Infection and Drug Resistance

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ORIGINAL RESEARCH

The resistance mechanism of *Escherichia coli* induced by ampicillin in laboratory

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Mengchen Li[1]
Qiaoli Liu[1]
Yanli Teng[1]
Liuyang Ou[1]
Yuanlin Xi[1]
Shuaiyin Chen[1,*]
Guangcai Duan[1,2,*]

[1]Department of Epidemiology, College of Public Health, Zhengzhou University, Zhengzhou, Henan, People's Republic of China; [2]Henan Collaborative Innovation Center of Molecular Diagnosis and Laboratory Medicine, Xinxiang Medical College, Xinxiang, Henan, People's Republic of China

*These authors contributed equally to this work

Background: Multi-drug-resistant *Escherichia coli* poses a great threat to human health, especially resistant to ampicillin (AMP), but the mechanism of drug resistance is not very clear.
Purpose: To understand the mechanism of resistance of *E. coli* to beta-lactam antibiotics by inducing drug resistance of sensitive bacteria in laboratory.
Methods: Clinical sensitive *E. coli* strain was induced into resistance strain by 1/2 minimum inhibitive concentration (MIC) induced trails of AMP. The drug resistance spectrum was measured by modified K-B susceptibility test. Whole-genome sequencing analysis was used to analyze primary sensitive strain, and resequencing was used to analyze induced strains. Protein tertiary structure encoded by the gene containing single nucleotide polymorphism (SNP) was analyzed by bioinformatics.
Results: After 315 hrs induced, the MIC value of *E. coli 15743* reached to 256 μg/mL, 64 times higher than that of the sensitive bacteria. During the induction process, the bacterial resistance process is divided into two stages. The rate of drug resistance occurs rapidly before reaching the critical concentration of 32 μg/mL, and then the resistance rate slows down. Sequencing of the genome of resistant strain showed that *E. coli 15743* drug-resistant strain with the MIC values of 32 and 256 μg/mL contained four and eight non-synonymous SNPs, respectively. These non-synonymous SNPs were distributed in the genes of *frdD, ftsI, acrB, OmpD, marR, VgrG*, and *envZ.*
Conclusion: These studies will improve our understanding of the molecular mechanism of AMP resistance of *E. coli*, and may provide the basis for prevention and control of multi-drug-resistant bacteria and generation of new antibiotics to treat *E. coli* infection.
Keywords: *Escherichia coli*, ampicillin, drug resistance

Introduction

Pathogenic *Escherichia coli* often causes diarrhea, sepsis, and other clinical symptoms, and is still one of the main intestinal pathogens affecting human and animal health. Ampicillin (AMP), a semi-synthetic β-lactam antibiotics, is widely used to treat of human and livestock *E. coli* infection, but recently its resistance rate has increased.[1–3] AMP works on the active replicating stage of bacteria, inhibiting the synthesis of bacterial cell wall. Bacteria often resist such an antibiotics in the following ways: encodes β-lactamase, changes the target protein in cell wall, reduces the permeability of outer membrane, and increases the expression of drug efflux pump. Antibacterial drugs are used by animals and then spread to the environment via excreta, which not only makes the environment polluted, but also brings great harm to human health and the sustainable development of the breeding industry.[4,5]

Correspondence: Shuaiyin Chen; Guangcai Duan
Department of Epidemiology, College of Public Health, Zhengzhou University, No.100 Kexue Avenue, Zhengzhou, Henan 450001, People's Republic of China
Tel +86 1 352 340 8394
Fax +86 6 778 1453
Email sychen@zzu.edu.cn; gcduan@yeah.net

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Whole-genome sequencing (WGS) has been shown to guide the prevention and control of bacterial resistance.[6] Single nucleotide polymorphism (SNP) mainly refers to the DNA sequence polymorphism caused by the variation of a single nucleotide at the genomic level, and the resequencing analysis to screen the different SNPs can more directly study drug resistance. We simulated the process of clinical antibiotics in organisms by using the method of AMP laboratory induction, and explored the relationship between the degree of drug resistance and the mutation site. Screening for non-synonymous single nucleotide polymorphism (non-SNP) between drug-resistant and susceptible strains to understand the role of non-SNP in drug-resistant strains. The purpose of this study is to understand the law and mechanism of drug resistance of *E. coli*, provide new targets for the development of new antibiotics, make the rational use of antibiotics, and solve the multiple occurrence and treatment of multi-drug resistance of *E. coli* in clinical practice.

Materials and methods

Bacterial isolates and reagent

The *E. coli* strain used in this study (*E. coli 15743*) was isolated from a stool specimen from a patient at a hospital in Suixian, Henan Province, China, in 2015. Characterization of this strain by Kirby Bauer (K-B) paper diffusion method showed that the strain was sensitive to eight classes of 20 antibiotics. *E. coli ATCC 25922* was used as a control for our study.

M-H broth medium and M-H solid medium (Oxoid company, UK), Pharmaceutical sensitive paper (Hangzhou Binhe microbial company, Hangzhou, China), AMP standard products (Chinese drug identification Institute, Beijing, China), DNA extraction kit (Shanghai Laifeng Biotech company, Shanghai, China). Illumina Hiseq was done at Shanghai Lingen Biotechnology Co., Ltd.

The *E. coli* used in the experiment was specifically isolated for this study. The study was approved by the Life Science Ethics Committee of Zhengzhou University, and patient also signed written informed consent.

Induction process

Minimum inhibitory concentration (MIC) was determined by microbroth dilution method.[7–9] The strain of *E. coli* (isolated from clinical and have MIC value) that is sensitive to AMP was cultured in MH solid medium, 37°C culture after 18–24 hrs, pick a single colony in 8 mL M-H liquid medium for amplification of bacteria. The above bacteria solution was cultured in M-H liquid medium containing 1/2MIC AMP, respectively, and the concentration of AMP was continuously increased during the subculture process. When the concentration of antibiotics reached 16 μg/mL, 8 μg/mL was increased each time, and each concentration was subcultured twice. When the value of the MIC change of a drug was greater than or equal to four times MIC before and after induction, it was considered that the MIC change after induction had significant significance.[10] The culture medium of M-H broth without antibiotics was used as the control during the whole process.

Multilocus sequence typing (MLST) of *E. coli* strains were classified by seven pairs of housekeeping genes containing *adk, fumC, gyrB, icd, mdh, purA*, and *recA*.

Susceptibility testing

Kirby Bauer paper diffusion method was used to screen the *E. coli* which was sensitive to eight kinds of antibiotics, including aminoglycosides, penicillins, cephalosporins, tetracycline, β-lactamase inhibitors, carbamates, sulfonamides, and quinolones. The induced strains were repeated using drug sensitivity test. The data interpretation was performed in accordance with the Clinical and Laboratory Standards Institute 2016 guidelines.[11]

First, we induced *E. coli* resistance to AMP, by culturing *E. coli* with gradually increase the concentration of AMP (2, 4, 8, 16, 32, 64, 128, and 256 μg/mL). After we obtained resistance strain, we compared the resistance spectrum of 20 antibiotics between the induced strain (*E. coli 15743-256*, induced at 256 μg/mL) and the original strain (*E. coli 15743*) by performing drug sensitivity tests. The bacterial suspension was spread onto an agar plate, with a small circular pieces paper containing different antibiotics, and cultured at 37°C for 16–20 hrs. Antimicrobial ring diameter was measured.

WGS and resequencing analysis

The strains at MIC values of 32 and 256 were named as *E. coli 15743-32* and *E. coli 15743-256*, respectively. Whole-genome analysis was performed on the primary sensitive strains, and resequencing was performed on induced resistant strains. The results of resequencing were compared with those of the original map. Screening non-SNPs that may affect protein function.

Sequencing was performed by Shanghai ling'en Biotechnology Co. Ltd. (Shanghai, China). Illumina

Hiseq combined with third-generation sequencing technology was used to complete genomic sequencing of the strains in this project.

RT-PCR

Remove the reverse transcribed DNA from the 4°C freezer and prepare the desired concentration of the reagent according to the instructions. Turn on the ABI Fast7500 instrument, set 95°C for 30 s, react for 40 cycles, 95°C for 3 s, 60°C for 30 s, and dissolve the curve for 95°C for 15 s, 60°C for 60 s, and 95°C for 15 s. Add the sample to the 8-row EP tube, three replicate wells per sample, and remove the bubbles by centrifugation. The average CT value of each sample was recorded after the reaction was completed. The relative expression level of the gene of interest was calculated using $2^{-\Delta\Delta CT}$. (ΔCT = CT value of the target gene-CT value of the internal reference gene. ΔΔCT = experimental sample ΔCT - control group ΔCT.)

Bioinformatics analysis

SWISS-MODEL software was used to analyze the amino acid sequence of the protein encoded before and after gene mutation, and predict protein tertiary structure.[12,13]

Statistical analysis

SPSS17.0 was used for simple linear regression analysis and the regression equation was tested. Size of a test was 0.05 (α=0.05).

Results

Drug susceptibility test results

Our data showed that *E. coli 15743* was sensitive to 20 different antibiotics. After induction, *E. coli 15743-256* was resistant to AMP, piperacillin, cefuroxime, cefazolin, cefoxitin, AMP/sulbactam, amoxicillin/clavulanic acid, piperacillin/tazobactam, and aztreonam, but still sensitive to remaining 11 antibiotics (Table 1, Note that Intermediaries were also defined as drug resistance). Our results indicated that original sensitive *E. coli* was not only induced resistant to AMP, but also resistant to a variety of other antibiotics and became multi-drug resistant during induction.

The occurrence of drug resistance (determined by MIC value) during induction

To study kinetics of drug resistance, we cultured *E. coli* with increasing concentration of AMP for different periods and measured MIC at each concentration as indicated in Table 2. Regression analysis was performed on the MIC

Table 1 Antibacterial ring diameter of *Escherichia coli*

Antibiotics	*15743*	*15743-256*	CLSI criterion (mm)		
			R	I	S
Gentamicin	20	18	≤12	13–14	≥15
Tobramycin	19	19	≤12	13–14	≥15
Ampicillin	19	7	≤13	14–16	≥17
Piperacillin	26	18	≤17	18–20	≥21
Cefepime	31	22	≤14	15–17	≥18
Cefuroxime	21	8	≤14	15–17	≥18
Ceftazidime	23	16	≤14	15–17	≥18
Cefoperazone	30	22	≤15	16–20	≥21
Cefazolin	25	8	≤19	20–22	≥23
Cefoxitin	24	8	≤14	15–17	≥18
Tetracycline	20	15	≤11	12–14	≥15
Ampicillin/sulbactam	24	8	≤11	12–14	≥15
Amoxicillin/clavulanic acid	22	8	≤13	14–17	≥18
Peracillin/tazobartan	24	16	≤17	18–20	≥21
Meropenem	35	27	≤19	20–22	≥23
Imipenem	32	25	≤19	20–22	≥23
Aztreonam	28	16	≤17	18–20	≥21
Sulfamethoxazole	26	19	≤10	11–15	≥16
Evofloxacin	27	26	≤13	14–16	≥17
Ciprofloxacin	29	25	≤15	16–20	≥21

Abbreviations: R, resistance; I, intermediaries; S, sensitivity.

Table 2 The MIC value of *E. coli 15743* over time and induced concentration

Induced concentration (μg/mL)	Time (hrs)	MIC (μg/mL)
2	5	4
4	21	8
8	39	16
16	63	32
32	81	48
64	113	64
128	187	128
256	315	256

value and induction time using SPSS 17.0. The regression equation was y=1.0435lnx−0.7316. The fitting effect of the equation was evaluated, R^2=0.9605, P<0.05. The MIC value reaching 32 μg/mL is the critical value, and the MIC value increased faster before reaching 32 μg/mL than after (Table 2).

Meanwhile, the part with MIC value less than or equal to 32 μg/mL was selected for regression analysis, and the regression equation was y=0.0358x+1.2812. The fitting effect of the equation was evaluated, R^2=0.991, P<0.05. The MIC value of *E. coli 15743* increased with the increase of induction concentration and induction time (Figure 1).

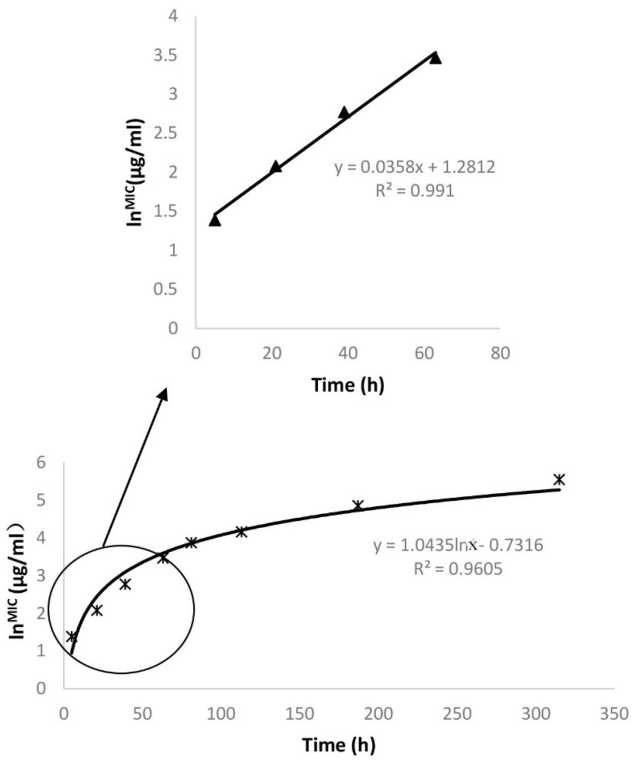

Figure 1 The change of MIC value over time.
Abbreviation: MIC, minimum inhibitiveconcentration.

MLST results

To demonstrate that the induced strain (*E. coli 15743-256*) was indeed derived from the original strain (*E. coli 15743*), we performed MLST of above two strains. Genomic DNA was extracted by bacterial DNA extraction kit, PCR amplified, and sequenced by Sangon Biotech (Shanghai) Co., Ltd. Blast searching of NCBI database indicated that these two strains have identical, MLST type, *adk*-13, *fumC*-363, *gyrB*-302, *icd*-97, *mdh*-17, *purA*-94, and *recA*-93. Our data indicate that the induction process was not contaminated, and the resistant strain *E. coli 15743-256* was derived from the sensitive strain *E. coli 15743.*

Whole-genome analysis

E. coli 15743 contained 4408 genes, 22 rRNA, and 85 tRNA. The gene density was 0.945 kb, the GC content was 51.7%, the gene percentage was 88.3%, the intergenic region length was 545,151, the intergenic region GC content was 42.6%, and the intergenic region accounted for 11.7% of the genome. The characteristics of *E. coli 15743* genomes are summarized in Figure 2. *E. coli 15743* did not contain plasmids.

The genome map of the strain includes distribution of genes on the chains of justice and antisense, functional classification of Clusters of Orthologous Groups of proteins (COG), GC content, genome island, and homologous genes, which can fully display the features of the genome.

COG

The functional classification of COG of *E. coli 15743* showed that most genes were related to amino acid transport and metabolism, carbohydrate transport and metabolism, energy production and conversion, general function prediction only, inorganic ion transport and metabolism, and cell envelope biogenesis (Figure 3).

Non-SNPs

To determine whether there was a change in the *E. coli* genome after induction of the original strain, we performed a genome-wide sequencing of the induced resistant strains (*E. coli 15743-32* and *E. coli 15743-256*) and analyzed the number of mutations and the site of the mutation.

Compared to the original *E. coli* strain (*E. coli 15743*), there were nine non-SNPs in two induced drug-resistant strains, including three shared non-SNPs, which were present in the genes *orf00819, orf01200,* and *orf02235*. Other non-SNPs were present in the genes *orf01916, orf00490,*

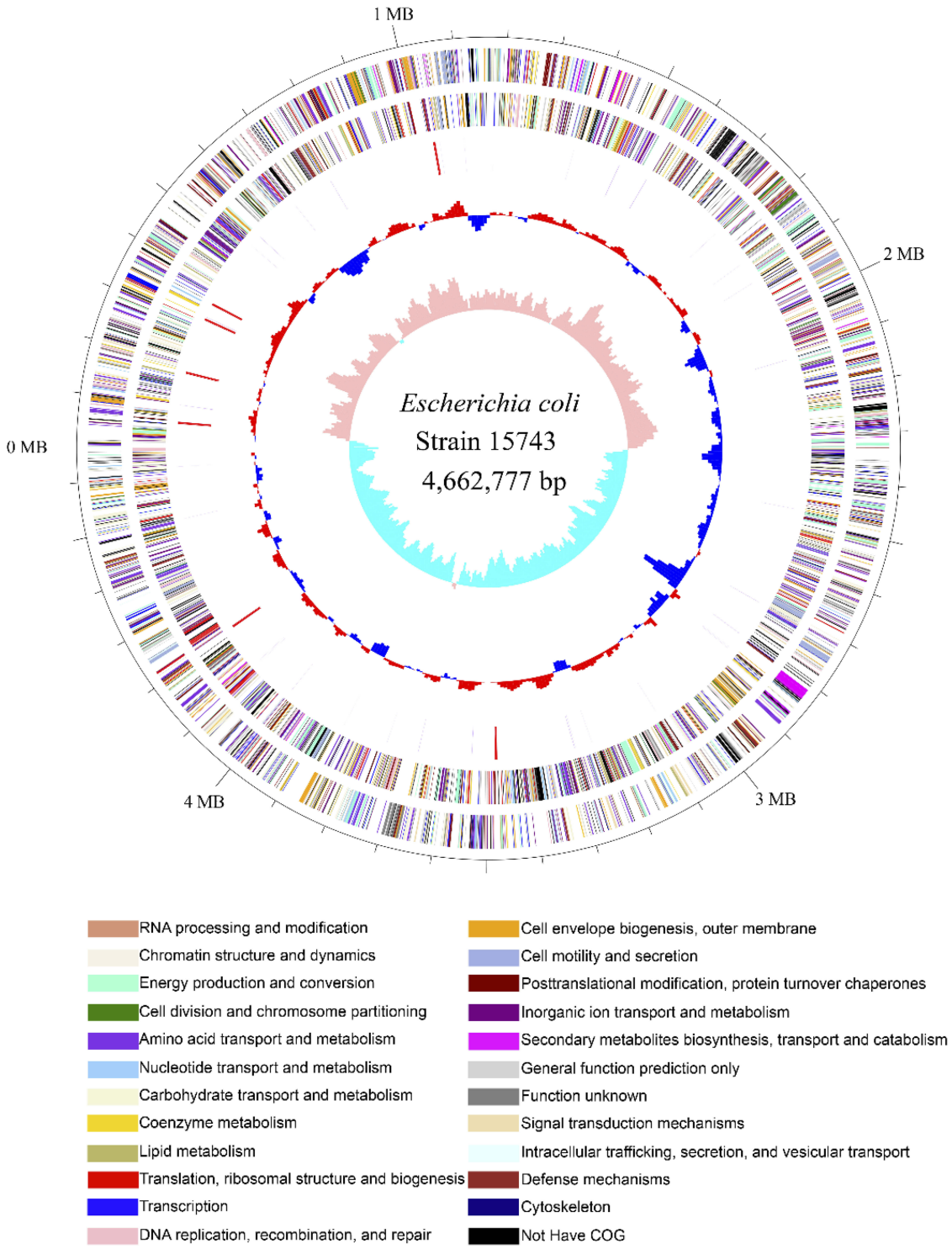

Figure 2 The genomic map of *E. coli 15743*.

Notes: The outermost circle of the circle map is the genome-sized logo, each scale is 0.1 Mp. The second and third circles are CDS on the positive and negative chains, and the different colors indicate different COG classifications of the CDS. The fourth circle is rRNA or tRNA. The fifth circle is the GC content, and the outward red part indicates that the GC content in the region is higher than the whole-genome average GC content. The higher the peak value indicates the greater the difference from the average GC content, and the inward blue portion indicates that the GC content in the region is low. For the whole-genome average GC content, a higher peak indicates a greater difference from the average GC content. The innermost circle is the GC skew value. The specific algorithm is G−C or G+C. When the value is positive in the biological sense, the positive chain tends to transcribe CDS. When it is negative, the negative chain tends to transcribe CDS.

Abbreviation: COG, Clusters of Orthologous Groupsof proteins.

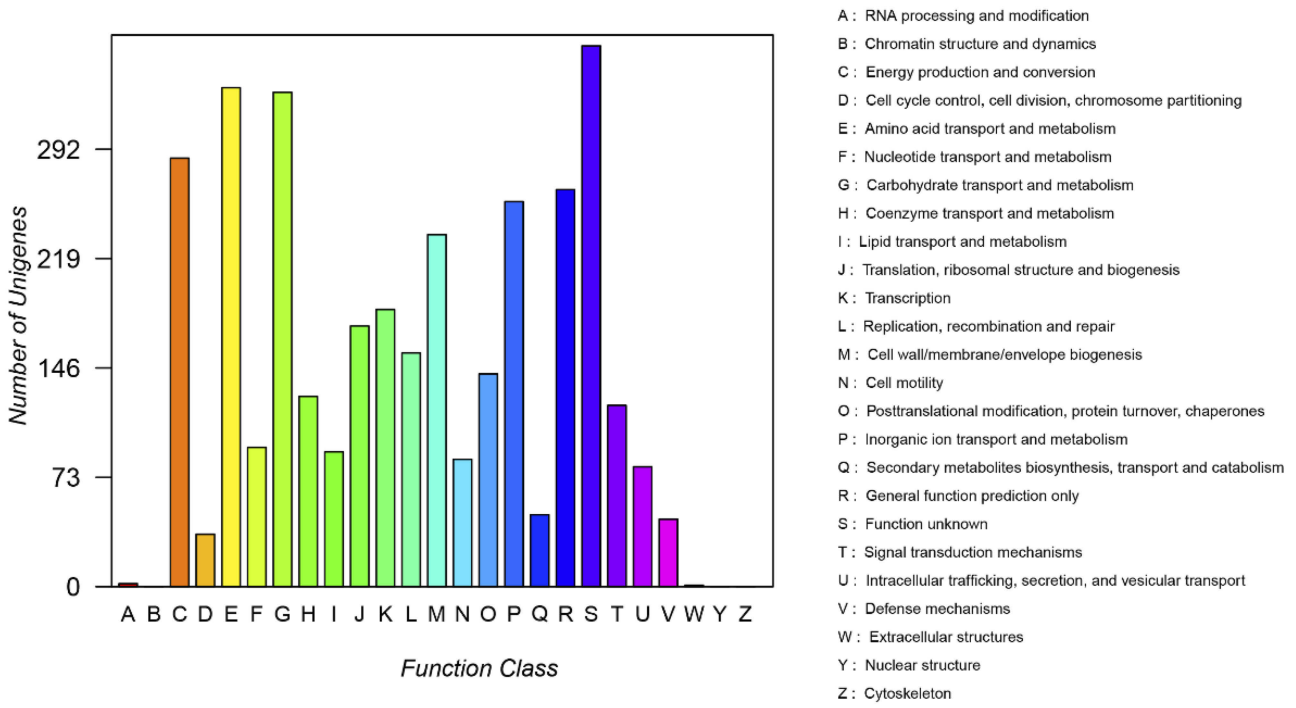

Figure 3 The functional classification of COG of E. coli *15743*.
Abbreviation: COG, Clusters of Orthologous Groups of proteins.

orf03479, orf04094. Three non-SNPs mutations occurred in the *orf03479* gene, and only one SNP mutation occurred in each of the remaining genes. Three non-SNPs were in genes that encode cell membrane proteins. Three were in genes with unknown functions. One was related to the transport and metabolism of inorganic ion, one was related to transcription, and one was related to signal transduction mechanisms (Table 3).

Our data showed that there were four non-SNPs in *E. coli 15743-32*, which were on four genes. There were eight non-SNPs in *E. coli 15743-256*, spread across six genes. The functional classification of COG showed that most genes were related to amino acid transport and metabolism, carbohydrate transport and metabolism, energy production and conversion, general function prediction only, inorganic ion transport and metabolism, and cell envelope biogenesis.

Table 3 The non-SNPs analysis results of *E. coli 15743-32* and *E. coli 15743-256*

Location	Gene-id	Position	Base	Codon	Aa	COG	Gene
E. coli 15743-32	*orf00819*	899,435	G→A	GTT→ATT	Val→Ile	M	*ftsI*
	orf01200	1,302,112	C→T	TCG→TTG	Ser→Leu	*P*	*acrB*
	orf01916	2,031,602	C→T	GCC→GTC	Ala→Val	M	*OmpD*
	orf02235	2,368,737	C→A	GCA→GAA	Ala→Glu	K	*marR*
E. coli 15743-256	*orf00490*	541,538	T→A	GTC→GAC	Val→Asp	M	*frdD*
	orf00819	899,435	G→A	GTT→ATT	Val→Ile	M	*ftsI*
	orf01200	1,302,112	C→T	TCG→TTG	Ser→Leu	*P*	*acrB*
	orf02235	2,368,737	C→A	GCA→GAA	Ala→Glu	K	*marR*
	orf03479	3,681,194	G→C	AGG→AGC	Arg→Ser	S	*VgrG*
	orf03479	3,681,453	C→T	CTC→TTC	Leu→Phe	S	*VgrG*
	orf03479	3,681,480	C→G	CGG→GGG	Arg→Gly	S	*VgrG*
	orf04094	4,314,203	C→A	CGT→AGT	Arg→Ser	T	*envZ*

Abbreviations: Ala, alanine; Asp, aspartic acid; Glu, glutamic acid; Ile, isoleucine; Ser, serine; Val, valine; Thr, threonine; Lys, lysine; HIS, histidine; Gln, glutamine; Asn, asparagine; C, energy production and conversion; M, cell wall or envelope biogenesis; S, unknown function.

RT-PCR

Whole-genome re-sequencing *E. coli 15743-32* and *E. coli 15743-256*, fluorescent real-time quantitative PCR detection of consensus genes. The genes in which non-SNPs occur were screened, and *E. coli 15743-32* and *E. coli 15743-256* had three identical genes (*orf00819, orf01200, orf02235*), and the expression levels of these genes in each generation strain are shown in Figure 4A–C, respectively.

RT-PCR showed that the *orf01200, orf00819, orf02235* genes showed high expression in resistant strains (*E. coli 15743-32, E. coli 15743-64, E. coli 15743-128, E. coli 15743-256*).

Protein structure prediction

The tertiary structure changes only in proteins encoded by genes *orf01200* and *orf04094*, and the predicted results are shown in Figures 5 and 6.

Before the mutation of *orf01200*, 2hrt.1.A was selected as reference template protein (Figure 5A). The model range of residual infrastructure was 2-1033, the sequence similarity was 0.59, and the template coverage was 1.00. After the mutation of *orf01200*, 1iwg.1.A was selected as reference template protein (Figure 5B). The model range of residual infrastructure was 7-1036, the sequence similarity was 0.59, and the template coverage was 1.00.

Before the mutation of *orf04094*, 4cti.1.B was selected as reference template protein (Figure 6A). The model range of residual infrastructure was 184–436, the sequence similarity was 0.56, and the template coverage was 0.59. After the mutation of *orf04094*, 3ib7.1.A was selected as reference template protein (Figure 6B). The model range of residual infrastructure was 10–262, the sequence similarity was 0.33, and the template coverage was 0.91.

Discussion

Regression analysis of MIC and induction time showed that the MIC value of the strain increased with the increase of exogenous antibiotic pressure and the induction time. Liu et al, showed that during the induction of *E. coli* resistance by imipenem, the MIC value increased with time.[14] Even when the induced concentration reached 128 times the MIC value of the primary strain, the induction was continued, and the MIC value continued to increase with induction. Consistent with the results of this study, the MIC value of *E. coli* increased with time and induced concentration. It shows that if the dose is not limited, the resistance of the strain will become more and more serious.

AMP was induced to *E. coli* 15743 for 63 hrs (MIC reached 32 μg/mL), and the MIC value was eight times that of the susceptible strain. Prior to this, the MIC value

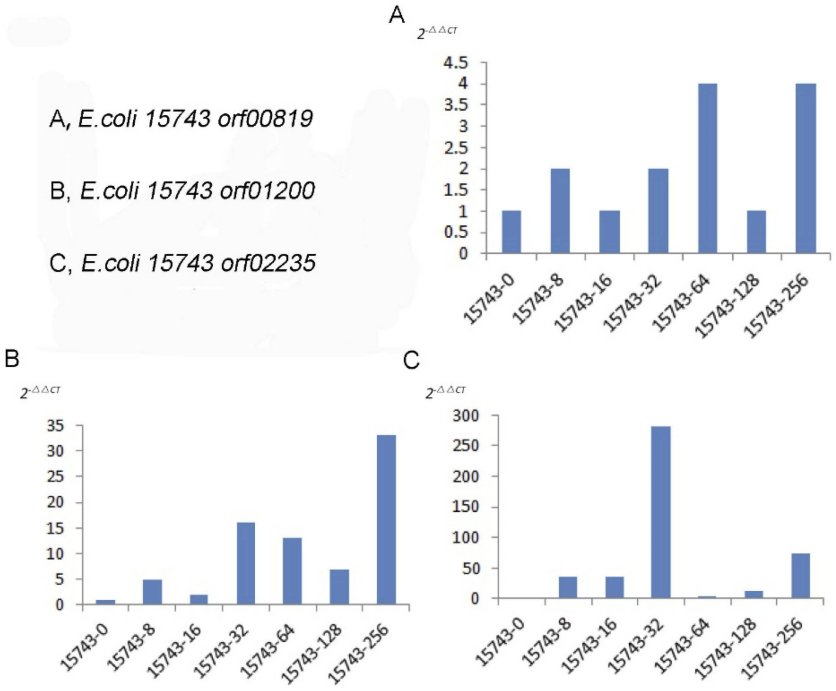

Figure 4 Results of mRNA expression in different generations of strains.

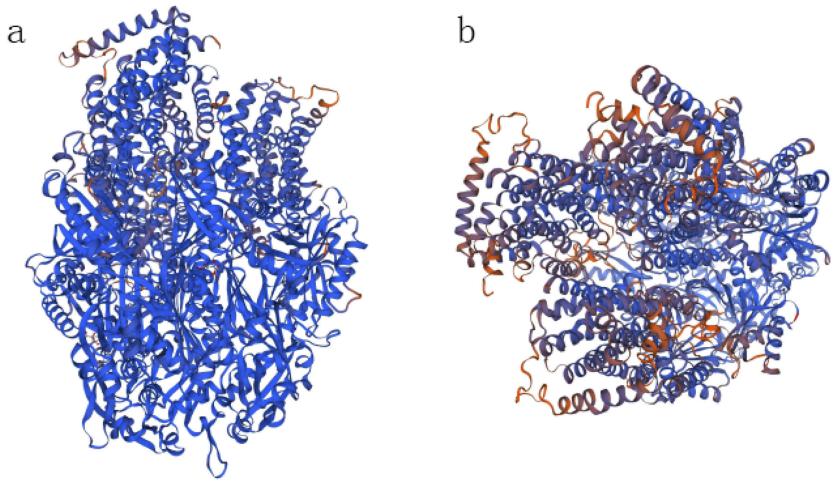

Figure 5 The tertiary structure of the protein encoded by *orf01200* gene.
Notes: (**A**) Before the mutation; (**B**) after the mutation.

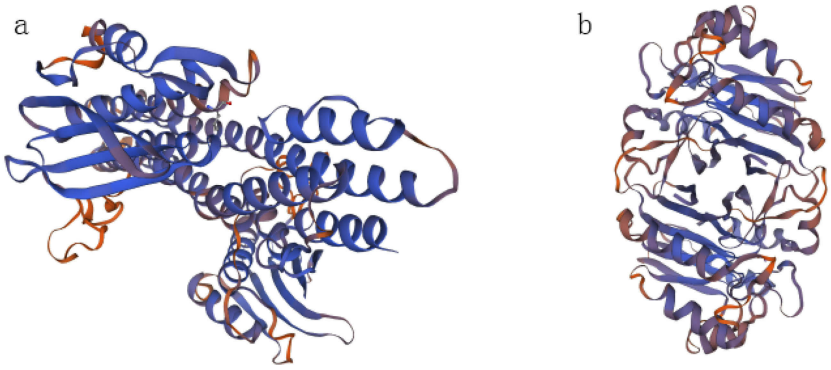

Figure 6 The tertiary structure of the protein encoded by *orf04094* gene.
Notes: (**A**) Before the mutation; (**B**), after the mutation.

increased rapidly, whereas when induced to a MIC value of 32 μg/mL, the induction continued and the growth rate of the MIC value decreased. Considering bacterial resistance can occur shortly before reaching the drug resistance threshold (MIC value of 32 μg/mL). After reaching the critical value, the bacteria may be lazy and grow slowly, but the MIC value continues to increase. It is also believed that this strain activates certain resistance mechanisms and changes the drug resistance status of the bacteria.

Zhang et al, showed that chloramphenicol induced sensitive *Shigella* to the drug-resistant state, and its drug resistance spectrum would change.[10] As a result, *Shigella* was not only resistant to chloramphenicol, but also resistant to other types of antibiotics. Consistent with the results of this study, the drug resistance spectrum of *E. coli* was amplified after induction. The results showed that *E. coli 15743-256* was not only resistant to AMP, but also to piperacillin, cefuroxime, cefazolin, cefoxitin, AMP/sulbactam, amoxicillin/clavulanic acid, piperacillin/tazobactam, and aztreonam were also resistant. It is considered that during the induction of *E. coli* by AMP, the expression system of AcrAB-TolC is activated, or more than one of the multiple efflux pump systems is activated, and there are other resistance mechanisms other than the efflux mechanism.

The molecular mechanism of bacterial resistance is still unclear. In order to investigate the specific molecular mechanism of *E. coli* resistance to AMP, bacterial WGS analysis was performed. The sequencing results were compared with the reference sequence, and 20 SNPs were screened from the sequence of *E. coli 15743-32*, 4 of which were non-synonymous SNPs. Twenty-six SNPs were screened from the *E. coli 15743-256* strain, eight of which were non-synonymous SNPs. Xiang et al, showed

that the resistance level of mutant strains was higher than that of non-mutant strains, and there was a quantitative reaction between point mutations and bacterial resistance levels, and multiple gene mutations could enhance the resistance of bacteria to antibiotics.[15] Consistent with the results of this study, the number of mutant genes in *E. coli 15743-32* was less than *E. coli 15743-256*, indicating that the number of mutations may be related to the degree of drug resistance, and the more mutation sites, the higher the degree of drug resistance.

After sequencing, the non-SNPs screened in this experiment were distributed in the genes of *orf00490, orf00819, orf01916, orf01200, orf02235, orf03479,* and *orf04094*. Among them, genes *orf00490, orf00819,* and *orf01916* are involved in cell wall synthesis. The annotations in KEGG are fumarate reductase subunit D (*frdD*), cell division protein ftsI (penicillin-binding protein 3) and porin outer membrane protein OmpD, respectively. Studies have shown that the *frd* gene encodes a FRD enzyme to catalyze the conversion between fumarate reductase and succinate dehydrogenase.[16] It has also been found that amplification of the *frdD* gene using a plasmid vector can increase the yield of succinic acid.[17,18] In combination with this study, it is considered that the *frdD* gene is involved in certain metabolic pathways, perhaps associated with AMP resistance. In *E. coli*, the main targets of β-lactam antibiotics are PBP1 (maintaining cell morphology), PBP2 (maintaining *E. coli* tension and rod shape), and PBP3 (related to bacterial division). PBP3 is a core component of cell division proteins that catalyze the cross-linking of cell wall peptidoglycans during cell division.[19–22] Studies have shown that down-regulation of OmpD protein and *OmpD* gene expression in bacterial biofilms leads to decreased cell membrane permeability and increased resistance to antibiotics.[23,24] Consistent with the results of this study, the *OmpD* gene mutation initiates a mechanism of bacterial resistance to β-lactam antibiotics, and the decrease in *E. coli* cell membrane permeability is one of the reasons for the increased resistance to AMP. It is considered that these changes in the function of proteins encoded by genes involved in cell wall synthesis affect the resistance of bacteria to AMP.

Genes *orf04094, orf01200, orf02235* are annotated in KEGG as osmotic pressure sensor histidine kinase (*envZ*), multi-drug efflux pump gene (*acrB*), and multi-drug resistance protein involved in transcriptional regulation (*marR*). In recent years, the active efflux mechanism is the main reason for the multiple drug resistance of bacteria.[25–27] Since most of the effluent system transports substrates widely, and many active effluent systems can exist in the same bacteria, this system can lead to bacterial resistance to various antibacterial drugs with completely different structures, namely multiple resistance. In Marlen Adler's study, mutations in the *ftsI* gene alone did not increase antibiotic resistance, whereas *ftsI* and *envZ* gene mutations increased the MIC of antibiotics multiple times. Cohen et al, demonstrated that the function of the inhibitory protein encoded by the mutated *MarR* gene would be reduced, and the effect of the bacteria on the multiple resistance of antibiotics was small when the *MarR* mutation was only detected.[28] Merric et al, found that *E. coli* showed only low levels of multi-drug resistance when the *MarR* gene was mutated.[29] The results of this study showed that multiple genes were simultaneously mutated and *E. coli* resistance to AMP increased.

Gene *orf03479* is annotated as valine glycine repeat G (*VgrG*) protein in KEGG. The Type VI Secretion System (T6SS) is a phage-related system that exists in many bacterial pathogens, such as *E. coli, Pseudomonas aeruginosa*, and *Burkholderia cenocepacia*. The effector factors can be secreted to the extracellular of bacteria, and the protein secretion system is closely related to virulence of pathogenic bacteria. Wang Jianfeng et al, showed that *VgrG* gene mutation affects the toxicity and drug resistance of bacteria, but the function of glutamate valine repeat protein is still unclear.[30] This study considers that the *VgrG* gene may be associated with AMP resistance, and its mechanism needs further investigation.

In summary, the COG function of these mutant genes is related to the origin of cell membranes, transport and metabolism of inorganic ions, transcription and signal transduction mechanisms. Studies have shown that under antibiotic stress, bacteria can take both active defense and passive defense to ensure their survival.[31] In passive defense, bacteria make itself dormant, reduce the vitality of life and block the combination of antibiotics and target to reduce the killing effect of antibiotics. In active defense, they increase the activity of efflux pump to increase the efflux of antibiotics and reduce the accumulation of antibiotics in bacteria, thereby reducing the killing effect of antibiotics on bacteria. This study suggests that the resistance of *E. coli* to AMP is a combination of active defense systems and passive defense systems. Drug resistance can occur shortly before the bacterial MIC value reaches the drug resistance threshold. Genes *frdD, ftsI, acrB, OmpD, marR, VgrG,* and *envZ* are associated with AMP

resistance. These studies will help to improve the molecular mechanism of *E. coli* resistant to β-lactam antibiotics, and provide a research basis for the prevention and control of multi-drug-resistant bacteria and the targets of new antibiotics.

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Author contributions

All authors contributed to data analysis, drafting or revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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