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Molecular Characterization of a Multidrug Resistance IncF Plasmid from the Globally Disseminated Escherichia coli ST131 Clone

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Abstract

Escherichia coli sequence type 131 (E. coli ST131) is a recently emerged and globally disseminated multidrug resistant clone associated with urinary tract and bloodstream infections. Plasmids represent a major vehicle for the carriage of antibiotic resistance genes in E. coli ST131. In this study, we determined the complete sequence and performed a comprehensive annotation of pEC958, an IncF plasmid from the E. coli ST131 reference strain EC958. Plasmid pEC958 is 135.6 kb in size, harbours two replicons (RepFIA and RepFII) and contains 12 antibiotic resistance genes (including the blaCTX-M-15 gene). We also carried out hyper-saturated transposon mutagenesis and multiplexed transposon directed insertion-site sequencing (TraDIS) to investigate the biology of pEC958. TraDIS data showed that while only the RepFII replicon was required for pEC958 replication, the RepFIA replicon contains genes essential for its partitioning. Thus, our data provides direct evidence that the RepFIA and RepFII replicons in pEC958 cooperate to ensure their stable inheritance. The gene encoding the antitoxin component (ccdA) of the post-segregational killing system CcdAB was also protected from mutagenesis, demonstrating this system is active. Sequence comparison with a global collection of ST131 strains suggest that IncF represents the most common type of plasmid in this clone, and underscores the need to understand its evolution and contribution to the spread of antibiotic resistance genes in E. coli ST131.

Introduction

Escherichia coli sequence type 131 (E. coli ST131) is a recently emerged and globally disseminated multidrug resistant clone associated with urinary tract and bloodstream infections [1, 2]. E. coli ST131 was originally identified in 2008 as a major clone linked to the spread of the CTX-M-15 extended-spectrum β-lactamase (ESBL)-resistance gene [3–5]. Since then, E. coli ST131 has also been strongly associated with fluoroquinolone resistance, as well as co-resistance to aminoglycosides.
and trimethoprim-sulfamethoxazole [6, 7]. Recent analyses of the global epidemiology of E. coli ST131 using whole genome sequencing has revealed the CTX-M-15 allele is highly prevalent within a fluoroquinolone resistant-FimH30 (H30) ST131 sublineage [8] and demonstrated a significant role for recombination in the evolution of this E. coli lineage [9].

As observed for most other multidrug resistant Enterobacteriaceae pathogens, plasmids are the major vehicles for carriage of antibiotic resistance genes in E. coli ST131. Multiple plasmids from a range of incompatibility groups and containing various combinations of antibiotic resistance genes, conjugal transfer genes and other cargo genes have been described in E. coli ST131 strains [2]. This includes the IncF plasmids pEK499, pEK516 [10], pGUE-NDM [11], pC15-1a [12], pJJ1886-5 [13], pEC_B24, pEC_L8, pEC_L46 [14], pJIE186-2 [15], as well as the IncN plasmid pECN580 [16], the IncX plasmid pJIE143 [17] and the IncI plasmid pEK204 [10].

E. coli EC958 represents one of the best-characterised genome-sequenced E. coli ST131 strains [18]. E. coli EC958 is a phylogenetic group B2, CTX-M-15 positive, fluoroquinolone resistant, H30 E. coli ST131 strain [19]. The strain belongs to the pulse field gel electrophoresis defined United Kingdom (UK) epidemic strain A [20], and the recently defined ST131 Clade C2/H30-Rx sublineage [8, 9]. E. coli EC958 contains multiple genes associated with the virulence of extra-intestinal E. coli, including type 1 fimbriae which are required for adherence to and invasion of human bladder cells, the formation of intracellular bacterial communities, and colonization of the mouse bladder [19, 21]. In animal models, E. coli EC958 causes acute and chronic urinary tract infection (UTI) [21] and impairment of uterine contractility [22]. E. coli EC958 is also resistant to the bactericidal action of human serum, and the complement of genes that define this phenotype have been comprehensively defined [23].

E. coli EC958 contains a large IncF plasmid (pEC958—HG941719) containing multiple antibiotic resistance genes. Here we describe the full annotation of pEC958, and demonstrate that genes encoded on pEC958 are common among other Clade C2/H30-Rx ST131 strains. Plasmid pEC958 contains two replicons, and we show that both replicons contribute to its maintenance in E. coli EC958.

Materials and Methods

Bacterial strains and growth conditions

E. coli EC958 is a UTI strain originally isolated in the UK in 2005 [19]. E. coli TOP10 has been described previously [24]. E. coli strains were stored in 15% glycerol at -80°C and routinely cultured at 37°C on solid or in liquid Lysogeny Broth (LB) medium.

Antimicrobial susceptibility testing

The minimal inhibitory concentrations (MICs) were determined by Etest (bioMérieux Australia) on Mueller-Hinton agar at 37°C. The procedure and interpretation of MIC were performed as recommended by the manufacturer using CLSI breakpoints [25].

Molecular methods

Plasmid DNA purification was performed using the PureLink HiPure Plasmid Filter MidiPrep Kit (Life Technologies). E. coli TOP10 electro-competent cells were prepared as previously described [23] and pEC958 plasmid DNA was transformed into TOP10 in a 2 mm cuvette using a BioRad GenePulser set to 2.5 kV, 25 mF and 200 Ω. Cells were resuspended in 1 mL SOC medium and incubated at 37°C for 2 hours, then selected on LB agar plates supplemented with ampicillin 100 μg/mL.
In silico replicon sequence typing (RST)

The FAB formula for IncF plasmids (IncF RST scheme [26]) was identified in silico using the online pMLST tool (http://cge.cbs.dtu.dk/services/pMLST/) [27]. The pEC958 information was uploaded to the pMLST database (http://pubmlst.org/).

Annotation of pEC958

The sequence of plasmid pEC958 (emb|HG941719) [18] was manually curated in Artemis [28] using BLAST and literature searches. Antibiotic resistance genes were named in accordance with ResFinder 1.4 [29] and confirmed manually by BLAST and literature searches.

TraDIS analyses

The TraDIS sequence data used in this work was generated from a previously published study that examined essential genes in EC958 (BioProject number PRJNA189704; input A and B samples) [23]. The short reads were mapped to the pEC958 sequence using Maq version 0.7.1 [30]. Counts of insertion per gene and insertion index were calculated as previously described [23].

Phylogenetic tree building

The maximum-likelihood phylogenetic tree of EC958_A0140 homologs was built using the PhyML v3.0 online tool [31]. The tree used the WAG model for amino acid substitution and branch supports were calculated using approximate likelihood-ratio test (aLRT) [32].

Visualization

The read counts and insertion sites from TraDIS were visualized using Artemis version 15.0 [28]. The circular genome diagram was generated by DNAplotter [33] and linear genetic diagrams were constructed using Easyfig version 2.1 [34]. Circos [35] and Circoletto [36] were used to generate the sequence comparison figure. Sequence comparisons of pEC958 against ST131 strains were generated using BLAST Ring Image Generator (BRIG) [37].

Results

Characteristics of plasmid pEC958

The plasmid pEC958 is a 135,600 bp circular DNA molecule containing 142 coding sequences (CDSs) and 10 pseudogenes (Fig 1). The most closely related plasmid to pEC958 is pEK499 (99% identity covering 85% of pEC958; pEK499 lacks the second transfer region present in pEC958, which accounts for the remaining 15% of pEC958) (Fig 2). In silico replicon sequence typing identified pEC958 as a hybrid plasmid containing both IncFII and IncFIA replicons (FAB formula F2:A1:B-).

The RepFIA replicon

The 6,509bp RepFIA replicon in pEC958 is 99% identical to the corresponding region on the F-plasmid (nt 45922 to 52516, accession no. NC_002483.1) and 100% identical to two other plasmids isolated from E. coli ST131 strains, pEK499 (NC_013122.1 [10]) and pJJ1886_5 (NC_022651.1 [13]) (Fig 2). As observed in many other RepFIA sequences, this region does not contain the repC gene (replication initiation) found on the F-plasmid. The first region of RepFIA in pEC958 contains two rfsF sites (the target sequences of the site-specific resolvase ResD [38]), followed by the oriV-1 origin of replication, ccdAB genes (post-segregational...
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Fig 1. Circular representation of plasmid pEC958. The two outer rings show the coding sequences (CDSs) on the forward and reverse strand of the plasmid. Each CDS is colour-coded by its predicted function as shown in the figure. The grey ring depicts mobile elements identified on the plasmid. The two inner rings represent the GC plot and GC skew graph, respectively.

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killing), and resD (multimer resolution). The second region of RepFIA in pEC958 contains the replication repE gene (RepFIA) with its upstream sequences ssiA (single strand initiation) and oriV-2 (including the DnaA boxes, A/T rich region and four iterons), and the downstream incC iterons (incompatibility and copy-number control). The third region of RepFIA in pEC958 contains the sopAB partition genes and their target centromere-like sopC sequence.
This is the only partition system found on pEC958. Although this RepFIA replicon contains two origins of replication (oriV-1 and oriV-2), replication is predicted to start unidirectionally from oriV-2 because the bidirectional replication from oriV-1 is known to require the missing RepC protein [39, 40].

The RepFII replicon

The second replicon in pEC958, RepFII (4,068 bp), is 99% identical to the IncFII replicon in the *Shigella flexneri* 2b plasmid R100 (accession no. NC_002134.1, [41]) and 100% identical to the RepFII replicon in the *E. coli* ST131 plasmid pEK499 (Fig 1). This replicon encodes the essential gene for its replication, repA1, which is regulated by the negative regulator RepA2 (CopB), the non-coding RNA copA and the regulatory leading peptide RepA6 [42–44]. The pEC958 RepFII origin of replication (ori) is located between repA1 and repA4, consistent with previous descriptions for the initiation of DNA replication from this replicon [42, 45–47]. The repA4 region is important for plasmid stability and contains the ter sites for replication termination [48]. The pEC958 RepII replicon contains the tir (transfer inhibition protein [49]) and the type II toxin-antitoxin system pemI/pemK [50, 51] downstream of repA4.

The transfer region of pEC958 is not functional

The transfer (tra) region of pEC958 is disrupted by a composite mobile element flanked by IS26a and IS26b, carrying blaTEM gene (Fig 1). The first half of this tra region is 100% identical to the corresponding region on pEK499 (F2:A1:B-), and 99% identical to the corresponding region of several other IncF plasmids including pJ1886_5 (F2:A1:B-), pEC_L46 (F2:A1:B-), pEC_L8 (F2:A1:B-), pEFC36a (F2:A1:B-) and pChi7122-2 (F11:A1:B-). In contrast, the second half of the pEC958 tra region is 100% identical to pC15-1a (F2:A1:B-), R100 (F2:A1:B-), pHN3A11 (F2:A1:B-), pFOS-HK151325 (F2:A1:B-), pXZ (F2:A1:B-), pHK23a (F2:A1:B-), pHK01 (F2:A1:B-) and pEG356 (F2:A1:B-). However, the pEC958 conjugation system is missing three genes, namely trbI, traW and traU. TrbI is an inner membrane protein that affects pilus retraction [52]; TraW is required for F-pilus assembly [52]; and mutations in traU significantly reduce plasmid transfer proficiency [53]. Despite repeated attempts, we were unable to demonstrate conjugative transfer of pEC958 to recipient strains, supporting the bioinformatic prediction that its conjugation system is non-functional (data not shown).

Toxin-antitoxin systems

The pEC958 plasmid encodes four toxin-antitoxin (TA) systems: the hok/sok system, the ccdAB system encoded within RepFIA, the pemIK system encoded within RepFII and the vagDC system. The hok/sok locus encodes a type I TA system including a "host killing" (hok) transmembrane protein that damages the cell membrane, a "modulation of killing" (mok) and a "suppression of killing" (sok) antisense RNA that inhibits translation of mok [54]. Both ccdAB and pemIK belong to type II TA system where the toxin protein is inactivated by direct interaction with the antitoxin protein. The ccdB gene encodes for a gyrase inhibitor toxin [55] that kills the cell in the absence of the CcdA anti-toxin, which is unstable and degraded by the Lon protease [56]. PemK is a sequence-specific endoribonuclease that cleaves mRNAs to inhibit protein synthesis [50] whereas PemI blocks the endoribonuclease activity and is also subjected to Lon proteolysis [57].

There are two identical copies of the vagDC genes in pEC958. Sequence analysis of VagD revealed a PIN_VapC-FitB (cd09881) domain found in toxins of many bacterial TA systems. VagC contains an antitoxin-MazE (pfam04014) domain. The vagDC genes have been shown to
be involved in plasmid stability in *Salmonella* Dublin, where VagD inhibits cell division and VagC modulates the activity of VagD [58].

**Mobile genetic elements and antibiotic resistance genes**

The majority of mobile genetic elements and antibiotic resistant genes in pEC958 cluster in two regions: an 8-kb region in the middle of the *tra* region, and a 41-kb region located immediately downstream of the RepFII replicon (Fig 1). Plasmid pEC958 contains eight IS26 elements (named IS26a-IS26h), two IS1 elements, one ISEc23 element and one group II intron (E.c.I11, found outside of the two regions) (Fig 3). IS26a and IS26b are located at the two ends of the 8-kb region, flanking ISEc1, a remnant of Tn3, which includes the *bla*TEM-1 gene, and a partial sequence of Tn21. The beginning of the 41-kb region contains a partial sequence of Tn5403 followed by IS26c. The region between IS26c and IS26d contains a cluster of 6 genes (EC958_A0096 to EC958_A0101) predicted to encode a series of ABC transporters and an iron permease. Downstream of IS26d is a class I integron In54 [59] with gene cassettes consisting of *dfrA17, aadA5* and *sul1*, encoding trimethoprim, streptomycin and sulfonamide resistance, respectively. The *mphR-mrx-mph(A)* operon encoding resistance to macrolides is located between IS6100 and IS26e. Immediately after IS26e is the *blaCTX-M-15* gene encoding cepotaxime resistance. Located between IS26f and IS26g are *catB4A* (non-functional; disrupted by IS26f), *blaOXA-1* (beta-lactam resistance) and *aac(6′)-Ib-cr* (fluoroquinolone and aminoglycoside resistance). After IS26g lies Tn1721, which harbours tetE and tet(A), encoding resistance to tetracycline. The end of the 41-kb region contains a partial sequence of Tn5403 and IS26h.

**Functional characterization of antibiotic resistance genes on pEC958**

To investigate the antibiotic resistance phenotypes conferred by plasmid pEC958, we transformed the plasmid into *E. coli* TOP10. Table 1 shows the resistance profile of wild-type EC958 (which contains pEC958) compared to TOP10(pEC958). EC958 is resistant to 11 of the 18 antibiotics tested, five of which are fully transferable via pEC958. EC958 is resistant to the cephalosporin cefoxitin and the three third-generation cephalosporins tested (cefotaxime, ceftazidime and cefpodoxime). These phenotypes, however, were not fully transferred to TOP10 by pEC958. TOP10(pEC958) had elevated MICs to cefoxitin, cepotaxime, cefazidime and cefpodoxime (MIC of 6, 1.5, 1.5 and 8.0 μg/mL, respectively) compared to the background strain TOP10 (MIC of 4, 0.047, 0.38 and 0.25 μg/mL, respectively), but these MICs were still 6–10 fold lower than those of the EC958 wild-type strain. This suggests that *blaCTX-M-15* on pEC958 plasmid does not mediate the full resistance against third-generation cephalosporins. This is...
consistent with previous reports of lower resistance to cephalosporins in strains where the $bla_{CTX-M-15}$ is separated by IS26 from its promoter within the ISEcp1 element [60–63]. The other resistance phenotypes not transferred were for quinolones and fluoroquinolones. Chromosomal mutations in $gyrA$ (S83L, D87N, A828S) and $parC$ (S80I, E84V, A192V, A471G, D475E, Q481H) genes are likely to mediate these phenotypes, even though the plasmid carries $aac(6\prime)-Ib-cr$ [64–66].

### Genes required for the stable maintenance of pEC958

In order to gain insights into molecular mechanisms of plasmid stability, we analyzed the TraDIS data from a saturated transposon mutant library of EC958 [23] against the complete sequence of pEC958 to identify genes required for plasmid stability. We used a total of 12 million transposon-tagged reads, of which 901,588 reads (7.4%) were mapped to plasmid pEC958, identifying 27,317 unique insertion sites (i.e. one insertion site every 4.96 bp). To devise a biological threshold for the identification of genes required for the stable maintenance of pEC958, the insertion index (number of mapped reads normalized by gene length) of each plasmid gene was calculated and compared with the $sopAB$ genes, which are known to be essential for plasmid partitioning (Fig 4).

A total of 9 genetic elements were identified to be required for the stable maintenance of pEC958. They are the $ccdA$, $sopA$ and $sopB$ genes in RepFIA; the $copA$, $repA6$, $repAI$, $repA4$ genes and the oriV region in RepFII; and the hypothetical gene EC958_A0140. Our results indicate that replication of pEC958 is initiated at the oriV of RepFII and requires at least the $copA$, $repA6$, $repAI$, $repA4$ genes. While RepFIA is not essential for replication, it is required for partitioning ($sopAB$) of pEC958 into daughter cells. Our data also demonstrated that the $ccdAB$ TA system located within RepFIA is functional.

### Table 1. Antibiotic resistance profiles (MIC, μg/mL) of EC958 and its pEC958 transformant in E. coli TOP10.

| Antibiotics                          | EC958     | TOP10     | TOP10 + pEC958 |
|--------------------------------------|-----------|-----------|----------------|
| Ampicillin                           | ≥256 (R)  | 2 (S)     | ≥256 (R)       |
| Amoxicillin/Clavulanic Acid          | 24 (R)    | 3 (S)     | 16 (R)         |
| Aztreonam                            | 2 (S)     | 0.094 (S) | 1 (S)          |
| Cefotaxime                           | 48 (R)    | 4 (S)     | 6 (S)          |
| Cefotaxime                           | 12 (R)    | 0.047 (S) | 1.5 (I)        |
| Ceftazidime                          | 16 (R)    | 0.38 (S)  | 1.5 (S)        |
| Cefpodoxime                          | 48 (R)    | 0.25 (R)  | 8.0 (R)        |
| Imipenem                             | 0.25 (S)  | 0.19 (S)  | 0.25 (S)       |
| Meropenem                            | 0.125 (S) | 0.064 (S) | 0.094 (S)      |
| Nalidixic Acid                       | ≥256 (R)  | 0.38 (S)  | 0.50 (S)       |
| Ciprofloxacin                        | ≥32 (R)   | 0.003 (S) | 0.008 (S)      |
| Sulfamethoxazole/Trimethoprim        | ≥32 (R)   | 0.032 (S) | ≥32 (R)        |
| Kanamycin                            | ≥256 (R)  | 2 (S)     | ≥256 (R)       |
| Amikacin                             | 24 (I)    | 3 (S)     | 24 (I)         |
| Tetracycline                         | ≥256 (R)  | 0.5 (S)   | 192 (R)        |
| Tigecycline                          | 0.38 (S)  | 0.094 (S) | 0.38 (S)       |
| Fosfomycin                           | 0.5 (S)   | 0.38 (S)  | 0.38 (S)       |
| Nitrofurantoin                       | 4 (S)     | 0.094 (S) | 0.094 (S)      |

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EC958_A0140 represents a novel gene associated with plasmid maintenance. We screened the NCBI complete plasmid sequence database and identified 17 other plasmids that also contain this gene (Fig 5). All of these plasmids were IncF type except for pECL_A (non-typable), and several were also isolated from E. coli ST131 strains (pJJ1886_5, pEK499, pEC_L8 and pEC_L46). Bioinformatic analysis of EC958_A0140 did not yield any clues regarding its function, and thus further work is required to confirm its role in plasmid stability.

pEC958-like plasmid sequences are highly prevalent in ST131

The prevalence of pEC958-like plasmid sequences was assessed in a previously described global collection of 97 E. coli ST131 strains [9]. Fig 6 shows the overview of plasmid sequences from 97 ST131 strains plus four complete ST131 plasmids available on GenBank in comparison with the pEC958 sequence. There are 20 strains and 2 database plasmids (pEK499 and pJJ1886_5) that contain more than 70% of pEC958 gene content, all of which belong to the clade C sublineage C2 (40%) (Fig 6 and S1 Table). Twelve out of these 20 strains (plus pEK499) also harbor all 9 pEC958 essential genes identified above.

In silico replicon sequence typing of IncF plasmids was also performed on the 97 strains. Table 2 shows the 8 most common FAB types found within this collection. The FAB formula of pEC958, F2:A1:B-, is also the most common replicon type, accounting for 20.6% of all 97 E. coli ST131 strains, or 27.8% of clade C strains, all of which also belong to subclade C2. The second most common type is F1:A2:B20, of which 17 are in subclade C1 and 1 is in clade A. In
terms of individual replicons, FIB is present in 100% of clade A and B strains, while FII is most common in clade C (87.5%; S1 Table). Based on our sequence analysis, 3/97 strains do not harbor an IncF plasmid.

Discussion

Our study presents a full annotation of pEC958, a multi-drug resistance plasmid in the well-characterized E. coli ST131 strain EC958 [18, 19, 23]. In addition, we identified genes required for the maintenance and stability of pEC958. Although IncF plasmids are extremely successful in the E. coli ST131 clonal lineage [67], this is the first study to examine the biology of an IncF plasmid in its native host using TraDIS [68]. The replication and stability of IncF plasmids (F-plasmid, R1, and R100) has been well documented [39, 47, 69, 70]. Here we provide insights into the interplay between two replicons in order to achieve stable maintenance of the circular plasmid DNA on which they co-exist.

The data analysis in this study used a straight cut-off based on the insertion index of the sopAB genes, which encode the partitioning system of pEC958. Mutation of sopAB is known to cause destabilization of IncF plasmids and thus they represent characterised genes involved in plasmid stability [71, 72]. This deviation from the model-based approach, in which the cut-off is defined as the intercept of two distribution models representing essential and non-essential genes [23], was chosen because of two reasons: (i) the number of genes on plasmid is insufficient to build two distribution models; and (ii) the cut-off previously defined using chromosomal data is not applicable because of the higher insertion frequency on the plasmid (i.e. one insertion every 4.96 bp compared to every 9.92 bp on the chromosome). In the case of the well-characterised IncF system, use of a straight cut-off assumed that any gene with an insertion index lower than the sopAB genes would have a similar or stronger effect on plasmid stability.

The stable maintenance of large plasmids such as pEC958 is achieved by the contribution of multiple factors, including systems involved in replication, partitioning and toxin-antitoxin production. Using the strategy outlined, we aimed to identify genes that when mutated caused destabilization of plasmid pEC958—thus they must play a role in plasmid stability.
Fig 6. BRIG image depicting the presence of pEC958-like sequences in E. coli ST131 strains. The strains are coloured according to their previously defined phylogenetic relationship: red (Clade A), orange (Clade B) and green (Clade C) [9]. The degree of coloured shading indicates the level of identity according to BLASTn between pEC958 (nucleotide position highlighted on the inner circle) and the draft Illumina assemblies of the E. coli ST131 strains [9]. BLASTn matches are coloured based on a nucleotide identity of between 70% and 90% (dark shading = high identity, light shading = low identity). Blank spaces in each ring represent BLASTn matches to pEC958 with less than 70% nucleotide identity, or pEC958 regions with no BLAST matches. Four strains originally characterised as ST131 but later shown to be ST95 are shown in black. Highlighted on the outer ring are the RepFII and RepFIA replicons, as well as antibiotic resistance genes, transposons and IS elements.

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Our results showed that RepFII, particularly the \textit{copA}, \textit{repA1}, \textit{repA4} genes and \textit{oriV} region, is required for the replication of pEC958. This is consistent with previous studies on the function of RepFII in the IncFII plasmid R100 [41]. In contrast to R100, the RepFII region on pEC958 does not contain its own intrinsic partition system (\textit{stb} locus on R100 [73, 74]). Furthermore, we could not identify any region that resembles a partition site (centromere-like) elsewhere on pEC958 other than within the RepFIA region. Thus, it is reasonable to assume that the \textit{sopAB} genes in the RepFIA region [75, 76] represent the only active partition system on pEC958. Indeed, our transposon mutagenesis revealed a very low insertion index for both \textit{sopA} and \textit{sopB}, confirming the requirement of these two genes for pEC958 partitioning and allowing us to use these genes as a reference threshold to identify biologically significant genes required for plasmid maintenance.

Using TraDIS, we were able to demonstrate that none of the known replication genes in RepFIA are required for pEC958 replication. This included the \textit{oriV-1} of RepFIA, which was not expected to be functional due to the absence of the \textit{repC} gene [40]. The \textit{oriV-2} and its associated genes in RepFIA appear to be intact yet dispensable in pEC958. Similar behavior has been reported in the dual-replicon plasmid pCG86, which contains an active RepFII replicon and an inactive (but intact) RepFIB replicon [77]. This is consistent with a previously proposed model for plasmid speciation, in which the existence of co-integrate plasmids (such as pEC958) allows one replicon to be relaxed and free to accumulate mutations whilst the other replicon is constrained by evolutionary pressure to maintain its replication function [78].

The RepFIA also carries one toxin-antitoxin system \textit{ccdAB} in which the antitoxin CcdA is protected from transposon mutagenesis, indicating that the system is active in pEC958. There are three other TA systems in pEC958, none of which were required for plasmid stability under the conditions tested in this study. Others have suggested that TA systems are more than just plasmid maintenance systems; they can also function as a stress-response system [79, 80], as a programmed cell-death network [81], or as a reversible bacteriostasis system (i.e. induction of dormancy or persistence) [82, 83]. It is conceivable that the redundancy of TA systems on pEC958 is linked to other functions that provide a fitness advantage to its host.

Plasmids of several different incompatibility types have been identified in \textit{E. coli} ST131, including IncF, IncI1, IncN, IncA/C and \textit{pir}-type [2]. Our data demonstrates that IncF plasmids...
are the most common plasmid type in *E. coli* ST131, and is in accordance with previous studies [2, 4]. To investigate the prevalence of pEC958 sequences in our strain collection, we used genome sequence data to evaluate the prevalence of pEC958 genes and to perform *in silico* IncF replicon sequence typing. We identified 20 strains (including EC958) that contained more than 70% of the genes identified on pEC958, suggesting that many ST131 strains carry very similar plasmids. We also identified 20 strains that possess the F2:A1:B- plasmid replicon formula, 17 of which contain >70% of pEC958 genes. Taken together, our data demonstrate that pEC958 belongs to the most common group of IncF plasmids found in *E. coli* ST131.

The overall success of IncFII plasmids extends beyond the carriage of *bla*<sub>CTX-M-15</sub> in *E. coli* ST131. IncFII plasmids that have acquired the *bla*<sub>NDM-1</sub> gene (thus conferring carbapenem resistance) have been described in the ST131 lineage [11, 84], but strain EC958 was isolated prior to the discovery of NDM determinants and we did not find any NDM determinants in the 97 ST131 strain collection. The IncFII<sub>k</sub> plasmid, a replicon type originally found in *Klebsiella* [26], has also been found in KPC-producing ST131 strains in the USA and China [85, 86]. The evolution and continual gain of new antimicrobial resistance determinants in IncFII plasmids represents a major challenge for our understanding of plasmid biology and the spread of antibiotic resistance genes. Here, we shed novel insight into our knowledge of plasmid replication by providing direct evidence that the RepFIA and RepFII replicons in pEC958 cooperate to ensure their stable inheritance. The combination of replication from RepFII and partition from RepFIA may represent a co-evolutionary adaptation for this common plasmid type.

**Supporting Information**

S1 Table. The presence/absence of pEC958 coding sequences in *E. coli* ST131 strains. (XLSX)

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**Author Contributions**

Conceived and designed the experiments: MDP MU SAB MAS. Performed the experiments: MDP KMP SS SH MU. Analyzed the data: MDP BMF MSC NLBZ MU. Contributed reagents/materials/analysis tools: MSC MU SAB. Wrote the paper: MDP MU SAB MAS.

**References**

1. Rogers BA, Sidjabat HE, Paterson DL. *Escherichia coli* O25b-ST131: a pandemic, multiresistant, community-associated strain. *J Antimicrob Chemother*. 2011; 66(1):1–14. doi: 10.1093/jac/dkq415 PMID: 21081548
2. Nicolas-Chanoine MH, Bertrand X, Madec JY. *Escherichia coli* ST131, an intriguing clonal group. *Clin Microbiol Rev*. 2014; 27(3):543–74. doi: 10.1128/CMR.00125-13 PMID: 24982321
3. Nicolas-Chanoine MH, Blanco J, Leflon-Guibout V, Demarty R, Alonso MP, Canica MM, et al. Intercontinental emergence of *Escherichia coli* clone O25:H4-ST131 producing CTX-M-15. *J Antimicrob Chemother*. 2008; 61(3):273–81. PMID: 18977311
4. Coque TM, Novais A, Carattoli A, Poirel L, Pitout J, Peixe L, et al. Dissemination of clonally related *Escherichia coli* strains expressing extended-spectrum β-lactamase CTX-M-15. *Emerg Infect Dis*. 2008; 14(2):195–200. doi: 10.3201/eid1402.070350 PMID: 18258110
5. Lau SH, Kaufmann ME, Livermore DM, Woodford N, Willshaw GA, Cheasty T, et al. UK epidemic *Escherichia coli* strains A-E, with CTX-M-15 β-lactamase, all belong to the international O25:H4-ST131 clone. *J Antimicrob Chemother*. 2008; 62(6):1241–4. doi: 10.1093/jac/dkn380 PMID: 18779256
6. Johnson JR, Johnston B, Clabots C, Kuskowski MA, Pendyala S, Debroy C, et al. *Escherichia coli* sequence type ST131 as an emerging fluoroquinolone-resistant uropathogen among renal transplant
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11. Bonnin RA, Poirel L, Carattoli A, Nordmann P. Characterization of an IncFII plasmid encoding NDM-1. Int J Antimicrob Agents. 2010; 35(5):444–50. doi: 10.1016/j.ijantimicag.2009.12.012 PMID: 20188525

12. Price LB, Johnson JR, Aziz M, Clabots C, Johnston B, Tchesnokova V, et al. The epidemic of extended-spectrum β-lactamase-producing \textit{Escherichia coli} ST131 is driven by a single highly pathogenic subclone. \textit{H30-Rx. MBio}. 2013; 4(6):e00377–13. doi: 10.1128/mBio.00377-13 PMID: 23435742

13. Smet A, Van Nieuwerburgh F, Vandekerckhove TT, Martel A, Deforce D, Butaye P, et al. Complete nucleotide sequence of \textit{H30-Rx. MBio}. 2013; 4(6):e00377–13. doi: 10.1128/mBio.00377-13 PMID: 23435742

14. Boyd DA, Tyler S, Christianson S, McGeer A, Muller MP, Willey BM, et al. Complete nucleotide sequences of plasmids pEK204, pEK499, and pEK516, encoding CTX-M enzymes in three major \textit{Escherichia coli} lineages from the United Kingdom, all belonging to the international O25:H4-ST131 clone. Antimicrob Agents Chemother. 2009; 53(10):4472–82. doi: 10.1128/AAC.00888-09 PMID: 19667243

15. Zong Z. Complete sequence of \textit{pJJE186-2}, a plasmid carrying multiple virulence factors from a subclone, \textit{H30-Rx}. Antimicrob Agents Chemother. 2010; 54(1):3758–64. PMID: 15588431

16. Chen L, Hu H, Chavda KD, Zhao S, Liu R, Liang H, et al. Complete sequence of a KPC-producing IncN multidrug-resistant plasmid from an epidemic \textit{Escherichia coli} sequence type 131 strain in China. Antimicrob Agents Chemother. 2014; 58(4):2422–5. doi: 10.1128/AAC.02587-13 PMID: 24395232

17. Partridge SR, Ellem JA, Tetu SG, Zong Z, Paulsen IT, Iredell JR. Complete sequence of pJJE143, a p-type plasmid carrying \textit{ISCpl1}-bla\textsubscript{CTX-M-15} from an \textit{Escherichia coli} ST131 isolate. Antimicrob Agents Chemother. 2011; 55(12):5933–5. doi: 10.1128/AAC.00839-11 PMID: 21911569

18. Forde BM, Ben Zakour NL, Stanton-Cook M, Phan MD, Totsika M, Peters KM, et al. The complete genome sequence of \textit{Escherichia coli} EC958: a high quality reference sequence for the globally disseminated multidrug resistant \textit{E. coli} O25b:H4-ST131 clone. PLoS One. 2014; 9(8):e104400. doi: 10.1371/journal.pone.0011202 PMID: 20585456

19. Totsika M, Beatson SA, Sarkar S, Phan MD, Petty NK, Bachmann N, et al. Insights into a multidrug resistant \textit{Escherichia coli} pathogen of the globally disseminated ST131 lineage: genome analysis and virulence mechanisms. PLoS One. 2011; 6(10):e26578. doi: 10.1371/journal.pone.0026578 PMID: 21920220

20. Lau SH, Reddy S, Cheesbrough J, Bolton FJ, Wilshaw G, Cheasty T, et al. Major uropathogenic \textit{Escherichia coli} strain isolated in the northwest of England identified by multilocus sequence typing. J Clin Microbiol. 2008; 46(3):1076–80. doi: 10.1128/JCM.02065-07 PMID: 18199778

21. Totsika M, Kostakiotu M, Hannan TJ, Upton M, Beatson SA, Janetka JW, et al. A FimH inhibitor prevents acute bladder infection and treats chronic cystitis caused by multidrug-resistant uropathogenic \textit{Escherichia coli} ST131. J Infect Dis. 2013.

22. Floyd RV, Upton M, Hultgren SJ, Wray S, Burdyga TV, Winstanley C. \textit{Escherichia coli}-mediated impairment of ureteric contractility is uropathogenic \textit{E. coli} specific. J Infect Dis. 2012; 206(10):1589–96. doi: 10.1093/infdis/jis554 PMID: 23002447

23. Phan M-D, Peters KM, Sarkar S, Lukowski SW, Allsopp LP, Moriel DG, et al. The Serum Resistance of a Globally Disseminated Multidrug Resistant Uropathogenic \textit{Escherichia coli} Clone. PLoS Genet. 2013; 9(10):e1003834. doi: 10.1371/journal.pgen.1003834 PMID: 24098145
24. Durfee T, Nelson R, Baldwin S, Plunkett G 3rd, Burland V, Mau B, et al. The complete genome sequence of *Escherichia coli* DH10B: insights into the biology of a laboratory workhorse. J Bacteriol. 2008; 190(7):2597–606. doi: 10.1128/JB.01695-07 PMID: 18245285

25. CLSI. Performance standards for antimicrobial susceptibility testing; Twenty-fourth informational supplement. Wayne, PA: Clinical and Laboratory Standards Institute. 2014; CLSI document M100-S24.

26. Lane D, de Feyter R, Kennedy M, Phua SH, Semon D. D protein of miniF plasmid acts as a repressor of virulence and resistance determinants. J Antimicrob Chemother. 2010; 65(12):2518–29. doi: 10.1093/jac/dkq347 PMID: 20935300

27. Carattoli A, Zankari E, Garcia-Fernandez A, Voldby Larsen M, Lund O, Villa L, et al. In silico detection and typing of Plasmids using Plasmid Finder and Plasmid Multilocus Sequence Typing. Antimicrob Agents Chemother. 2014; 58(7):3895–903. doi: 10.1128/AAC.02412-14 PMID: 24777092

28. Carver T, Harris SR, Berrimian M, Parkhill J, McQuillan JA. Artemis: an integrated platform for visualization and analysis of high-throughput sequence-based experimental data. Bioinformatics. 2012; 28(4):464–9. doi: 10.1093/bioinformatics/btr261 PMID: 22782487

29. Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, et al. Identification of acquired antimicrobial resistance genes. J Antimicrob Chemother. 2012; 67(11):2640–4. doi: 10.1093/jac/dkq347 PMID: 22782487

30. Li H, Ruan J, Durbin R. Mapping short DNA sequencing reads and calling variants using mapping quality scores. Genome Res. 2008; 18(11):1851–8. doi: 10.1101/gr.078212.108 PMID: 18714091

31. Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel O. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Systematic biology. 2010; 59(3):307–21. doi: 10.1038/sysbio/syq010 PMID: 20525638

32. Anisimova M, Gascuel O. Approximate likelihood-ratio test for branches: A fast, accurate, and powerful alternative. Systematic biology. 2006; 55(4):539–52. PMID: 16785212

33. Carver T, Thomson N, Bleasby A, Berrimian M, Parkhill J. DNAPlotter: circular and linear interactive genome visualization. Bioinformatics. 2009; 25(1):119–20. doi: 10.1093/bioinformatics/btn261 PMID: 18990721

34. Sullivan MJ, Petty NK, Beatson SA. Easyfig: a genome comparison visualizer. Bioinformatics. 2011; 27(7):1009–10. doi: 10.1093/bioinformatics/btr039 PMID: 21278367

35. Krzywinski M, Schein J, Birol I, Connors J, Gascoyne R, Horsman D, et al. Circos: an information aesthetic for comparative genomics. Genome Res. 2009; 19(9):1639–45. doi: 10.1101/gr.092759.109 PMID: 19541911

36. Darzentas N. Circoletto: visualizing sequence similarity with Circos. Bioinformatics. 2010; 26(20):2620–1. doi: 10.1093/bioinformatics/btq484 PMID: 20736339

37. Alikhan NF, Petty NK, Ben Zakour NL, Beatson SA. BLAST Ring Image Generator (BRIG): simple prokaryote genome comparisons. BMC Genomics. 2011; 12:402. doi: 10.1186/1471-2164-12-402 PMID: 21824423

38. Lane D, de Feyter R, Kennedy M, Phua SH, Semon D. D protein of miniF plasmid acts as a repressor of transcription and as a site-specific resolvase. Nucleic Acids Res. 1986; 14(24):9713. doi: 10.1093/nar/14.24.9713 PMID: 21824423

39. Kline BC. A review of mini-F plasmid maintenance. Plasmid. 1985; 14(1):1–16. PMID: 3898165

40. Lane HE. Replication and incompatibility of F and plasmids in the IncFII Group. Plasmid. 1981; 5(1):100–26. PMID: 7012871

41. Womble DD, Rownd RH. Genetic and physical map of plasmid NR1: comparison with other IncFII antibiotic resistance plasmids. Microbiol. Rev. 1988; 52(4):433–51. PMID: 3070319

42. Womble DD, Rownd RH. Regulation of IncFII plasmid DNA replication. A quantitative model for control of plasmid NR1 replication in the bacterial cell division cycle. J Mol Biol. 1986; 192(3):529–47. PMID: 2435919

43. Wu R, Wang X, Womble DD, Rownd RH. Suppression of replication-deficient mutants of IncFII plasmid NR1 can occur by two different mechanisms that increase expression of the *repA1* gene. J Bacteriol. 1993; 175(10):3161–73. PMID: 8491730

44. Wu R, Wang X, Womble DD, Rownd RH. Expression of the *repA1* gene of IncFII plasmid NR1 is translationally coupled to expression of an overlapping leader peptide. J Bacteriol. 1992; 174(23):7620–8. PMID: 1447133

45. Masai H, Kaziro Y, Arai K. Definition of *oriR*, the minimum DNA segment essential for initiation of R1 plasmid replication in vitro. Proc Natl Acad Sci U S A. 1983; 80(22):6814–8. PMID: 6316326

46. Ohtsubo E, Feingold J, Ohtsubo H, Mickel S, Bauer W. Unidirectional replication in *Escherichia coli* of three small plasmids derived from R factor R12. Plasmid. 1977; 1(1):8–18. PMID: 375272
47. Silver L, Chandler M, de la Tour EB, Caro L. Origin and direction of replication of the drug resistance plasmid R100.1 and of a resistance transfer factor derivative in synchronized cultures. J Bacteriol. 1977; 131(3):929–42. PMID: 330504

48. Jiang T, Min YN, Liu W, Wombie DD, Rownd RH. Insertion and deletion mutations in the repA4 region of the IncFII plasmid NR1 cause unstable inheritance. J Bacteriol. 1993; 175(7):3530–8. PMID: 8396115

49. Tanimoto K, Iino T, Ohtsubo H, Ohtsubo E. Identification of a gene, tir of R100, functionally homologous to the F3 gene of F in the inhibition of RP4 transfer. Mol Gen Genet. 1985; 198(2):356–7. PMID: 3920479

50. Zhang J, Zhang Y, Zhu L, Suzuki M, Inouye M. Interference of mRNA function by sequence-specific endoribonuclease PemK. J Biol Chem. 2004; 279(20):20678–84. PMID: 15020402

51. Tsuchimoto S, Ohtsubo H, Ohtsubo E. Two genes, pemK and pemL, responsible for stable maintenance of resistance plasmid R100. J Bacteriol. 1988; 170(4):1461–6. PMID: 2823464

52. Maneewannakul S, Maneewannakul K, Ippehl-Ihler K. Characterization, localization, and sequence of F transfer region products: the pilus assembly gene product TraW and a new product, TraL. J Bacteriol. 1992; 174(17):5567–74. PMID: 1355084

53. Moore D, Maneewannakul K, Maneewannakul S, Wu JH, Ippehl-Ihler K, Bradley DE. Characterization of the F-plasmid conjugative transfer gene traU. J Bacteriol. 1990; 172(8):4263–70. PMID: 2198250

54. Gerdes K, Wagner EG. RNA antitoxins. Curr Opin Microbiol. 2007; 10(2):117–24. PMID: 17376733

55. Miki T, Park JA, Nagao K, Murayama N, Horuchi T. Control of segregation of chromosomal DNA by sex factor F in Escherichia coli. Mutants of DNA gyrase subunit A suppress letD (cdcb) product growth inhibition. J Mol Biol. 1992; 225(1):39–52. PMID: 1316444

56. Van Melderen L, Bernard P, Couturier M. Lon-dependent proteolysis of CcdA is the key control for activation of CcdB in plasmid-free segregant bacteria. Mol Microbiol. 1994; 11(6):1151–7. PMID: 8022894

57. Tsuchimoto S, Nishimura Y, Ohtsubo E. The stable maintenance system pem of plasmid R100: degradation of PemI protein may allow PemK protein to inhibit cell growth. J Bacteriol. 1992; 174(13):4205–11. PMID: 1624414

58. Pullinger GD, Lax AJ. A Salmonella dublin virulence plasmid locus that affects bacterial growth under nutrient-limited conditions. Mol Microbiol. 1992; 6(12):1631–43. PMID: 1495391

59. Moura A, Soares M, Pereira C, Leitao N, Henriques I, Correia A. INTEGRALL: a database and search engine for integrons, integrases and gene cassettes. Bioinformatics. 2009; 25(8):1096–8. doi: 10.1093/bioinformatics/btp105 PMID: 19228805

60. Montesinos I, Rodriguez-Villalobos H, De Mendonca R, Bogaerts A, Deplano A, Glupczynski Y. Molecular characterization of plasmids encoding CTX-M-15 extended-spectrum β-lactamase associated with the ST131 Escherichia coli clone in Belgium. J Antimicrob Chemother. 2010; 65(8):1828–30. doi: 10.1093/jac/dkr041 PMID: 20934625

61. Woodford N, Ward ME, Kaufmann ME, Turton J, Fagan EJ, James D, et al. Community and hospital spread of Escherichia coli producing CTX-M-15 extended-spectrum β-lactamases in the UK. J Antimicrob Chemother. 2004; 54(4):735–43. PMID: 15347638

62. Partridge SR, Zong Z, Iredell JR. Recombination in IS26 and Tn2 in the evolution of multiresistance regions carrying blaCTX-M-15, on conjugative IncF plasmids from Escherichia coli. Antimicrob Agents Chemother. 2011; 55(11):4971–8. doi: 10.1128/AAC.00025-11 PMID: 21859935

63. Dhanji H, Patel R, Wall R, Dournith M, Patel B, Hope R, et al. Variation in the genetic environments of blaCTX-M-15 in Escherichia coli from the faeces of travellers returning to the United Kingdom. J Antimicrob Chemother. 2011; 66(5):1005–12. doi: 10.1093/jac/dkr041 PMID: 21393166

64. Gibreel TM, Dodgson AR, Cheesbrough J, Fox AJ, Bolton FJ, Upton M. Population structure, virulence potential and antibiotic susceptibility of uropathogenic Escherichia coli from Northwest England. J Antimicrob Chemother. 2012; 67(2):346–56. doi: 10.1093/jac/dkr451 PMID: 22028202

65. Jacoby GA. Mechanisms of resistance to quinolones. Clin Infect Dis. 2005; 41 Suppl 2:S120–6. PMID: 15942878

66. Drlica K, Zhao X. DNA gyrase, topoisomerase IV, and the 4-quinolones. Microbiology and molecular biology reviews: MMBR. 1997; 61(3):377–82. PMID: 9293187

67. Lanza VF, de Toro M, Garcia-Bacca MP, Mora A, Blanco J, Coque TM, et al. Plasmid flux in Escherichia coli ST131 sublineages, analyzed by Plasmid Constellation Network (PLACNET), a new method for plasmid reconstruction from whole genome sequences. PLoS Genet. 2014; 10(12):e1004766. doi: 10.1371/journal.pgen.1004766 PMID: 25522143

68. Langridge GC, Phan MD, Turner DJ, Perkins TT, Parts L, Haase J, et al. Simultaneous assay of every Salmonella Typhi gene using one million transposon mutants. Genome Res. 2009; 19(12):2308–16. doi: 10.1101/gr.097097.109 PMID: 19826075
69. Nordstrom K. Plasmid R1—replication and its control. Plasmid. 2006; 55(1):1–26. PMID: 16199086
70. Ohtsubo H, Ryder TB, Maeda Y, Armstrong K, Ohtsubo E. DNA replication of the resistance plasmid R100 and its control. Advances in biophysics. 1986; 21:115–33. PMID: 3019092
71. Austin S, Wierzbiicki A, Two mini-F-encoded proteins are essential for equipartition. Plasmid. 1983; 10(1):73–81. PMID: 6353453
72. Mori H, Kondo A, Ohshima A, Ogura T, Hiraga S. Structure and function of the F plasmid genes essential for partitioning. J Mol Biol. 1986; 192(1):1–15. PMID: 3029390
73. Miki T, Easton AM, Rownd RH. Cloning of replication, incompatibility, and stability functions of R plasmid NR1. J Bacteriol. 1984; 161(1):87–99. PMID: 6243631
74. Tabuchi A, Min YN, Kim CK, Fan YL, Wombol DD, Rownd RH. Genetic organization and nucleotide sequence of the stability locus of IncFII plasmid NR1. J Mol Biol. 1988; 202(3):511–25. PMID: 3172224
75. Vecchiarelli AG, Hwang LC, Mitsuuchi K. Cell-free study of F plasmid partition provides evidence for cargo transport by a diffusion-ratchet mechanism. Proc Natl Acad Sci U S A. 2013; 110(15):E1390–7. doi: 10.1073/pnas.1302745110 PMID: 23479605
76. Hatano T, Yamaichi Y, Niki H. Oscillating focus of SopA associated with filamentous structure guides partitioning of F plasmid. Mol Microbiol. 2007; 64(5):1198–213. PMID: 17542915
77. Maas R, Saadi S, Maas WK. Properties and incompatibility behavior of miniplasmids derived from the bireplicon plasmid pCG86. Mol Gen Genet. 1989; 218(2):190–8. PMID: 2674652
78. Sykora P. Macroevolution of plasmids: a model for plasmid speciation. Journal of theoretical biology. 1992; 159(1):53–65. PMID: 1291811
79. Buts L, Lah J, Dao-Thi MH, Wynn L, Loris R. Toxin-antitoxin modules as bacterial metabolic stress managers. Trends in biochemical sciences. 2005; 30(12):672–9. PMID: 16257530
80. Van Melderin L, Saavedra De Bast M. Bacterial toxin-antitoxin systems: more than selfish entities? PLoS Genet. 2009; 5(3):e1000437. doi: 10.1371/journal.pgen.1000437 PMID: 19325885
81. Amitai S, Yassin Y, Engelberg-Kulka H. MazF-mediated cell death in Escherichia coli: a point of no return. J Bacteriol. 2004; 186(24):8295–300. PMID: 15576778
82. Pedersen K, Christensen SK, Gerdes K. Rapid induction and reversal of a bacteriostatic condition by controlled expression of toxins and antitoxins. Mol Microbiol. 2002; 45(2):501–10. PMID: 12123459
83. Tripathi A, Baru B, Varadarajan R. Additional role for the ccd operon of F-plasmid as a transmissible persistence factor. Proc Natl Acad Sci U S A. 2012; 109(31):12497–502. doi: 10.1073/pnas.1121217109 PMID: 22802647
84. Flett J, Baranik A, Jezdebski R, Sitkiewicz I, Zabicka D, Melier A, et al. The first NDM metallo-β-lactamase-producing Enterobacteriaceae isolate in Poland: evolution of IncFII-type plasmids carrying the blaNDM-1 gene. Antimicrob Agents Chemother. 2014; 58(2):1203–7. doi: 10.1128/AAC.01197-13 PMID: 24247128
85. O’Hara JA, Hu F, Ahn C, Nelson J, Rivera JL, Pasculle AW, et al. Molecular epidemiology of KPC-producing Escherichia coli: occurrence of ST131-fimH30 subtype harboring pKpQIL-like IncFIIk plasmid. Antimicrob Agents Chemother. 2014; 58(7):4234–7. doi: 10.1128/AAC.02182-13 PMID: 24820082
86. Lou Z, Qi Y, Qian X, Yang W, Wei Z. Emergence of Klebsiella pneumoniae carbapenemase-producing Escherichia coli sequence type 131 in Hangzhou, China. Chinese medical journal. 2014; 127(3):528–31. PMID: 24451962