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## Case report

# An Unusual Carbapenem Resistant *Escherichia coli* Carrying Plasmid-mediated AmpC and Mutated *ompC* in A Patient with Recurrent Urinary Tract Infections



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## ABSTRACT

We describe a case of carbapenem resistant *E. coli* isolated from urine in an 87-year-old woman with recurrent urinary tract infections. Using whole genome sequencing (WGS), we identified the carbapenem resistance mechanism to be a combination of *ompC* porin loss and plasmid-mediated AmpC gene *bla*<sub>CMY-2</sub>, which was not detected by routine molecular and phenotypic carbapenemase assays. Our case raises a concern for the limitation of current CRE screening tools for emerging resistance mechanisms and demonstrates the utility of WGS as a better tool for characterization of CRE in the clinical setting.

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## Introduction

Carbapenem resistant *Enterobacteriales* (CRE) is a global concern in both the hospital and community setting. Patients with CRE infections are three times more likely to die compared to patients with non-CRE infections [1], with crude mortality estimated to be up to 70% [2]. Rapid and accurate identification of CRE is critical for infection prevention as well as guiding appropriate treatment. Once a patient has a confirmed CRE, additional precautions, including patient isolation, screening other at-risk patients, and tracking the spread of the CRE are warranted [2].

There are multiple resistance mechanisms for CRE including carbapenemase or  $\beta$ -lactamase with residual carbapenemase activity combined with concurrent porin deficiency [3,4]. The AmpC gene is mainly encoded in the chromosomal DNA of the many enteric bacteria including *Serratia*, *Citrobacter*, and *Enterobacter*, and its expression requires induction [5,6]. However, AmpC has increasingly been found constitutively expressed on plasmids in *Klebsiella pneumoniae* and *Escherichia coli* [5],

contributing to carbapenem resistance [7,8]. While modified Hodge test (MHT), modified carbapenem inactivation method (mCIM) [9], and PCR targeting specific carbapenemase genes are commonly used to characterize carbapenem resistance mechanisms [10], there are currently no commercially available tests for organisms with resistance due to plasmid-mediated AmpC (pAmpC). In the U.S., pAmpC is predominately found in *K. pneumoniae* and *E. coli*. One study found pAmpC-producing *K. pneumoniae* in 42% of hospitals surveyed between 1996 and 2000 [11]. Another study found that 17.85% of cefoxitin-nonsusceptible [12] and 8.5% of oxyimino- $\beta$ -lactam resistant *K. pneumoniae* [13] were AmpC producers. One children's hospital in Washington State noted an increase in the incidence of pAmpC-producing strains of *Enterobacteriales* between 1999 and 2007, with 39.2% of broad-spectrum  $\beta$ -lactam resistant *E. coli* isolates producing AmpC [14]. However, limited larger-scale surveillance data is available regarding pAmpC-producing *E. coli* in the U.S. Here we describe a case of carbapenem resistant *E. coli* isolated from urine where whole genome sequencing (WGS) identified a pAmpC.

## Case Report

An 87-year-old woman presented to the emergency department with a complicated medical history including dementia, chronic constipation, paroxysmal atrial fibrillation, hypertension, persistent atrial flutter, recurrent urinary tract infections, and urinary incontinence resulting in intermittent catheterization. Her

**Abbreviations:** AMR, Antimicrobial resistance; CRE, Carbapenem resistant *Enterobacteriales*; mCIM, Modified carbapenem inactivation method; MHT, Modified Hodge test; Omp, Outer membrane porin; pAmpC, Plasmid-mediated AmpC; WGS, Whole genome sequencing.

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**Table 1**

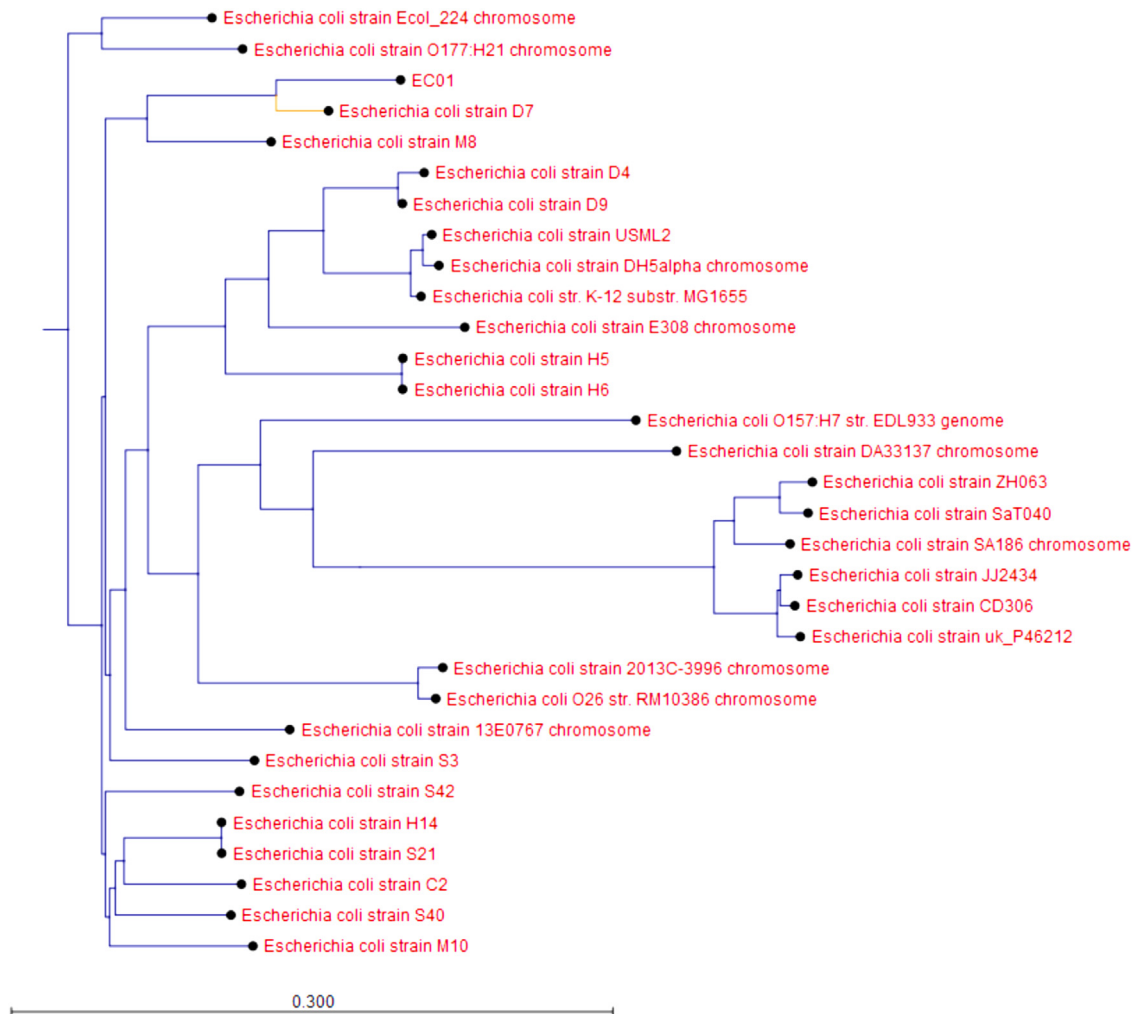
Antimicrobial Susceptibility Profile of EC01. Broth microdilution was used to determine susceptibility of EC01 to the antibiotics. The interpretive criteria were based on CLSI 2019 breakpoints. Cefazolin was used to predict susceptibility to oral cephalosporins.

Antimicrobial Agent	EC01	
	MIC (mcg/mL)	Interpretation
Amikacin	<= 4	S
Ampicillin	>256	R
Cefepime	16	R
Ceftazidime	>32	R
Ceftazidime/Avibactam	4	S
Ceftolozane/Tazobactam	>32	R
Ceftriaxone	>64	R
Ciprofloxacin	>4	R
Ertapenem	>4	R
Gentamicin	<= 1	S
Imipenem	>16	R
Levofloxacin	>8	R
Meropenem	8	R
Minocycline	1	S
Nitrofurantoin	<= 16	S
Oral Cephalosporins		R
Piperacillin + Tazobactam	>128	R
Tobramycin	<= 1	S
Trimethoprim/ Sulfamethoxazole	<= 1/20	S

current complaint was worsening abdominal pain and nausea. Upon examination, she was found to have cecal volvulus and underwent emergent exploratory laparotomy and large bowel resection. Her urine culture was positive for *Pseudomonas aeruginosa* and *E. coli* on hospital day 17. The patient was treated with nitrofurantoin and was discharged on hospital day 21 with improvement of symptoms.

The susceptibility of the *E. coli* isolate (designated EC01) was performed by broth microdilution method according to the Clinical and Laboratory Standards Institute (CLSI) reference using panels prepared in house. EC01 showed resistance to imipenem, ertapenem, and meropenem (Table 1). However, Xpert Carba-R (Cepheid, Sunnyvale, CA) did not detect IMP, VIM, NDM, KPC, or OXA-48 carbapenemase genes. Moreover, carbapenemase activity was not detected by routine phenotypic carbapenemase assays MHT or mCIM, suggesting alternative carbapenem resistance mechanisms.

WGS was performed using MiSeq (Illumina, San Diego, CA). Plasmid replicons, closely related strains, and antimicrobial resistance (AMR) genes were identified using Center for Genomic Epidemiology (CGE) tools (<http://www.genomicepidemiology.org/>). A Kmer tree was constructed using CLC Genomics Workbench (Qiagen, Valencia, CA, USA). Geneious (Biomatters, New Zealand) was used to perform sequence alignment analysis.



**Fig. 1.** EC01 is most closely related to *E. coli* strain D7. A Kmer tree was constructed using the most closely related strains as well as a large sampling of *E. coli* strains from around the world and derived from different sources.

WGS analysis revealed that EC01 harbored multiple chromosomal point mutations (parC p.S80I, parE p.S458A, gyrA p.S83L, gyrA p.D87N) conferring resistance to fluoroquinolones. EC01 contained an average of 2.42 copies of IncI1-type plasmids per bacteria, which carried a pAmpC gene *bla<sub>CMY-2</sub>*. The most closely related strain was *E. coli* strain D7 (NZ\_CP010150.1), with 99.4% pairwise identity and 97.1% coverage (Fig. 1).

To examine changes in the porins, the sequences of EC01 were aligned to wild-type *E. coli* strain K-12, which revealed that *ompC* in EC01 had a 12-bp deletion at position 524, a 12-bp insertion sequence (GGTACCATCGCT) at position 918, and a total of 44 point mutations throughout the gene. However, no point mutations, insertions, or deletions were identified in *ompF*.

## Discussion

WGS was instrumental in characterizing a urinary *E. coli* isolate that was carbapenem resistant, but not detected by traditional carbapenemase assays. The mechanism for resistance was found to be a combination of a  $\beta$ -lactamase and outer membrane porin mutation. The AmpC  $\beta$ -lactamase, *bla<sub>CMY-2</sub>*, was found in EC01, which has been previously implicated in neonatal meningitis [15] and was associated with ceftriaxone resistance [16].

The most closely related strain, *E. coli* strain D7 (NZ\_CP010150.1), was isolated from dog faeces in China. Interestingly, multiple studies have demonstrated shared plasmid-mediated *bla<sub>CMY-2</sub>* between humans and animals. One study found that humans and poultry meat shared *bla<sub>CMY-2</sub>*, suggesting a potential food-borne exposure [17]. Plasmid-mediated *bla<sub>CMY-2</sub>* was also identified in humans and their companion pets, with IncI1 as the most prominent plasmid type [18]. This plasmid was also found in our *E. coli* isolate, however, in our case, it is unclear whether the patient had any animal contact.

Outer membrane porin (Omp) mutations that decrease or obliterate the Omp protein level are often accompanied by AmpC production resulting in enhanced resistance to carbapenems [4,19]. For instance, decreased levels of OmpC and OmpF were associated with AmpC-mediated carbapenem resistant *Enterobacter aerogenes* [20]. We found multiple mutations and deletions in *ompC*. This highly mutated *ompC* is predicted to result in porin loss, which in conjunction with the plasmid-mediated *bla<sub>CMY-2</sub>*, explains the carbapenem resistance in our case. Notably this resistance mechanism (i.e., pAmpC + porin loss) has already been identified in both *K. pneumoniae* and *E. coli* previously [3,21]. However, the current prevalence of carbapenem resistant *E. coli* with this mechanism in the U.S. is still unknown.

Taken together, we identified an unusual carbapenem resistant *E. coli* with plasmid-mediated AmpC and porin loss, which was not detected by routine molecular and phenotypic carbapenemase assays currently used in clinical microbiology laboratories. As pAmpC can exhibit atypical susceptibility patterns and lead to confusion for clinicians, its timely and accurate detection is important for guiding appropriate treatment. In addition, close monitoring of pAmpC-mediated CRE is of great epidemiological value. Our case raises a concern for the limitation of current CRE screening tools and demonstrates the utility of WGS as a better method for characterization of CRE in the clinical setting.

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## CRedit authorship contribution statement

**Paige M.K. Larkin:** Conceptualization, Methodology, Investigation, Formal analysis, Writing – original draft, Writing – review & editing. **Susan Realegeno:** Investigation, Methodology, Writing – review & editing. **Kevin W. Ward:** Investigation, Methodology, Writing – review & editing. **Omai B. Garner:** Writing – review & editing. **Shangxin Yang:** Conceptualization, Methodology, Investigation, Formal analysis, Supervision, Writing – review & editing.

## Declaration of Competing Interest

All authors declared no conflict of interest.

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