - The distributions of pEK204-like and pEK499-like regions in ST131 assemblies.
Figure S10 - The distributions of pEK204-like regions in ST131 Clade C genome assemblies.

Data Access:
The following files are available on the FigShare project "Plasmids_ST131_resistome_2020":
1. The set of 794 AMR genes derived from [74] are available (with their protein sequence translation) at FigShare at doi: 10.6084/m9.figshare.11961402.
2. The AMR gene profiles per sample determined by their BLAST sequence similarity results against CARD are available at FigShare at doi: 10.6084/m9.figshare.11961612. This dataset includes the PlasmidFinder results. It also includes other AMR database comparisons (ARG-ANNOT, ResFinder, MegaRes, VFDB and VirulenceFinder).
3. The BLAST sequence similarity results for the *fim*, *pil, pap* and *ucl* operons' genes versus 4,071 *E. coli* ST131 assemblies from Decano & Downing (2019) are available at FigShare at doi: 10.6084/m9.figshare.11961711.
4. The genome sequences and annotation files for reference genomes NCTC13441, EC958 and SE15, along with the assembled contigs for 83972 and 3_2_53FAA are available at FigShare at doi: 10.6084/m9.figshare.11961813.
5. The 4,071 *E. coli* ST131 genome assemblies from Decano & Downing (2019) are available at FigShare at doi: 10.6084/m9.figshare.11962278 (the first 1,680 assemblies) and at doi: 10.6084/m9.figshare.11962557 (the second 2,391 assemblies).

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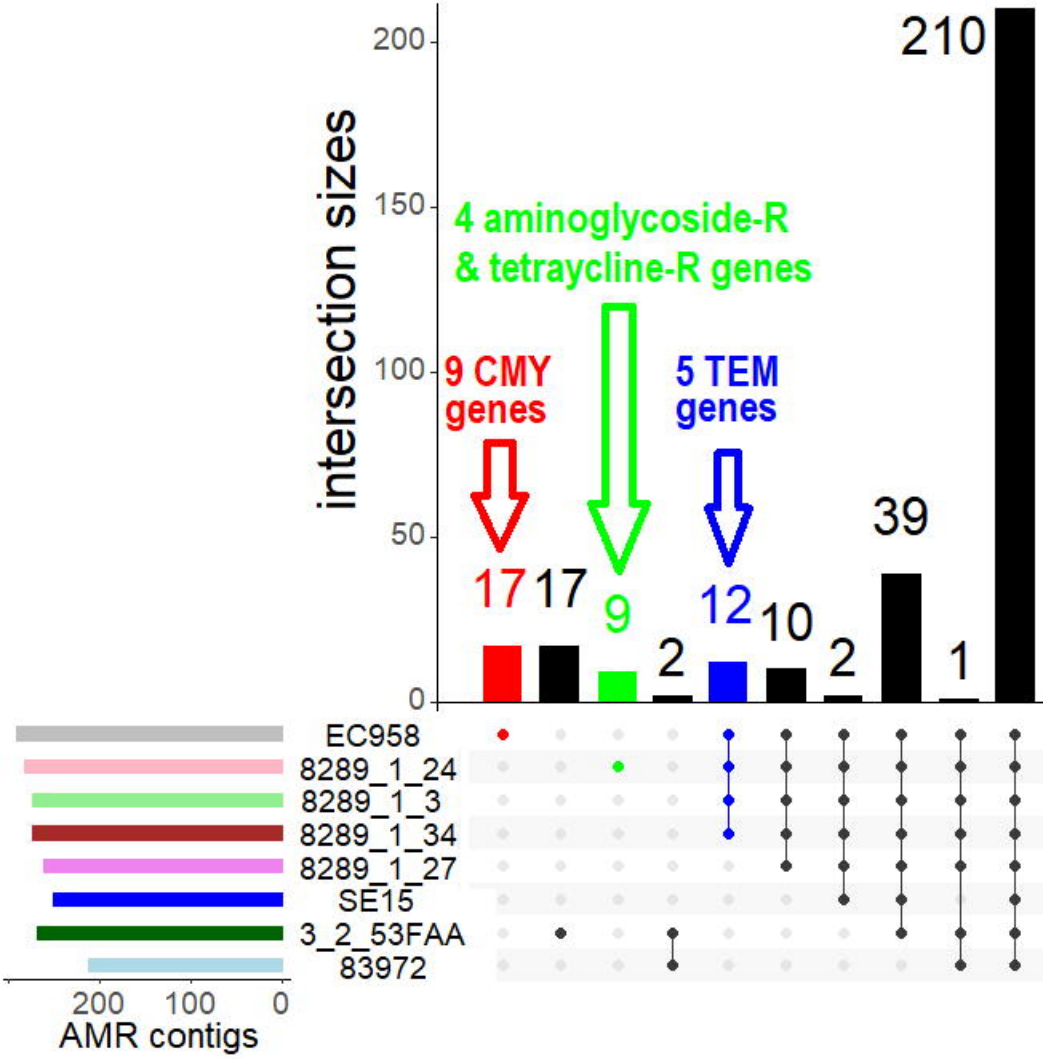
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150
100
50
0
210
4 aminoglycoside-R
& tetraycline-R genes
9 CMY
genes
5 TEM
genes
17
17
9
2
12
10
2
39
1
EC958
8289_1_24
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100
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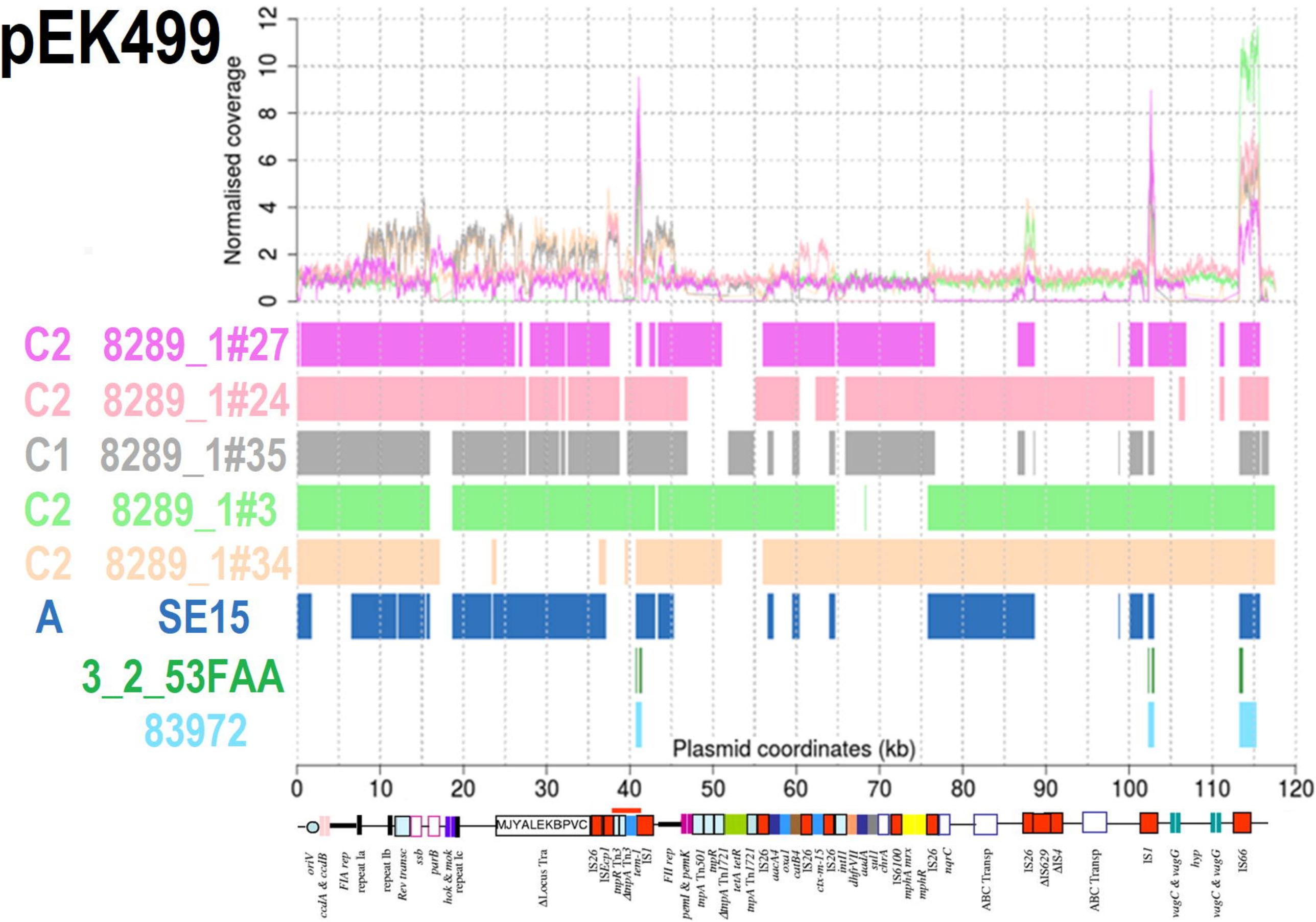
AMR contigs


pEK499
Normalised coverage
0
2
4
6
8
10
12
C2 8289_1#27
C2 8289_1#24
C1 8289_1#35
C2 8289_1#3
C2 8289_1#34
A SE15
3_2_53FAA
83972
Plasmid coordinates (kb)
0
10
20
30
40
50
60
70
80
90
100
110
120
MJYALEKBPVC
oriV
ccdA & ccdB
FIA rep
repeat Ia
repeat Ib
Rev transc
ssb
parB
hok & mok
repeat Ic
ΔLocus Tra
IS26
ISEcp1
mpR Tn3
ΔmpA Tn3
tem-1
IS1
FII rep
pemI & pemK
mpA Tn501
mpR
ΔmpA Tn1721
tetA tetR
mpA Tn1721
IS26
aacA4
oxa1
catB4
IS26
ctx-m-15
IS26
intI1
dhfrVII
aadA
sul1
chrA
IS6100
mphA mrx
mphR
IS26
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ΔIS629
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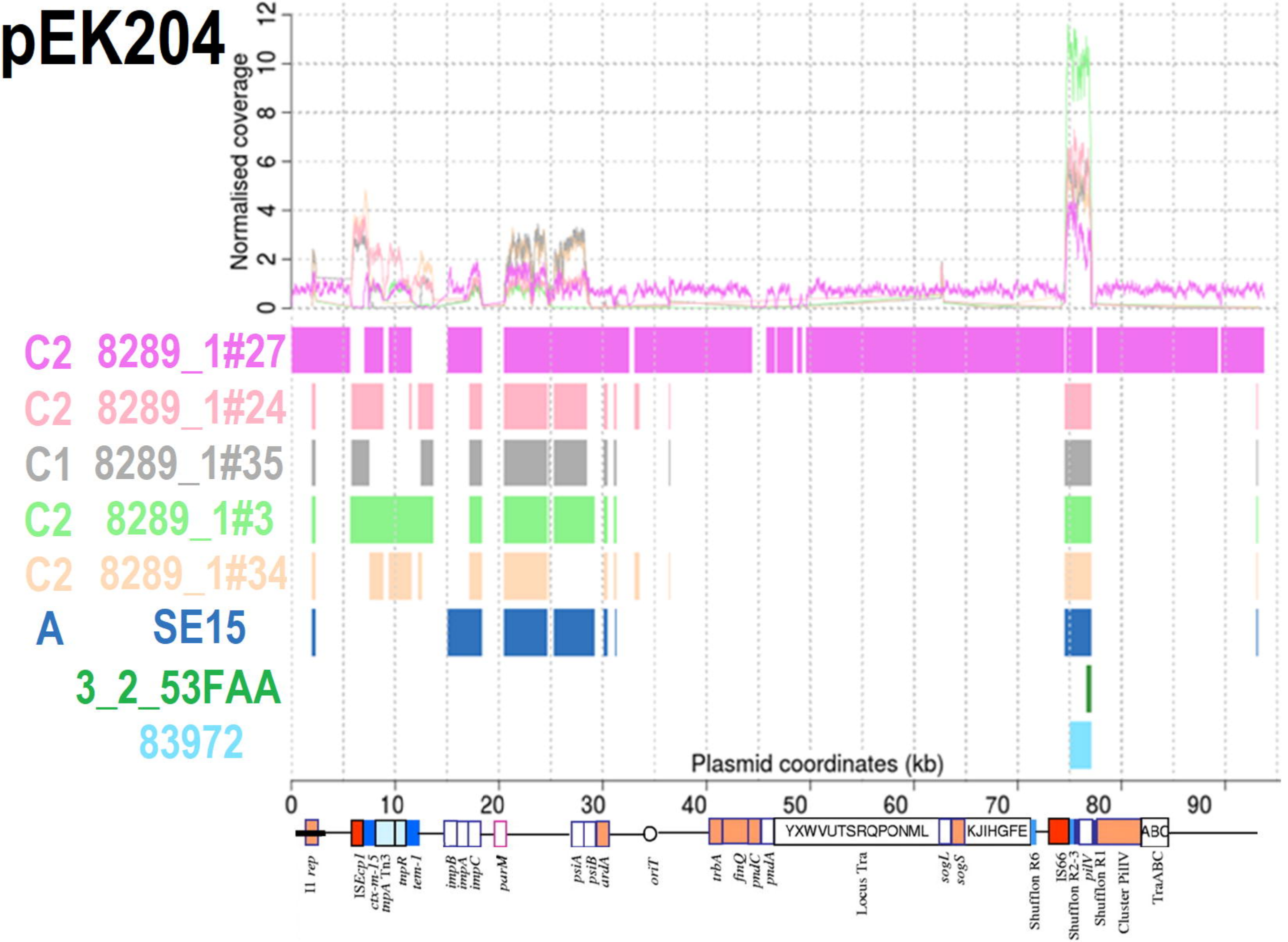
IS1
vagC & vagG
hyp
vagC & vagG
IS66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8
10
12
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C2 8289_1#3
C2 8289_1#34
A SE15
3_2_53FAA
83972
Plasmid coordinates (kb)
0
10
20
30
40
50
60
70
80
90
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KJIHGFE
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ISEcp1
ctx-m-15
mpA Tn3
mpR
tem-1
impB
impA
impC
parM
psiA
psiB
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oriT
trbA
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pndC
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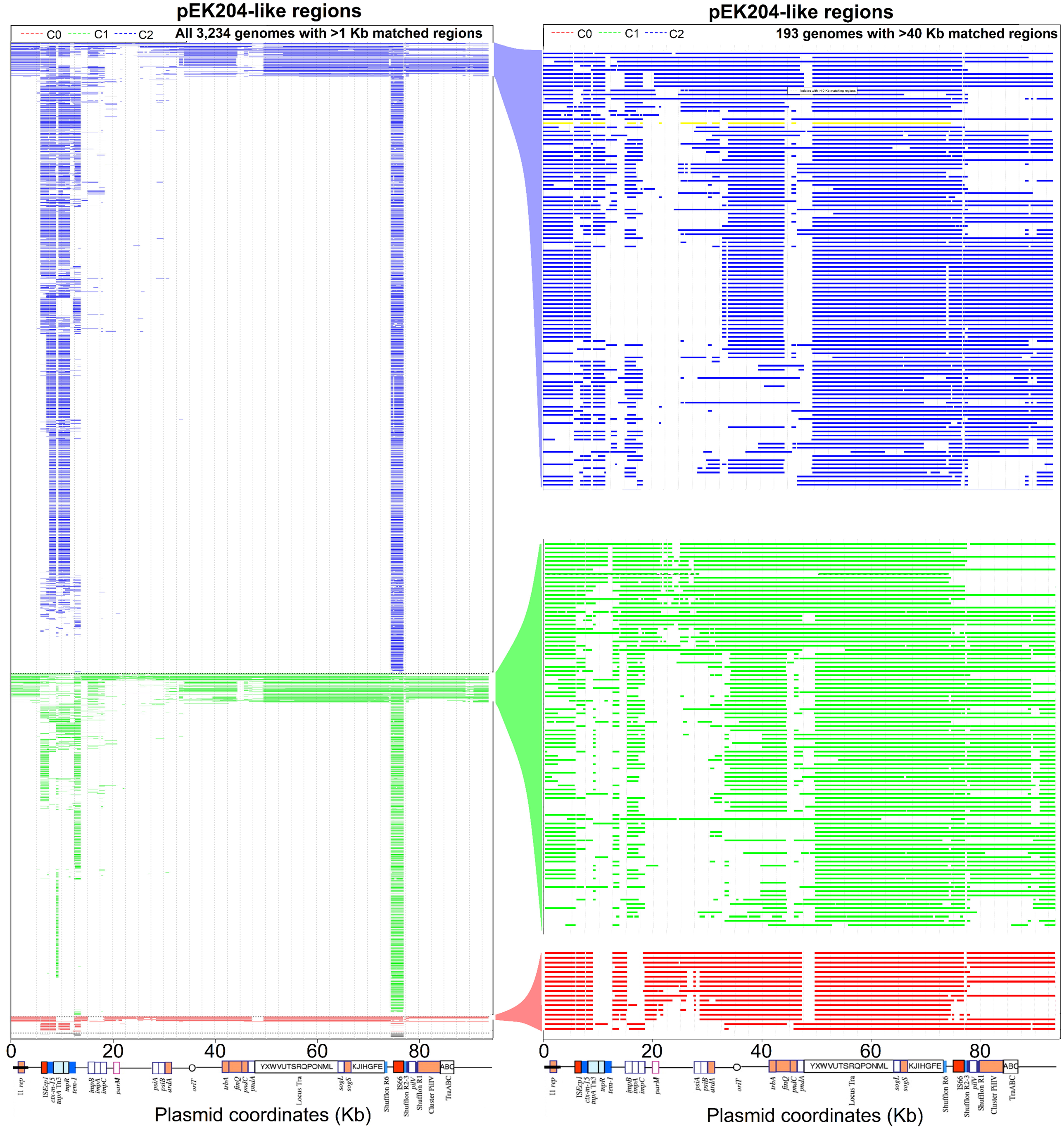
pEK204-like regions
C0
C1
C2
All 3,234 genomes with >1 Kb matched regions
0
20
40
60
80
I1 rep
ISEcp1
ctx-m-15
mpA Tn3
mpR
tem-1
impB
impA
impC
parM
psiA
psiB
ardA
oriT
trbA
finQ
pmdC
pmdA
YXWVUTSRQPONML
KJIHGFE
Locus Tra
sogL
sogS
Shufflon R6
IS66
Shufflon R2-3
pilV
Shufflon R1
Cluster PilIV
ABC
TraABC
Plasmid coordinates (Kb)
pEK204-like regions
C0
C1
C2
193 genomes with >40 Kb matched regions
Isolates with >40 Kb matching regions
0
20
40
60
80
I1 rep
ISEcp1
ctx-m-15
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mpR
tem-1
impB
impA
impC
parM
psiA
psiB
ardA
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trbA
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KJIHGFE
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ABC
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Plasmid coordinates (Kb)