Comparative Genomics of *Escherichia coli* Sequence Type 219 Clones From the Same Patient: Evolution of the IncI1 *bla*<sub>CMY</sub>-Carrying Plasmid *in Vivo*

Cheng-Yen Kao<sup>1†</sup>, Jenn-Wei Chen<sup>2</sup>, Tsung-Lin Liu<sup>3</sup>, Jing-Jou Yan<sup>4*‡</sup> and Jiunn-Jong Wu<sup>1*‡</sup>

This study investigates the evolution of an *Escherichia coli* sequence type 219 clone in a patient with recurrent urinary tract infection, comparing isolate EC974 obtained prior to antibiotic treatment and isolate EC1515 recovered after exposure to several β-lactam antibiotics (ceftriaxone, cefixime, and imipenem). EC974 had a smooth colony morphology, while EC1515 had a rough colony morphology on sheep blood agar. RAPD-PCR analysis suggested that both isolates belonged to the same clone. Antimicrobial susceptibility tests showed that EC1515 was more resistant to piperacillin/tazobactam, cefepime, cefpirome, and ertapenem than EC974.

Comparative genomic analysis was used to investigate the genetic changes of EC974 and EC1515 within the host, and showed three plasmids with replicons IncI1, P0111, and IncFII in both isolates. P0111-type plasmids pEC974-2 and pEC1515-2, contained the antibiotic resistance genes *aadA2*, *tetA*, and *drfA12*. IncFII-type plasmids pEC974-3 and pEC1515-3 contained the antibiotic resistance genes *bla*<sub>TEM</sub>-1, *aadA1*, *aadA22*, *sul3*, and *iniF*. Interestingly, *bla*<sub>CMY-111</sub> and *bla*<sub>CMY-4</sub> were found in very similar IncI1 plasmids that also contained *aadA22* and *aac(3)-IId*, from isolates EC974 (pEC974-1) and EC1515 (pEC1515-1), respectively. The results showed *in vivo* amino acid substitutions converting *bla*<sub>CMY-111</sub> to *bla*<sub>CMY-4</sub> (R221W and A238V substitutions). Conjugation experiments showed a high frequency of IncI1 and IncFII plasmid co-transference. Transconjugants and DH5α cells harboring *bla*<sub>CMY-4</sub> or *bla*<sub>CMY-111</sub> showed higher levels of resistance to ampicillin, amoxicillin, cefazolin, cefuroxime, cefotaxime, cefixime, and ceftazidine, but not piperacillin/tazobactam, cefpime, or ertapenem. All known genes (outer membrane proteins and extended-spectrum AmpC β-lactamases) involved in ETP resistance in *E. coli* were identical between EC974 and EC1515. This is the first study to identify the evolution of an IncI1 plasmid within the host, and to characterize *bla*<sub>CMY-111</sub> in *E. coli*.

**Keywords:** comparative genomics, *bla*<sub>CMY</sub>, conjugative plasmid, evolution, ertapenem

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<sup>1</sup> Department of Biotechnology and Laboratory Science in Medicine, School of Biomedical Science and Engineering, National Yang Ming University, Taipei, Taiwan, <sup>2</sup> Department of Microbiology and Immunology, College of Medicine, National Cheng Kung University, Tainan, Taiwan, <sup>3</sup> Department of Biotechnology and Bioindustry Sciences, National Cheng Kung University, Tainan, Taiwan, <sup>4</sup> Department of Pathology, Cheng Ching Hospital at Chung Kang, Taichung, Taiwan

<sup>†</sup> Present Address: Cheng-Yen Kao, Department of Medical Microbiology and Immunology, School of Medicine and Public Health, University of Wisconsin-Madison, Madison, WI, United States

<sup>‡</sup> These authors have contributed equally to this work.

<sup>*</sup>Correspondence: Jing-Jou Yan jing3767@gmail.com, Jiunn-Jong Wu jwu1019@ym.edu.tw

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INTRODUCTION

Enterobacteriaceae manifest resistance to third-generation cephalosporins by the production of extended-spectrum β-lactamases (ESBLs), chromosomal AmpC (cAmpCs), or plasmid-mediated AmpCs (pAmpCs) (Jacoby, 2009). However, AmpCs do not hydrolyze fourth-generation cephalosporins, such as ceftazidime (FEP) (Jacoby, 2009). Therefore, FEP is suggested for the treatment of infections caused by AmpC producers. Plasmid-mediated extended-spectrum AmpC β-lactamases (pESACs) with mutations in AmpC that enhance catalytic activity toward ceftazidime, derived from the most frequently detected pAmpC, blacMY−2, have been identified (Nordmann and Mammeri, 2007; Doi et al., 2009; Jacoby, 2009). Doi et al. showed a reduced susceptibility to FEP among Escherichia coli clinical isolates producing novel variants of blacMY−2, blacMY−33, and blacMY−44 (Doi et al., 2009). However, for these pESACs, structural information regarding their hydrolytic performance on different β-lactam antibiotics is yet to be characterized.

Among the β-lactams currently available, carbapenems are unique because they are relatively resistant to hydrolysis by most β-lactamases (Barry et al., 1985). Carbapenemases have thus been considered as the last resort of drugs for treating infections caused by multi-drug resistant Gram-negative bacilli. Carbapenem resistance in Enterobacteriaceae can arise by several mechanisms, including mutations that alter the expression and/or function of porins, or overexpression of active efflux pumps, while the greatest concern has been placed on acquired transferable carbapenemases (Temkin et al., 2014). blaOXA-type (blaOXA−48 and blaOXA−181) or blaNDM are the dominant carbapenemases found in E. coli (Nordmann et al., 2011; Temkin et al., 2014; Al-Agamy et al., 2017). Overproduction of class C β-lactamases, such as blacMY−2, or ESBL, can also lead to carbapenem resistance in E. coli, when combined with impermeability caused by porin (OmpC or OmpF) loss (Ma et al., 2013). Moreover, Dupont et al. reported that a Gly-63-Val substitution in OmpR, which codes for a regulatory protein involved in the control of OmpC/OmpF porin expression, caused the carbapenem resistance in E. coli (Dupont et al., 2017).

During evolution, the ability of bacteria to adapt to various hosts and environments has been favored by the acquisition of DNA elements through horizontal gene transfer or spontaneous mutations (Frost et al., 2005; Ilyas et al., 2017). The accumulation of antibiotic resistance, loss of nonessential genes, metabolic alterations, and virulence factor attenuation all occur when pathogenic organisms adapt to the host (Viberg et al., 2017). Antibiotic exposures also facilitate the genomic evolution of antibiotic resistance-associated genes (Pires et al., 2015; Lin et al., 2016). Here, we report a clinical case that illustrates the dynamic nature of an IncI1 plasmid within the host, as blacMY−111 was changed to blacMY−4 in E. coli.

MATERIALS AND METHODS

Bacterial Strains and Culture Conditions

The bacterial strains and plasmids used in this study are described in Table 1. On day 1, an E. coli isolate (EC974) isolated from a 76-year-old woman with urinary tract infection (UTI) showed intermediate resistance to piperacillin/tazobactam (TZP) but was susceptible to ceftazidime (CZA), ceftazidime (FEP), and ertapenem (ETP). Once admitted, she was empirically started on ceftriaxone (CRO). After a week, the therapy was switched to cefoxime (CFM) and continued for 4 days. One hundred and six days later, the woman was hospitalized due to recurrent UTI and was treated with imipenem (IMP). Two days later, an E. coli strain (EC1515) showing resistance to TZP, CPO, FEP, and ETP was isolated. E. coli was grown on Luria-Bertani (LB) agar or in broth. Bacteria harboring antibiotic resistance determinants were grown in the presence of the appropriate antibiotics at the following concentrations: ampicillin (AMP, 100 µg/mL); rifampicin (RIF, 256 µg/mL). All strains were stored at −80 °C in LB broth containing 20% glycerol until testing.

Antibiotic Susceptibility Testing

Antibiotic susceptibility of the strains was determined by the disk diffusion method on Mueller-Hinton (MH) agar, based on the CLSI guidelines (Clinical and Laboratory Standards Institute, 2009). Susceptibility to AMP, amoxicillin (AMC), amikacin (AN), chloramphenicol (CAP), ceftazidime (CZA), CFM, ciprofloxacin (CIP), cefmetazole (CMZ), cefpodoxime (CPD), CPO, CRO, cefuroxime (CXM), ceftazolin (CZ), ETP, FEP, gentamycin (GN), IPM, lomefloxacin (LOM), levofloxacin (LVX), meropenem (MEM), tobramycin (NN), ceftriaxone (CRO), cefixime (CFM) and continued for 4 days. One hundred and six days later, the woman was hospitalized due to recurrent UTI and was treated with imipenem (IMP). Two days later, an E. coli strain (EC1515) showing resistance to TZP, CPO, FEP, and ETP was isolated. E. coli was grown on Luria-Bertani (LB) agar or in broth. Bacteria harboring antibiotic resistance determinants were grown in the presence of the appropriate antibiotics at the following concentrations: ampicillin (AMP, 100 µg/mL); rifampicin (RIF, 256 µg/mL). All strains were stored at −80 °C in LB broth containing 20% glycerol until testing.

Genome Sequencing, Assembly, Annotation, and Analysis

Genomic DNA for E. coli EC974 and EC1515 was prepared using the Qiagen DNeasy Blood and Tissue kit (California, USA), according to the manufacturer’s instructions. The genomes of EC974 and EC1515 were sequenced using the PacBio RS II platform (Pacific Biosciences, USA). HGAP3 in the SMRT analysis (v2.3) was used to assemble the PacBio data with default parameters, except the genome size was set as 5.5 Mb (Kao et al., 2017). HGAP3 analysis gave seven contigs for both strains. Contigs with a low coverage (< 20% of the mean) and/or shorter than 20 kb were discarded. This resulted in five and four contigs for the strains EC974 and EC1515, respectively. The remaining shortest contig of the strain EC974 was found highly similar to segments of another larger contig and there was a sudden drop in read coverage, so that contig was discarded. We therefore reported one chromosome and three plasmids for both strains.

For each contig, we aligned the first and last 20,000 bp using BLAT (v35, option: minMatch = 1) (Kent, 2002). Nearly perfect matches of lengths 4.6–16.6 kb were found for all contigs, suggesting their circular nature. We noted that the first 520 bp of the second largest contig of strain EC974 did not align well to the tail of the contig. The perfect match of ~4 kb which followed
suggested false assembly at the contig start. Therefore, the first 520 bp of the contig was trimmed. A similar observation was made for the second largest contig of strain EC1515, and the first 554 bp was trimmed.

To circularize the contigs, we trimmed redundant segments at both contig ends. Specifically, the mid-point of the nearly perfect match was determined and bases from either end to the mid-point were trimmed. In addition, we adjusted contig orientation and starting position to match those of a reference sequence. For each contig, a closest reference was found via alignment against the NCBI NR database using BLAST. For each sequence, the first base of that plasmid was not detected in our database (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch&BLAST_SPEC=MicrobialGenomes). The multilocus sequence typing (MLST), ResFinder, and PlasmidFinder databases (http://www.genomicepidemiology.org/) were used to find the sequence type (ST), serotype, antibiotic resistance genes, and the plasmid types present in the genomes. For each contig, a closest reference was found via alignment against the NCBI NR database using BLAST. 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AMP (100 µg/mL) were used to select for the \( \text{bla}_{\text{CMY}-4} \) or \( \text{bla}_{\text{CMY}-111} \) producers.

**RESULTS**

**Antibiotic Susceptibility and Clonal Relationship of EC974 and EC1515**

After CRO, CFM, and IMP treatments, EC1515 was isolated from a patient with recurrent UTI infection. Compared to EC974 from the same patient, EC1515 was more resistant to TZP, FEP, CPO, and ETP, than EC974 (Table 2), and had a rough colony morphology (Figure S1). To investigate whether the patient was recurrently infected by the same \( E. \ coli \) clone, the clonal relationships between the two isolates were analyzed by RAPD-PCR with primer 1254 (Pacheco et al., 1998). The results showed highly polymorphic profiles, distinguishing individual \( E. \ coli \) isolates (Figure S2). EC974 and EC1515 showing identical RAPD-PCR profiles were considered to be the same clone (Figure S2).

**Complete Genome Sequences of EC974 and EC1515 and Their Characteristics**

For the two \( E. \ coli \) strains EC974 and EC1515, PacBio sequencing generated 106,134 and 106,265 polymerase reads, and the total numbers of bases were 1,475,613,460 and 1,581,950,763, respectively. The N50 lengths of the 159,569 and 177,682 subreads were 12,365 and 12,090 bp, respectively. For each strain, the data was assembled using HGAP3 and the resulting contigs were circularized. Mean read coverage across the genome were 147X and 198X, respectively for strains EC974 and EC1515.

We reported one chromosome and three plasmids for both strains and their lengths were shown in Table 3.

### TABLE 2 | Antibiotic susceptibility of \( E. \ coli \) isolates EC974 and EC1515.

| Isolate | Date  | Antibiotic susceptibility |
|---------|-------|--------------------------|
|         | AMP  | AMC | TZP | CZ  | CMX | CMZ | CAZ | CRO | CPD | CFM | FEP | CPO | IPM | ETP | MEM | LVX | CIP | LOM | GN | NN | AN | TE | CAP | SXT |
| EC974   | 960824 | R   | R   | I   | R   | R   | R   | R   | S   | S   | S   | S   | S   | I   | R   | S   | S   | S   | R   | R   | R   | R   |
| EC1515  | 961210 | R   | R   | R   | R   | R   | R   | R   | R   | R   | SDD | R   | S   | R   | S   | S   | I   | R   | S   | S   | S   | R   | R   | R   |

Antibiotic susceptibility tests were performed in duplicate. AMP, ampicillin; AMC, amoxicillin; TZP, piperacillin/tazobactam; CZ, cefazolin; CMX, cefuroxime; CMZ, cefmetazole; CAZ, ceftazidime; CRO, ceftriaxone; CPD, cefpodoxime; CFM, cefixime; FEP, cefepime; CPO, ceftizoxime; IPM, imipenem; ETP, ertapenem; MEM, meropenem; LVX, levofloxacin; CIP, ciprofloxacin; LOM, lomefloxacin; GN, gentamycin; NN, tobramycin; AN, amikacin; TE, tetracycline; CAP, chloramphenicol; SXT, cotrimoxazole. S, sensitive; SDD, susceptible-dose dependent; I, intermediate resistance; R, resistant.

### TABLE 3 | Characteristics of chromosomes and plasmids in isolates EC974 and EC1515.

**Characteristics**

**CHROMOSOMES**

| Characteristics | Size (bp) | GC contents (%) | ORF (n) | RNA (n) | Antimicrobial resistance genes |
|-----------------|----------|-----------------|--------|--------|-------------------------------|
| EC974           | 5,168,339 | 50.24           | 4906   | 112    | \( \text{bla}_{\text{TEM}-1}, \text{cmlA1}, \text{sul}3, \text{tetM}, \text{mefB}, \text{aadA1} \) |
| EC1515          | 5,167,364 | 50.24           | 4911   | 112    | \( \text{bla}_{\text{TEM}-1}, \text{cmlA1}, \text{sul}3, \text{tetM}, \text{mefB}, \text{aadA1} \) |

**PLASMIDS**

| Characteristics | Size (bp) | Replicon | ORF | Conjugative related genes | High similarity plasmid (gene bank accession) | Antimicrobial resistance genes |
|-----------------|----------|----------|-----|--------------------------|---------------------------------------------|-----------------------------|
| EC974           | pEC974-1 | IncI1    | 123 | +                        | Escherichia coli strain TVGHEC01 plasmid (LC019731) | \( \text{aad}22, \text{aac(3)-IId}, \text{bla}_{\text{CMY}-111} \) |
|                 | pEC974-2 | P0111    | 105 | -                        | Escherichia coli strain EHS30-1, plasmid pEHS30-1 (KX772391) | \( \text{aad}2, \text{tetA}, \text{dfrA}12 \) |
|                 | pEC974-3 | IncFII   | 94  | +                        | Escherichia coli strain V423 plasmid pV423-b (LC056646) | \( \text{bla}_{\text{TEM}-1}, \text{aadA}1, \text{aadA}22, \text{su}3, \text{inuF} \) |
| EC1515          | pEC1515-1 | IncI1    | 123 | +                        | Escherichia coli strain TVGHEC01 plasmid (LC019731) | \( \text{aad}22, \text{aac(3)-IId}, \text{bla}_{\text{CMY}-4} \) |
|                 | pEC1515-2 | P0111    | 102 | -                        | Escherichia coli strain EHS30-1, plasmid pEHS30-1 (KX772391) | \( \text{aad}2, \text{tetA}, \text{dfrA}12 \) |
|                 | pEC1515-3 | IncFII   | 92  | +                        | Escherichia coli strain V423 plasmid pV423-b (LC056646) | \( \text{bla}_{\text{TEM}-1}, \text{aadA}1, \text{aadA}22, \text{su}3, \text{inuF} \) |
showed that the EC974 and EC1515 genomes had a size of 5,168,339 and 5,167,364 bp, respectively (Table 3). The GC contents of the two strains were both 50.24% (Table 3). RAST annotation results showed that the EC974 genome contained 4,906 coding sequences and 112 RNA genes, and the EC1515 genome contained 4,911 coding sequences and 112 RNA genes (Table 3). Moreover, 61% of the protein coding sequences were functionally annotated by the RAST server into 610 and 609 subsystems in EC974 and EC1515 strains, respectively.

Serotype was determined by the genotype of fliC, wzc, and wzy, and the results showed that both strains belonged to the O51-H34 serotype. Antimicrobial resistance genes against β-lactams (blaTEM-1), penicillin (cmiA1), sulphonamide (sul3), tetracycline (tetM), macrolide (mefB) and aminoglycoside (aadA1) were identified on both chromosome sequences (Table 3). The MLST scheme of both isolates based on the sequences of seven housekeeping genes (gyrB53, recA42, mdh24, purA1, icd58, adk58, fumC53) confirmed that both isolates belonged to the ST219 type. Microbial genome BLAST results showed that the genome sequence of EC974 was highly similar to EC1515 (coverage 100%, identity 99%), DSM 103246 (coverage 99%, identity 99%, ST553), Santai (coverage 99%, identity 99%, ST773), RM12579 (coverage 99%, identity 99%, ST553), 2013C-92%, identity 99%, ST773), Santai (coverage 99%, identity 99%, ST773), RM12579 (coverage 99%, identity 99%, ST553), and CB9615 (coverage 99%, identity 99%, ST553), among 1,593 E. coli genomes in the database.

We further compared the genomes of the two strains using MUMMER and showed the 1-to-1 alignments allowing rearrangements in Table 4. The results revealed that only two large-scale rearrangements on the chromosome: one 973 bp deletion (Chromosome: 941793-942765 of strain EC974) and one 1,881 bp inversion (Chromosome: 3045861-3047678 of strain EC974) on the chromosome. For the largest plasmid, we observed one 532 bp insertion (pEC1515-1: 17188-17719 of strain EC1515) and one 326 bp deletion (pEC974-1: 17974-17973 of strain EC974). No large-scale rearrangement was observed on the other two plasmids. The shortest plasmids of the two strains were perfectly identical. MUMMER also revealed 12 SNPs and 35 small INDELS. Of the 12 SNPs, 9 and 3 resided in the chromosome and plasmid 1, respectively (Table 5 and Table S1). Among the 12 SNPs, eight were missense mutations and the rest were either intergenic (three) or synonymous (one) (Table 5). A majority of small INDELS were likely false due to the error-prone nature of PacBio data. Consistently, we found that many small INDELS occurred at loci of homopolymers and the assembly qualities were low. Of the 35 small INDELS, only 8 were of a high quality (quality ≥ 30) (Table 5). Among the 8 high quality small INDELS, four resulted in a frame shift while the rest resided in intergenic regions (Table 5). Taken together, these results showed the evolutionary relationships between EC974 and EC1515.

According to our annotation, the large deleted segments on chromosome contained two mobile element genes. The left boundary of the inversion on chromosome does not break any gene, but the right one broke a gene coding for phage tail fiber protein. The inverted segments contained two genes for phage tail fiber assembly. On the largest plasmid, the insertion did not break any gene and no gene was annotated in the inserted segment. The deletion broke a gene coding for InclI plasmid conjugative transfer pilus-tip adhesin protein, PilIV.

We next examined the genes associated with repair systems in EC974 and EC1515 to determine whether EC1515 was a hypermutator. The results showed that the base excision repair system (uracil-DNA glycosylase, endonuclease III), nucleotide excision repair system (UvrABC), mismatch repair (MutS/LH), and repair related genes (recA, recF, radA, and radC) were identical in EC974 and EC1515. However, RecC, an ATP-dependent DNA helicase involved in DNA repair, showed an amino acid substitution in EC1515 (Table 5).

The numbers of plasmid in isolates EC974 and EC1515 were verified by Kado-Liu’s method (Figure S3). The results showed three plasmids with replicons IncI1, P0111, and IncFII were found in both isolates (Table 3). Moreover, a high similarity of plasmid sequences between EC974 and EC1515 was observed (Table 4). These results excluded the possibility of lateral acquisition of plasmids and indicated the evolution of plasmids within the host. Conjugation-related genes were identified in IncI1- and IncFII plasmids. P0111-type plasmids pEC974-2 and pEC1515-2 contained the antibiotic resistance genes aadA2, tetA, and drfA12, which confer resistance to aminoglycosides, tetracycline, and trimethoprim, respectively (Table 3). IncFII-type plasmids pEC974-3 and pEC1515-3 contained antibiotic resistance genes blatem-1, aadA1, aadA22, sul3, and inaF. Interestingly, aadA22, acc(3)-IId, and blaCMY–111 were found in IncI1-plasmid pEC974-1, and aadA22, acc(3)-IId, and blaCMY–4 were identified in pEC1515-1 (Table 3). blaCMY–4 was different from blaCMY–111 by W221R and V238A substitutions. Both blaCMY genes were located downstream of an ISectI family, IS1380, an element commonly associated with blaCMY genes (Seifert et al., 2013).

**Resistant Gene Transfer and Plasmid Analysis**

blaCMY–111 was first identified in *Serratia marcescens* strain A4Y201 (Boyd et al., 2015); however, the function of blaCMY–111 in conferring antibiotic resistance is still unclear. Transfer of antibiotic resistance phenotypes in pEC974-1 and pEC1515-1 was determined by conjugation tests, and the plasmid sizes
present in parental isolates and transconjugants were verified by Kado and Liu’s methods (Figure S3). We randomly selected 10 transconjugants derived from EC974 and EC1515, and the results revealed that all transconjugants contained two plasmids, in IncI1- and IncFII groups (Figure S3). Transconjugants harboring bla\textsuperscript{CMY-4} (EC1515-TG) or bla\textsuperscript{CMY-111} (EC974-TG) showed higher level resistance to AMP, AMC, CZ, CXM, CTX, CFM, and CAZ, but not TZP, FEP, or ETP (Table 6). We further cloned bla\textsuperscript{CMY-4} and bla\textsuperscript{CMY-111} genes into pACYC177 to verify their resistance phenotypes. Compared to the DH5\textalpha{} cells, DH5\textalpha{}-bla\textsuperscript{CMY-4} and DH5\textalpha{}-bla\textsuperscript{CMY-111} were highly resistant to AMP, AMC, CZ, CXM, CTX, CFM, and CAZ (Table 6). These results indicated that bla\textsuperscript{CMY-4} and bla\textsuperscript{CMY-111} showed similar antibiotic resistance characteristics.

**Mechanisms Associated With Ertapenem Resistance in EC1515**

To investigate the mechanisms contributing to ETP resistance in EC1515, the nucleotide sequences and protein levels of OmpA, OmpC, OmpF, and OmpR were analyzed. The western blot results showed that the expression of OmpA, OmpC, and OmpF was similar between EC974 and EC1515 (data not shown), and the results were similar to our previous report (Yan et al., 2010). Sequences of ompA, ompC, ompF, and ompR were identical between EC974 and EC1515. Moreover, the promoter region and sequence of the chromosome-borne, extended-spectrum AmpC (ESAC) \(\beta\)-lactamase were identical between EC974 and EC1515.

**DISCUSSION**

This study characterized genome-wide and phenotypic changes that have occurred in the \(E.\ coli\) ST219 clone after nearly 4 months of chronic carriage and antibiotic treatments. Recurrent UTI is one of the most common bacterial infections in older women and children and poses a major clinical challenge worldwide (Guglietta, 2017). Tapiainen et al. showed that recurrent infection isolates were found to form biofilms effectively (Tapiainen et al., 2014). Moreover, the adhesiveness and invasiveness on
the cells were significantly higher for K. pneumoniae recurrent strains than for initial colonized strains (Lin et al., 2014). Here, we showed that the patient with a recurrent UTI was infected by the same ST219 clone, retrievable as isolates EC974 and EC1515. Moreover, EC974 and EC1515 displayed different colony morphologies, with a rougher colony morphology of EC974 compared to EC1515 (Figure S1). These results indicate evolution of EC974 in vivo, with phenotype changes, during adaptation to the host and exposure to antibiotics.

One of the strategies that bacteria adopt to survive stress conditions is a change in morphology. Such changes were observed in bacteria on exposure to toxic organic compounds in conditions is a change in morphology. Such changes were involved in cell aggregation, biofilm, and colony morphology adaptation by selecting for advantageous mutations, consistent with the results shown in Figure S3. It is possible that there are slightly different versions of that plasmid, resulting in ambiguous assemblies at those small INDEL loci. We also found that the inserted segment on the largest plasmid of strain EC1515 was highly similar to a contig segment of EC974, but that contig was retrieved as isolates EC974 and EC1515, compared with EC974, that arose during a 4-month treatment regimen. Here, we revealed 12 SNPs and 8 INDELs (quality ≥ 30) in EC1515, compared with EC974, that arose during a 4-month persistent infection within host (Table 5 and Table S1). The mutation rate in EC1515 was higher, when compared with a previous report (1.1 per genome/year) (Reeves et al., 2011). Our results suggest that antibiotic exposure not only imposes a selective challenge to bacterial cells but also accelerates the rate of adaptation by selecting for advantageous mutations, consistent with a previous report (Long et al., 2016). Moreover, our comparative sequence results revealed a SNP of the RecQ protein (repair-related protein) in EC1515 (Table 5). Yang et al. reported that the mutation of RecQ resulted in a mutator phenotype in E. coli cells (Yang et al., 2004). However, the mutation rate in EC974 and EC1515 remains to be determined.

| Strains | Antibiotic susceptibility (diameter, mm) |
|---------|----------------------------------------|
|         | AMP | AMC | Tzp | Cz  | Cxm | CaZ | Cfm | Ctx | Fep | IpM | ETP | MEM | Lvx | Cip | Gn  | An  | Sxt |
| EC974   | R (6) | R (7) | I (18) | R (6) | R (6) | R (6) | R (6) | S (25) | S (25) | S (23) | S (20) | S (18) | I (17) | S (25) | S (24) | R (6) |
| EC1515  | R (6) | R (6) | R (10) | R (6) | R (6) | R (6) | R (6) | SDD (19) | S (26) | R (17) | S (29) | S (18) | I (17) | S (26) | S (26) | R (6) |
| C600    | S (16) | S (23) | S (35) | S (31) | S (30) | S (36) | S (27) | S (42) | S (42) | S (36) | S (40) | S (42) | S (32) | S (35) | S (25) | S (32) |
| EC974-TCG | R (6) | R (7) | S (22) | R (6) | R (6) | R (14) | R (6) | R (17) | S (35) | S (32) | S (31) | S (29) | S (33) | S (36) | S (27) | S (28) | S (28) |
| EC1515-TCG | R (6) | R (7) | S (26) | R (6) | R (6) | R (10) | R (6) | R (12) | S (35) | S (35) | S (34) | S (30) | S (37) | S (28) | S (29) | S (29) |
| DH5α   | S (20) | S (26) | S (36) | S (31) | S (30) | S (35) | S (30) | S (42) | S (40) | S (36) | S (45) | S (39) | S (32) | S (34) | S (27) | S (28) | S (37) |
| DH5α-pACYC177 | R (6) | R (7) | S (30) | S (29) | S (33) | S (40) | S (34) | S (48) | S (41) | S (43) | S (40) | S (44) | S (37) | S (40) | S (33) | S (35) |
| DH5α-blacMYR111 | R (6) | R (6) | S (26) | R (6) | R (6) | R (8) | R (6) | R (12) | S (35) | S (29) | S (29) | S (32) | S (31) | S (33) | S (27) | S (29) | S (35) |
| DH5α-blacMYR4 | R (6) | R (6) | S (23) | R (6) | R (6) | R (6) | R (6) | R (10) | S (35) | S (30) | S (35) | S (34) | S (30) | S (33) | S (26) | S (28) | S (35) |

Antibiotic susceptibility tests were performed in duplicate.
AMP, Ampicillin; AMC, Amoxicillin; Tzp, Piperacillin/Tazobactam; Cz, Cefazolin; Cxm, Cefuroxime; CaZ, Cefazidime; Cfm, Cefoxime; Fep, Cefepime; IpM, Imipenem; ETP,厄他培南; MEM, Meropenem; Lvx, Levofloxacin; CIP, Ciprofloxacin; GN, Gentamicins; AN, Amikacin; SXT, Cotrimoxazole.
S, sensitive; SDD, susceptible-dose dependent; I, intermediate resistance; R, resistant.
ETP has become an important option for the treatment of E. coli infections. ETP-resistant E. coli strains may appear susceptible to IMP and MEP in routine susceptibility tests (Yan et al., 2010). Combinations of mutations in envZ, ftsI, mrdA, acrB, and acrR can cause high-level carbapenem resistance, independent of reduced ompCF expression in E. coli (Adler et al., 2016). However, all known genes involved in ETP resistance in E. coli were identical between EC974 and EC1515. We identified SNPs in two regulators (putative transcriptional repressor and BaeR) in EC1515 (Table 5). However, whether the SNPs in EC1515 regulators lead to differential gene expression between EC974 and EC1515 is unclear. The development of next generation sequencing technologies, whole genome sequence and RNA-seq, has provided a novel means to study host-pathogen interaction and bacterial evolution within-host. Therefore, whether the gene expression patterns in EC1515 are different from that in EC974 and thus lead to ETP resistance between EC974 and EC1515 is unclear. The development of next generation sequencing technologies, whole genome sequence and RNA-seq, has provided a novel means to study host-pathogen interaction and bacterial evolution within-host. Therefore, whether the gene expression patterns in EC1515 are different from that in EC974 and thus lead to ETP resistance remain to be clarified by RNA-seq. Moreover, the results raised the possibility that ETP resistance may be caused by a combination of several mutations. In summary, we first identified an IncI1-plasmid carrying blaCMY−111 in E. coli and observed its evolution to blaCMY−4 in vivo.

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AUTHOR CONTRIBUTIONS

C-YK, J-WC, and T-LL designed the study, performed analyses and wrote the manuscript. C-YK and J-WC performed bioinformatics analyses. J-JY and J-JW participated in, coordinated, and supervised the study. All authors approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb. 2018.01518/full#supplementary-material

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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