Clinical Microbiology and Infectious Diseases

Research Article ISSN: 2398-8096

Genomic differences between carbapenem-susceptible and carbapenem-resistant Escherichia coli analyzed by whole-genome sequencing

Qiong Wu, Jianqiang Wang, Yungai Li, Yunqi Pan and Jin Tang*

Clinical Laboratory Department, Shanghai 6th People's Hospital Affiliated to Shanghai Jiaotong University School of Medicine, China

Abstract

The widespread resistance to carbapenem via *Enterobacteriaceae* is a major public health concern. To investigate the reason for the change of *Escherichia coli* from carbapenem-susceptible to carbapenem-resistant status after antibiotic therapy, whole-genome sequencing was applied for the analysis of genetic difference between the two isolates. The genome of the two isolates of *Escherichia coli* with distinct resistant phenotypes recovered from one patient was sequenced and compared. Single-nucleotide polymorphisms and insertions/deletions were analyzed using software MUMmer (http://mummer.sourceforge.net/). The homology of chromosomes, plasmid 1, and plasmid 2 between the two isolates was very high (>99.5%). However, the coverage ratio of plasmid 3 was only 7.13%. This plasmid harbored the IMP-4 carbapenemase-coding gene. The upstream of *bla*IMP-4 was the plasmid mobilization relaxosome protein Mobe gene and the downstream were class 1 integrom integrase IntI1 and IS6 family transposase IS15DIV, which may be related to carbapenemase gene capture. Single-nucleotide mutation, insertion, and deletion could not result in the development of resistance to carbapenem, whereas acquisition of the IMP-4-encoding gene may be the reason for changes in resistance to carbapenem.

Introduction

The abundance of multidrug-resistant Gram-negative bacteria has increased alarmingly in the last two decades worldwide. In particular, the emergence of carbapenem-resistant *Enterobacteriaceae* (CRE) has become a new challenge in the treatment of infectious diseases [1,2]. In China, the isolation ratio of CRE has increased five-fold from 2005 to 2014 [3]. The mechanism of resistance to carbapenem in *Enterobacteriaceae* is mainly mediated by the production of carbapenemases, which are capable of hydrolyzing the carbapenems, and production of an extended-spectrum β-lactamase (ESBL) and/or AmpC cephalosporinase (AmpC) in conjunction with membrane impermeability or active drug efflux pumps [4-6]. CRE is difficult to control in the clinical setting because of the multiple acquisition pathways. These include the endogenous pathway through antibiotic selective pressure on intestinal microbiota and exogenous pathway through horizontal transmission or through a combination of these factors [5]. Responding to the threat of antimicrobial resistance (AMR), which includes surveillance and stewardship, has been designated as a strategic priority worldwide. For improved monitoring, detection, and screening of AMR, it is important to determine the AMR genes that are prevalent, genes that are moving around, and those that pose the greatest threat. In recent years, whole-genome sequencing (WGS) has been gradually used for the genotype-based diagnosis of AMR [7-9]. WGS has great potential for epidemiological tracking and understanding the development of resistance via experimental evolution. DNA analysis also offers the opportunity to construct databases that record genes of interest, the mobile elements that move these genes, and the cells or species that acquire such genes [10].

In this study, two isolates of *Escherichia coli (E. coli)* were collected from one patient prior to and after antimicrobial therapy; one isolate was susceptible to carbapenem, whereas the other was resistant. We performed WGS to identify the genetic heterogeneity within the isolates that result in change of resistance to carbapenem.

Materials and methods

Patient and bacterial isolates

One isolate of *E. coli* E41-2, which was susceptible to carbapenem, was recovered from sputum. After 10 days of antimicrobial therapy, a carbapenem-resistant *E. coli* E41-1 was also isolated. The isolates were identified by Vitek2-Compact. The minimum inhibitory concentration of the two isolates to antibiotics is listed in Table 1. The crude enzyme extracts of the two isolates were gathered using the repetitive freeze-thawing method. Imipenem hydration activity was determined through the modified Hodge test.

Genome sequencing, gene annotation, and protein classification

The genome of *E. coli* was sequenced using a PacBio RS II platform and Illumina HiSeq X10 system (Illumina, SanDiego, CA, USA) at the Beijing Genomics Institute (Shenzhen, China). Seven databases, namely KEGG (Kyoto Encyclopedia of Genes and Genomes), COG (Clusters

Correspondence to: Jin Tang, Clinical Laboratory Department, Shanghai 6th People's Hospital Affiliated to Shanghai Jiaotong University School of Medicine, Shanghai, China, E-mail: sh6hospital@126.com

Key words: *carbapenem-resistant Enterobacteriaceae, whole-genome sequencing analysis, plasmid-encoded carbapenemase*

Received: April 12, 2021; **Accepted:** May 17, 2021; **Published:** May 25, 2021

of Orthologous Groups), NR (Non-Redundant Protein Database), Swiss-Prot [11], GO (Gene Ontology), TrEMBL, and EggNOG were used for general function annotation. Four databases were used for pathogenicity and drug resistance analysis.

Single-nucleotide polymorphisms (SNP) analysis

Using the alignment software MUMmer (http://mummer.sourceforge.net/), each query sequence was aligned with the reference sequence. The variation sites between the query sequence and reference sequence were identified and filtered preliminarily to detect potential SNP sites. Credible SNP can be obtained by filtering the SNP located in repeat regions.

Insertion/deletion analysis

Using the LASTZ software (http://www.bx.psu.edu/miller_lab/dist/README.lastz-1.02.00/), the reference sequence and query sequence were aligned to obtain the alignment results. The alignment results were verified with BWA (http://bio-bwa.sourceforge.net/) and samtools (http://samtools.sourceforge.net/).

Results

AMR

Both isolates of *E. coli* were susceptible to aminoglycoside. Following antibiotic therapy, one isolate became resistant to imipenem, ertapenem, and cefotetan. The crude enzyme of E41-1 hydrates imipenem, whereas that of E41-2 does not (Figure 1).

AMR determinants

The E41-1 complete genome (accession number: CP028483-CP028486) and E41-2 draft genome (accession number: PZPQ00000000) were submitted to the National Center for Biotechnology Information. The genome sequencing analysis showed that the carbapenem-resistant *E. coli* E41-1 harbored 49 types of AMR determinants. The resistance genes are listed in Table 2. Beta-lactamase gene *bla*CTX-M, *bla*TEM-1, and *bla*EC were associated with resistance to cephalosporin. There were 28 multidrug resistance efflux pump genes identified in the E41-1 genome. These genes encode the cell division transporter system that controls substance transport, including aminoglycoside, tigecycline, fluoroquinolone, beta-lactam, tetracycline, and fosfomycin.

Table 1. Antimicrobial susceptibility patterns of the two isolates of *E. coli*

Antimicrobial agents	*E. coli* E41-2	*E. coli* E41-1
Amikacin	≤ 2	4
Gentamicin	≤ 1	≤ 1
Nitrofurantoin	≤ 16	≤ 16
Tobramycin	≤ 1	2
Piperacillin/tazobactam	≤ 4	8
Ampicillin/sulbactam	≥ 32	≥ 32
Sulfamethoxazole/trimethoprim	≥ 320	≥ 320
Imipenem	≤ 1	≥ 16
Levofloxacin	≥ 8	≥ 8
Ampicillin	≥ 32	≥ 32
Aztreonam	≥ 64	≥ 64
Ceftazidime	≥ 64	≥ 64
Ciprofloxacin	≥ 4	≥ 4
Ceftriaxone	≥ 64	≥ 64
Cefotetan	≤ 4	≥ 64
Ertapenem	≤ 0.5	≥ 8
Cefepime	16	≥ 64

Figure 1. The hydration activity of imipenem was determined using the modified Hodge test

Common SNP in the two isolates

There were 134 point mutations between these two isolates of *E. coli*. However, only 74 points were nonsynonymous mutations (Table 3). Two points of SNP were related to antibiotic resistance; one was the membrane protein E41-1GL001858 and the other was class A ESBL CTX-M-14 (E41-1GL003892). Phenotype comparison showed that these two point mutations did not alter the resistance in the two isolates. The genome chromosome and the three plasmids circos of E41-1 are shown in Figures 2-5.

Insertion/deletion between the two isolates

Compared with E41-2, there were 12 nucleotide changes in E41-1 (nine insertion and three deletion sites). Two deletion sites and one insertion site were on the chromosome, while the other two insertion sites were located on the plasmid (Table 4). In E41-1, there was a large insertion fragment (49,097 bp) in plasmid 3 (GenBank accession no. CP028486.1) compared with E41-2. From the analysis of the coverage ratio of the E41-2 genome compared with E41-1 (Table 5), the homology of chromosome, plasmid 1, and plasmid 2 between the two isolates was very high (>99.5%). However, the coverage ratio of plasmid 3 was only 7.13%. This plasmid harbored the IMP-4 carbapenemase-coding gene and led to a change in resistance to carbapenem (Figure 6). The upstream of *bla*IMP-4 was the plasmid mobilization relaxosome protein Mobe gene and the downstream were class 1 integrom integrase IntI1 and IS6 family transposase IS15DIV, which may be related to carbapenemase gene capture.

Discussion

The rapid increase in CRE has become a global public health crisis. The resistance type and multiple acquisition pathways of CRE may affect its control in hospitals. CRE are classified into two groups: carbapenemase-producing *Enterobacteriaceae* (CP-CRE) and non-carbapenemase-producing *Enterobacteriaceae* (non-CP-CRE). Non-CP-CRE arise through mechanisms other than carbapenemase

Table 2. Antimicrobial resistance genes in *E. coli* E41-1 sequenced by whole-genome analysis

Gene_id of E41-1	Identity	Resistance Type	Antibiotic Resistance	Description
E41-1GL000027	99.47	emrd	--	Multidrug resistance efflux pump.
E41-1GL000207	100	mdtf	doxorubicin, erythromycin	Resistance-nodulation-cell division transporter system. Multidrug resistance efflux pump.
E41-1GL000208	100	mdte	doxorubicin, erythromycin	Resistance-nodulation-cell division transporter system. Multidrug resistance efflux pump.
E41-1GL000327	40.27	vanra	vancomycin, teicoplanin	VanA type vancomycin resistance operon genes, which can synthesize peptidoglycan with modified C-terminal D-Ala-D-Ala to D-alanine--D-lactate.
E41-1GL000338	53.44	pbp1a	penicillin	The enzyme has a penicillin-insensitive transglycosylase N-terminal domain (formation of linear glycan strands) and a penicillin-sensitive transpeptidase C-terminal domain (cross-linking of the peptide subunits).
E41-1GL000456	88.87	acrb	aminoglycoside, glycylcycline, macrolide, beta-lactam, acriflavin	Resistance-nodulation-cell division transporter system. Multidrug resistance efflux pump.
E41-1GL000457	68.53	acra	aminoglycoside, glycylcycline, macrolide, beta-lactam, acriflavin	Resistance-nodulation-cell division transporter system. Multidrug resistance efflux pump.
E41-1GL000661	100	baca	bacitracin	Undecaprenyl pyrophosphate phosphatase, which consists in the sequestration of Undecaprenyl pyrophosphate.
E41-1GL000686	100	tolc	aminoglycoside, glycylcycline, macrolide, beta-lactam, acriflavin	Resistance-nodulation-cell division transporter system. Multidrug resistance efflux pump.
E41-1GL001374	65.95	acrb	aminoglycoside, glycylcycline, macrolide, beta-lactam, acriflavin	Resistance-nodulation-cell division transporter system. Multidrug resistance efflux pump.
E41-1GL001565	99.39	arna	polymyxin	Bifunctional enzyme that catalyzes the oxidative decarboxylation of UDP-glucuronic acid (UDP-GlcUA) to UDP-4- keto-arabinose (UDP-Ara4O) and the addition of a formyl group to UDP-4-amino-4-deoxy-L-arabinose (UDP-L- Ara4N) to form UDP-L-4-formamido-arabinose (UDP-L-Ara4FN). The modified arabinose is attached to lipid A and is required for resistance to polymyxin and cationic antimicrobial peptides.
E41-1GL001638	100	bcr	--	--
E41-1GL001902	40.28	vanrb	vancomycin	VanB type vancomycin resistance operon genes, which can synthesize peptidoglycan with modified C-terminal D-Ala-D-Ala to D-alanine--D-lactate.
E41-1GL001931	100	emre	aminoglycoside	Multidrug resistance efflux pump.
E41-1GL002260	100	mdtk	enoxacin, norfloxacin	Major facilitator superfamily transporter. Multidrug resistance efflux pump.
E41-1GL002524	45.28	smec	fluoroquinolone	Resistance-nodulation-cell division transporter system. Multidrug resistance efflux pump.
E41-1GL002525	56.84	mexb	aminoglycoside, tigecycline, fluoroquinolone, beta-lactam, tetracycline	Resistance-nodulation-cell division transporter system. Multidrug resistance efflux pump.
E41-1GL002526	52.49	acra	aminoglycoside, glycylcycline, macrolide, beta-lactam, acriflavin	Resistance-nodulation-cell division transporter system. Multidrug resistance efflux pump.
E41-1GL002898	100	mdth	deoxycholate, fosfomycin	Major facilitator superfamily transporter. Multidrug resistance efflux pump.
E41-1GL002911	100	mdtg	deoxycholate, fosfomycin	Major facilitator superfamily transporter. Multidrug resistance efflux pump.
E41-1GL003172	99.69	macb	macrolide	Resistance-nodulation-cell division transporter system. Multidrug resistance efflux pump. Macrolide-specific efflux system.
E41-1GL003209	100	mdfa	--	--
E41-1GL003411	59.9	pbp2	penicillin	The enzyme has a penicillin-insensitive transglycosylase N-terminal domain (formation of linear glycan strands) and a penicillin-sensitive transpeptidase C-terminal domain (cross-linking of the peptide subunits).
E41-1GL003487	45.26	oprm	aminoglycoside, tigecycline, fluoroquinolone, beta-lactam, tetracycline	Resistance-nodulation-cell division transporter system. Multidrug resistance efflux pump.
E41-1GL003551	76.05	rosa	fosmidomycin	Efflux pump/potassium antiporter system. RosA: Major facilitator superfamily transporter. RosB: Potassium antiporter.
E41-1GL003552	80.11	rosb	fosmidomycin	Efflux pump/potassium antiporter system. RosA: Major facilitator superfamily transporter. RosB: Potassium antiporter.
E41-1GL003567	100	acra	aminoglycoside, glycylcycline, macrolide, beta-lactam, acriflavin	Resistance-nodulation-cell division transporter system. Multidrug resistance efflux pump.
E41-1GL003568	99.9	acrb	aminoglycoside, glycylcycline, macrolide, beta-lactam, acriflavin	Resistance-nodulation-cell division transporter system. Multidrug resistance efflux pump.
E41-1GL003571	41.38	catb3	chloramphenicol	Group B chloramphenicol acetyltransferase, which can inactivate chloramphenicol. Also referred to as xenobiotic acetyltransferase.
E41-1GL003752	66.45	tet34	tetracycline	Xanthine-guanine phosphoribosyl transferase. Mechanism detail unknown.
E41-1GL003876	53.96	pbp1b	penicillin	The enzyme has a penicillin-insensitive transglycosylase N-terminal domain (formation of linear glycan strands) and a penicillin-sensitive transpeptidase C-terminal domain (cross-linking of the peptide subunits).
E41-1GL003892	100	bl2be_ctxm	monobactam, penicillin, cephalosporin_iii, ceftazidime, cephalosporin_ii, cephalosporin_i	Class A beta-lactamase. This enzyme breaks the beta-lactam antibiotic ring open and deactivates the molecule's antibacterial properties.
E41-1GL003988	99.63	ksga	kasugamycin	Specifically, dimethylates two adjacent adenosines in the loop of a conserved hairpin near the 3'-end of 16S rRNA in the 30S particle. Its inactivation leads to kasugamycin resistance.
E41-1GL004104	98.54	mdtm	chloramphenicol, acriflavine, norfloxacin	Major facilitator superfamily transporter. Multidrug resistance efflux pump.
E41-1GL004361	99.47	bl1_ec	cephalosporin	Class C beta-lactamase. This enzyme breaks the beta-lactam antibiotic ring open and deactivates the molecule's antibacterial properties.

E41-1GL004363	45.71	ykkc	na_antimicrobials	Small Multidrug Resistance (SMR) protein family. Multidrug resistance efflux pump, which consists of two proteins.
E41-1GL004441	99.42	mdtn	t_chloride, acriflavine, puromycin	Resistance-nodulation-cell division transporter system. Multidrug resistance efflux pump.
E41-1GL004442	99.27	mdto	t_chloride, acriflavine, puromycin	Resistance-nodulation-cell division transporter system. Multidrug resistance efflux pump.
E41-1GL004443	99.39	mdtp	t_chloride, acriflavine, puromycin	Resistance-nodulation-cell division transporter system. Multidrug resistance efflux pump.
E41-1GL004853	99.74	mdtl	chloramphenicol	Major facilitator superfamily transporter. Multidrug resistance efflux pump.
E41-1GL004871	100	bl2b_tem1	penicillin, cephalosporin_ii, cephalosporin_i	Class A beta-lactamase. This enzyme breaks the beta-lactam antibiotic ring open and deactivates the antibacterial properties of the molecule.
E41-1GL004876	100	teta	tetracycline	Major facilitator superfamily transporter, tetracycline efflux pump.
E41-1GL004878	99.58	aph6id	streptomycin	Aminoglycoside O-phosphotransferase, which modifies aminoglycosides by phosphorylation.
E41-1GL004879	100	aph33ib	streptomycin	Aminoglycoside O-phosphotransferase, which modifies aminoglycosides by phosphorylation.
E41-1GL004880	100	sul2	sulfonamide	Sulfonamide-resistant dihydropteroate synthase, which cannot be inhibited by sulfonamide.
E41-1GL004890	100	sul1	sulfonamide	Sulfonamide-resistant dihydropteroate synthase, which cannot be inhibited by sulfonamide.
E41-1GL004891	57.09	ant3ia	spectinomycin, streptomycin	Aminoglycoside O-nucleotidylyltransferase, which modifies aminoglycosides by adenylylation.
E41-1GL004974	99.73	tetc	tetracycline	Major facilitator superfamily transporter, tetracycline efflux pump.
E41-1GL005163	99.45	qnrs	fluoroquinolone	Pentapeptide repeat family, which protects DNA gyrase from the inhibition of quinolones.

Table 3. Nonsynonymous SNP between the two isolates of *E. coli*

Gene id of E41-1	Identity	GenBank accession no.	Description
E41-1GL000108	99.82	gi|481042149|ref|WP_001296525.1|	MULTISPECIES: L-lactate permease [*Enterobacteriaceae*]
E41-1GL000133	99.53	gi|446075989|ref|WP_000153844.1|	L-dehydroascorbate transporter large permease subunit [*Escherichia coli*]
E41-1GL000358	99.54	gi|431431112|gb|ELH12890.1|	inner membrane protein [*Escherichia coli* KTE165]
E41-1GL000580	100	gi|445944916|ref|WP_000022771.1|	MULTISPECIES: tagatose-1,6-bisphosphate aldolase [*Enterobacteriaceae*]
E41-1GL000806	100	gi|291291731|gb|ADD91702.1|	YeeS [*Escherichia coli*]
E41-1GL000807	100	gi|315295288|gb|EFU54618.1|	antirestriction protein [*Escherichia coli* MS 153-1]
E41-1GL000809	97.4	gi|828391439|gb|AKK51269.1|	yafZ [*Escherichia coli* PCN033]
E41-1GL000974	99.77	gi|481041972|ref|WP_001296348.1|	MULTISPECIES: purine permease [*Enterobacteriaceae*]
E41-1GL001050	100	gi|446276418|ref|WP_000354273.1|	VGR-related protein [*Escherichia coli*]
E41-1GL001242	99.82	gi|446803683|ref|WP_000880939.1|	MULTISPECIES: DNA repair protein RecN [*Enterobacteriaceae*]
E41-1GL001548	99.4	gi|226901115|gb|EEH87374.1|	protein yfbM [Escherichia spp. 3_2_53FAA]
E41-1GL001636	99.83	gi|446500210|ref|WP_000578064.1|	MULTISPECIES: ATP-dependent helicase [*Enterobacteriaceae*]
E41-1GL001858	99.75	gi|446777104|ref|WP_000854360.1|	MULTISPECIES: membrane protein [*Enterobacteriaceae*]
E41-1GL002182	99.28	gi|446097161|ref|WP_000175016.1|	MULTISPECIES: NAD(+) synthetase [*Enterobacteriaceae*]
E41-1GL002283	99.73	gi|446757826|ref|WP_000835082.1|	MULTISPECIES: anhydro-N-acetylmuramic acid kinase [*Enterobacteriaceae*]
E41-1GL002307	99.83	gi|754848490|ref|WP_042209487.1|	beta-glucuronidase [*Escherichia coli*]
E41-1GL002742	99.61	gi|553359248|gb|ESA86116.1|	Na+/H+ antiporter NhaB [*Escherichia coli* 907779]
E41-1GL002798	99.84	gi|446950005|ref|WP_001027261.1|	MULTISPECIES: Terminase large subunit from bacteriophage origin [*Enterobacteriaceae*]
E41-1GL002910	99.68	gi|384470350|gb|EIE54463.1|	lipid A biosynthesis lauroyl acyltransferase [*Escherichia coli* AI27]
E41-1GL003163	98.4	gi|323958196|gb|EGB53905.1|	AsnC family protein [*Escherichia coli* H263]
E41-1GL003821	100	gi|447062911|ref|WP_001140167.1|	MULTISPECIES: D-glycero-beta-D-manno-heptose 1,7-bisphosphate 7-phosphatase [*Enterobacteriaceae*]
E41-1GL003865	99.79	gi|446768152|ref|WP_000845408.1|	MULTISPECIES: ClC family H(+)/Cl(-) exchange transporter [*Enterobacteriaceae*]
E41-1GL003892	100	gi|486436156|ref|WP_001617865.1|	MULTISPECIES: class A extended-spectrum beta-lactamase CTX-M-14 [*Enterobacteriaceae*]
E41-1GL004642	99.78	gi|446502563|ref|WP_000580417.1|	MULTISPECIES: two-component sensor histidine kinase [Proteobacteria]
E41-1GL005042	99.53	gi|727409755|ref|WP_033817146.1|	protein ImpB [*Escherichia coli*]
E41-1GL005057	100	gi|831357709|emb|CEL26134.1|	ParB-like (plasmid) [*Escherichia coli*]
E41-1GL005058	100	gi|446768642|ref|WP_000845898.1|	MULTISPECIES: recombinase [*Enterobacteriaceae*]

Table 4. Indel analysis between the two isolates of *E. coli*

Gene ID of E41-2	Indel type	InDel_start	InDel_end	location	ref_start	ref_end	Base	gene_ID
Scaffold1	Insertion	183631	183632	Chromosome	2047633	2047633	C	E41-1GL001933
Scaffold11	Insertion	98520	98521	Chromosome	318091	318091	G	E41-1GL000300
Scaffold12	Deletion	171810	171810	Chromosome	4487177	4487178	C	E41-1GL004379
Scaffold15	Deletion	30739	30739	Chromosome	2883831	2883832	G	E41-1GL002810
Scaffold19	Deletion	12635	12635	Chromosome	2651515	2651516	C	E41-1GL002552
Scaffold3	Insertion	206329	206332	Chromosome	209633	209633	TTA	E41-1GL000197
Scaffold35	Insertion	948	949	Chromosome	833253	833253	A	E41-1GL000819
Scaffold6	Insertion	75123	75124	Chromosome	3859267	3859267	T	E41-1GL003766
Scaffold6	Insertion	75325	75326	Chromosome	3859385	3859385	T	E41-1GL003766
Scaffold88	Insertion	325	326	Plasmid2	20390	20390	C	E41-1GL005107
Scaffold24	Insertion	66053	66054	Plasmid2	40902	40902	C	E41-1GL005107
Scaffold104	Insertion	375	376	Plasmid1	109551	109551	G	E41-1GL000109

Table 5. The coverage ratio of the E41-2 genome compared with that of E41-1

ChrID	Reference (E41-1) size (bp)	Covered length (bp) E41-2	Coverage (%)
Chromosome	5022609	5022605	100
Plasmid1	128911	128903	99.99
Plasmid2	86657	86297	99.58
Plasmid3	52864	3767	7.13
Total	5291041	5241572	99.07

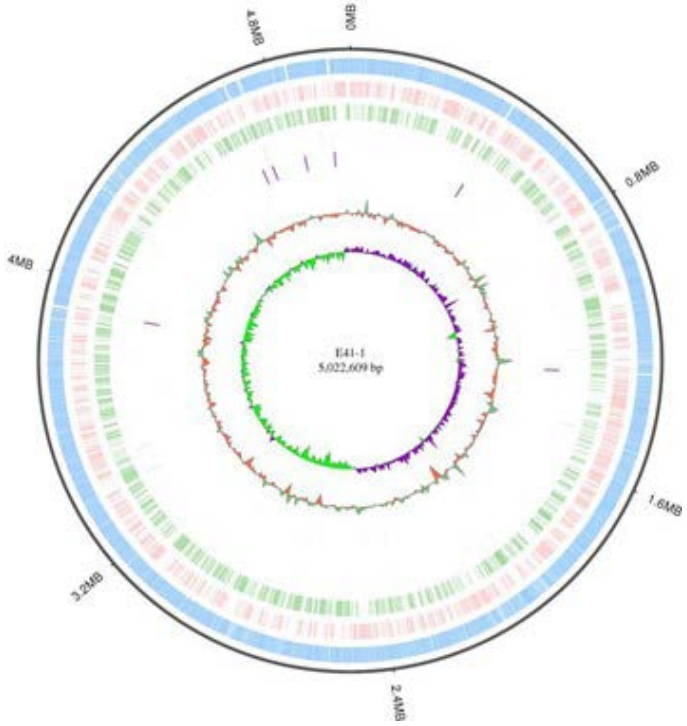

Figure 2. E41-1 genome chromosome circos

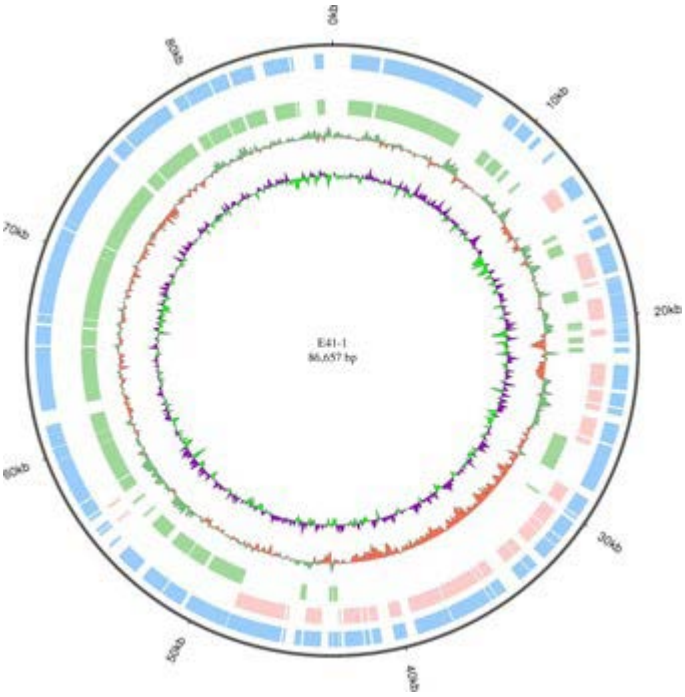

Figure 4. E41-1 plasmid plasmid 2 circos

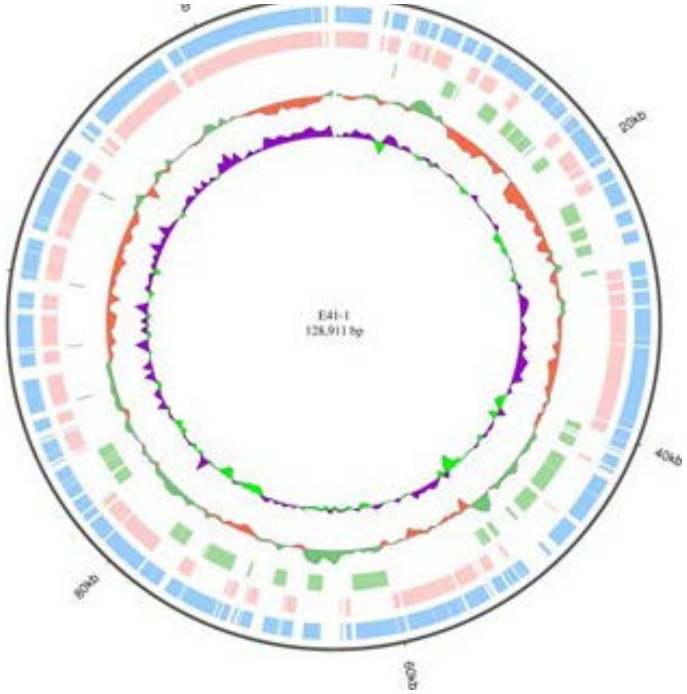

Figure 3. E41-1 plasmid plasmid 1 circos

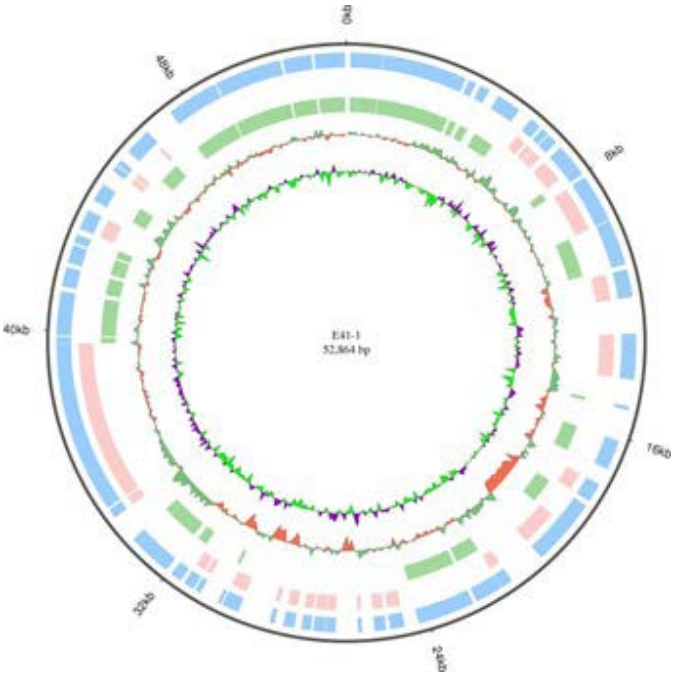

Figure 5. E41-1 plasmid plasmid 3 circos

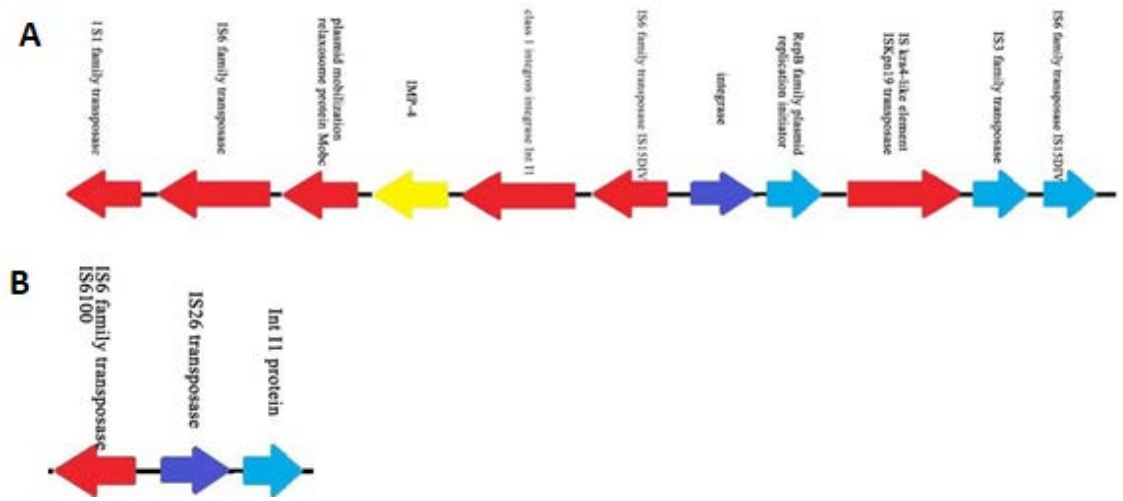

Figure 6. Structure of the E41-1 plasmid plasmid 3 **(A)** Features of the E41-1 plasmid plasmid 3 carrying the carbapenemase gene *bla*IMP-4 **(B)** Features of the E41-2 plasmid plasmid 3

production. These mechanisms most commonly include production of ESBLs and/or AmpCs, in combination with cell membrane alterations [12]. Importantly, exposure to antibiotics may result in bacterial genomic instability that increases mutation and genetic reassortment [13]. CP-CRE has the ability to produce three different categories of carbapenemases: (a) class A (serine carbapenemases, such aswwwwwww *Klebsiella pneumoniae* carbapenemase); (b) class B (metallo-blactamases, such as IMP, VIM, and NDM); and (c) class D (OXA carbapenemases, such as OXA-23 and OXA-48) [14]. These carbapenemase-coding genes are mostly acquired through plasmids or transposons.

In our study, carbapenem-susceptible and carbapenem-resistant *E. coli* isolates were recovered from a single patient during therapy. Prior to the antibiotic therapy, the *E. coli* isolated from the sputum was susceptible to carbapenem. Following the use of carbapenem to control the infection, the *E. coli* became resistant to carbapenem. Based on the comparison of the genomes of the two isolates by whole-genome sequencing analysis, the sequences of chromosome, plasmid 1, and plasmid 2 of E41-1 were highly homologous to those of E41-2, and the gene difference was located in plasmid 3. Thus, we concluded that single-nucleotide mutation, insertion, and deletion did not result in the development of resistance to carbapenem. In contrast, acquisition of the IMP-4-encoding gene may be responsible for changes in drug resistance. IMP-4 carbapenemase was first identified in *Acinetobacter* spp. in Hong Kong between 1994 and 1998 and its encoding gene was not associated with any plasmids [15]. In 2001, the occurrence of the *bla*IMP-4 gene on a conjugative plasmid in *Citrobacter youngae* was reported by Hawkey et al. [16]. Based on the results of our study, the *bla*IMP-4 gene was related to class 1 integron IntI1, IS6 family transpose, and plasmid mobilization relaxosome protein Mobe gene, which may have led to gene capture.

In recent years, WGS has been widely used in genomic research. It provides information on the arrangement of multiple AMR genes and associated genes. Because gene expression is influenced by numerous factors, detection of an AMR gene does not necessarily indicate that the isolate is resistant to some antibiotic. However, WGS is more sensitive and comprehensive than traditional methods and facilitates the identification of more determinants [17]. A total of 49 types of AMR genes were determined in the chromosome of E41-1. Of those, 30 AMR genes exhibited highly homologous identities to those of previously confirmed AMR genes (>99%). Most of the genes (acra, acrb, tolc, smec, mexb, emre, *et cl*) were efflux pump genes associated with multi-antibiotics, including aminoglycoside, tigecycline, fluoroquinolone, beta-lactam, tetracycline, and fosfomycin. In contrast, E41-1 was susceptible to aminoglycoside. Hence, even if the isolate harbors the emre gene, it may not be resistant to aminoglycoside. The phenotype of the isolate is not determined by a single AMR gene, but is affected by numerous factors. Unlike traditional biocuration, the AMR genes are constantly moving and mutating under selective pressures. New AMR mutations and horizontal transfer of AMR genes among pathogens will occur with the emergence of new threats from the environment and protoresistome [10,18,19]. We should develop proper analytical pipelines for the accurate detection of the resistome and subsequent accurate prediction of the antibiogram based on genomic and metagenomic data [20]. Thus, WGS can be employed inmodifying antibiotic usage strategies to optimize antimicrobial stewardship.

In principle, for the clinical prediction of antibiotic resistance by WGS, a comprehensive set of genetic determinants of the resistome needs to be identified for each species and further research should investigate new mechanisms of antimicrobial resistance. Recent studies demonstrated that WGS could be feasibly and effectively used for surveillance of antibiotic resistance and provides actionable results in infection control.

Significance and Impact of Study

Whole-genome sequencing was applied to analyze the genetic difference between the two isolates of *Escherichia coli*. We found that single-nucleotide mutation did not contribute to change in antibiotic resistance. Acquisition of a plasmid carrying a migratable gene cassette resulted in spreading of antibiotic resistance.

Acknowledgement

This study was supported by the National Natural Science Foundation of China (81101282).

Author contribution

Qiong Wu and Jun Hu contributed equally to this work. Qiong Wu and Jun Hu conceived and designed research. Jianqiang Wang, Yungai Li and Yunqi Pan conducted experiments. Rong Chen and Jin

Tang analyzed data. Qiong Wu and Jin Tang wrote the manuscript. All authors read and approved the manuscript.

References

1. Xu Y, Gu B, Huang M, Liu H, Xu T, et al. (2015) Epidemiology of carbapenem resistant Enterobacteriaceae (CRE) during 2000-2012 in Asia. *J Thorac Dis* 7: 376-385. [Crossref]
2. Iredell J, Brown J, Tagg K (2016) Antibiotic resistance in Enterobacteriaceae: mechanisms and clinical implications. *BMJ* 352: h6420. [Crossref]
3. Hu FP, Guo Y, Zhu DM, Wang F, Jiang XF, et al. (2016) Resistance trends among clinical isolates in China reported from CHINET surveillance of bacterial resistance, 2005-2014. *Clin Microbiol Infect* 22: S9-S14. [Crossref]
4. Livingstone D, Gill MJ, Wise R (1995) Mechanisms of resistance to the carbapenems. *J Antimicrobial Chemother* 35: 1-5.
5. Goodman KE, Simner PJ, Tamma PD, Milstone AM (2016) Infection control implications of heterogeneous resistance mechanisms in carbapenem-resistant Enterobacteriaceae (CRE). *Expert Rev Anti Infect Ther* 14: 95-108. [Crossref]
6. Yu H, Qu F, Shan B, Huang B, Jia W, et al. (2016) Detection of the mcr-1 Colistin Resistance Gene in Carbapenem-Resistant Enterobacteriaceae from Different Hospitals in China. *Antimicrob Agents Chemother* 60: 5033-5035. [Crossref]
7. Snitkin ES, Zelazny AM, Thomas PJ, Stock F; NISC Comparative Sequencing Program Group, et al. (2012) Tracking a hospital outbreak of carbapenem-resistant Klebsiella pneumoniae with whole-genome sequencing. *Sci Transl Med* 4: 148ra116. [Crossref]
8. Onori R, Gaiarsa S, Comandatore F, Pongolini S, Brisse S, et al. (2015) Tracking Nosocomial Klebsiella pneumoniae Infections and Outbreaks by Whole-Genome Analysis: Small-Scale Italian Scenario within a Single Hospital. *J Clin Microbiol* 53: 2861-2868. [Crossref]
9. Knudsen PK, Gammelsrud KW, Alfsnes K, Steinbakk M, Abrahamsen TG, et al. (2018) Transfer of a bla CTX-M-1-carrying plasmid between different Escherichia coli strains within the human gut explored by whole genome sequencing analyses. *Sci Rep* 8: 280. [Crossref]
10. Gillings MR, Paulsen IT, Tetu SG (2017) Genomics and the evolution of antibiotic resistance. *Ann N Y Acad Sci* 1388: 92-107. [Crossref]
11. Kanehisa M, Sato Y, Kawashima M, Furumichi M, Tanabe M (2016) KEGG as a reference resource for gene and protein annotation. *Nucleic Acids Res* 44: D457-462.
12. Rock C, Thom KA, Masnick M, Johnson JK, Harris AD, et al. (2014) Frequency of Klebsiella pneumoniae carbapenemase (KPC)-producing and non-KPC-producing Klebsiella species contamination of healthcare workers and the environment. *Infect Control Hosp Epidemiol* 35: 426-429. [Crossref]
13. Shapiro RS (2015) Antimicrobial-induced DNA damage and genomic instability in microbial pathogens. *PLoS Pathog* 11: e1004678. [Crossref]
14. AlTamimi M, AlSalamah A, AlKhulaifi M, AlAjlan H (2017) Comparison of phenotypic and PCR methods for detection of carbapenemases production by Enterobacteriaceae. *Saudi J Biol Sci* 24: 155-161. [Crossref]
15. Riccio ML, Franceschini N, Boschi L, Caravelli B, Cornaglia G, et al. (2000) Characterization of the metallo-beta-lactamase determinant of Acinetobacter baumannii AC-54/97 reveals the existence of bla(IMP) allelic variants carried by gene cassettes of different phylogeny. *Antimicrob Agents Chemother* 44: 1229-1235. [Crossref]
16. Hawkey PM, Xiong J, Ye H, Li H, M'Zali FH (2001) Occurrence of a new metallo-beta-lactamase IMP-4 carried on a conjugative plasmid in Citrobacter youngae from the People's Republic of China. *FEMS Microbiol Lett* 194: 53-57. [Crossref]
17. Su JQ, Cui L, Chen QL, An XL, Zhu YG (2017) Application of genomic technologies to measure and monitor antibiotic resistance in animals. *Ann N Y Acad Sci* 1388: 121-135. [Crossref]
18. Köser CU, Ellington MJ, Peacock SJ (2014) Whole-genome sequencing to control antimicrobial resistance. *Trends Genet* 30: 401-407. [Crossref]
19. Schürch AC, van Schaik W (2017) Challenges and opportunities for whole-genome sequencing-based surveillance of antibiotic resistance. *Ann N Y Acad Sci* 1388: 108-120. [Crossref]
20. McArthur AG, Tsang KK (2017) Antimicrobial resistance surveillance in the genomic age. *Ann N Y Acad Sci* 1388: 78-91. [Crossref]