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ORIGINAL RESEARCH

IS*Ecp1*-mediated transposition of chromosome-borne bla_{CMY-2} into an endogenous ColE1-like plasmid in *Escherichia coli*

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Background: CMY-2 is the most prevalent pAmpC β-lactamase, but the chromosomal bla_{CMY-2} gene transfer via horizontal transmission has been seldom reported. This study aimed to describe an IS*Ecp1*-mediated transposition of a chromosomal bla_{CMY-2} gene from *Escherichia coli* into a small endogenous ColE1-like plasmid, resulting in elevated resistance to extended-spectrum cephalosporins.

Methods: Three ESCs-resistant ST641 *E. coli* strains EC6413, EC4103 and EC5106 harbored the bla_{CMY-2} gene. S1- PFGE, I-*ceu* I-PFGE, Southern blotting and electroporation experiments were performed to investigate the location and transferability of bla_{CMY-2}. The genetic context and gene expression of bla_{CMY-2} in the original isolates and the corresponding electroporants were explored by PCR mapping, primer walking strategy and RT-qPCR.

Results: The bla_{CMY-2}-containing region (IS*Ecp1*-bla_{CMY-2}-Δ*blc*-Δ*yggR*-Δ*tnp1*-*orf7*-*orf8*-*orf9*-Δ*tnp2*-Δ*hsdR*) was transposed into endogenous ColE1-like plasmid pSC137 in the process of electroporation at very low frequencies (10^{-8}–10^{-9}). The transpositions resulted in novel larger bla_{CMY-2}-harboring ColE1-like plasmids with size of 14,845 bp, enabling increase in MICs of 2 to 8-fold for cefotaxime, ceftiofur, and ceftazidime in recipient strains over their respective original counterparts. Transcriptional level analysis revealed that the increased bla_{CMY-2} expression was correlated with elevated MIC values of cephalosporins. The bla_{CMY-2} transposition unit was identical to that in a clinical isolate *E. coli* TN44889 from France isolated in 2004.

Conclusions: Our results firstly demonstrated that IS*Ecp1* mediated a transposition of chromosome-borne bla_{CMY-2} into an endogenous ColE1-like plasmid by electroporation. Amplification of the bla_{CMY-2} gene facilitates the strain adaptation to a changed environment with an elevated antibiotic pressure.

Keywords: bla_{CMY-2}, chromosome-borne, ColE1-like plasmid, IS*Ecp1*-mediated transposition, extended-spectrum cephalosporin

Introduction

Third- and fourth-generation extended-spectrum cephalosporins (ESCs) are used to treat both intestinal and extraintestinal *Escherichia coli* infections in human and veterinary medicine.[1,2] However, resistance rates due to extended spectrum β-lactamases and plasmid-mediated AmpC (pAmpC) β-lactamases are increasing.[3] Moreover, pAmpC β-lactamases are active against cephamycins, especially cefoxitin, and are not inactivated by β-lactam/β-lactamase inhibitor combinations such as amoxicillin/clavulanic acid combination.[4]

CMY-2 is the most prevalent pAmpC β-lactamase and has been reported in *E. coli* worldwide.[5] This is largely due to the spread of IncA/C and IncI1 plasmids among *E. coli* from humans, animals, and even environmental sources.[6–8] In addition to IncA/C and IncI1

plasmids, insert sequence, IS*Ecp1*, also plays an important role in spread of bla_{CMY-2}.[9–11] IS*Ecp1* seems to mobilize the adjacent resistance genes through transposition by using a weakly related downstream sequence in combination with left inverted repeat (IRL).[12] The plasmid-borne bla_{CMY-2} most likely originated from the *Citrobacter freundii* chromosome by IS*Ecp1*-mediated transposition.[9,11]

Research on antimicrobial resistance plasmids has been mainly focused on large plasmids and the role that small plasmids play in resistance gene transfer is not clear. ColE1-like plasmids are small with sizes ranging from ~2 to ~10 kb and have their replication driven only by host-encoded proteins.[13,14] Rather surprisingly, prior to 2006, ColE1-like plasmids and other small plasmids were seldom implicated in the spread of antibiotic resistance.[15] However, during the last decade, ColE1-like plasmids were identified which disseminated resistance genes for β-lactams (bla_{CMY}, bla_{CTX-M}, bla_{IMP-8}, $bla_{OXA-181}$, $bla_{OXA-232}$, bla_{KPC-2}, bla_{GES-5} and bla_{BEL-1}), quinolones (*qnrB*, *qnrS*, and *aac-(6')-Ib-cr*) as well as kanamycin (*aph(3')-I*).[13,16–23] ColE1 plasmids are not self-transmissible but can be mobilized by a helper plasmid.[24] Furthermore, like many other non-conjugative plasmids, ColE1 plasmids are multiple copy plasmids in *E. coli*.[25] The high copy number could maintain their segregational stability in the absence of any active and specific segregation mechanism.[26] Therefore, once the resistance genes are acquired by ColE1-like plasmids, their mobility and high copy number may accelerate dissemination of these genes.

Numerous studies have indicated a greater probability of the spread of plasmid-borne bla_{CMY-2} genes. However, the chromosomal bla_{CMY-2} gene transfer through horizontal transmission has been seldom documented, except for an SXT/R391-like integrative conjugative element, which was implicated in the spread of chromosomal bla_{CMY-2} in *Proteus mirabilis*.[27] In our previous study, we found that the bla_{CMY-2} gene from three ESC-resistant ST641 *E. coli* strains could not be transferred by conjugation, indicating an alternative gene location of bla_{CMY-2} in these strains.[28] To determine the location of bla_{CMY-2} gene and its transferability in these *E. coli* strains, we conducted a series of experiments, including electroporation, gene location, plasmids analysis, and the genetic contexts of bla_{CMY-2}, and confirmed that the chromosomal locations of the bla_{CMY-2} genes could transfer into an endogenous ColE1-like plasmid through an IS*Ecp1*-mediated transposition.

Materials and methods

Bacterial strains

Three ESC-resistant *E. coli* strains EC6413, EC4103, and EC5106 were isolated from rectal swab samples from sows on a large farrowing farm in Southern China in August 2011 as previously reported.[28] They were identified as clonal ST641 *E. coli* strains, but only strains EC6413 and EC4103 belonged to the same *Xba*I-pulsed-field gel electrophoresis (PFGE)-type (>90% similarity).

Gene location and transfer of bla_{CMY-2}

We used S1-PFGE and I-*Ceu* I-PFGE to determine the genomic locations of the bla_{CMY-2} genes. Briefly, plasmid analysis was carried out in the three original isolates by DNA linearization with S1 nuclease (Takara, Dalian, China) followed by PFGE.[29] Total DNA was also digested with I-*Ceu* I (NEB, Ipswich, MA, USA) followed by PFGE.[30] Southern blotting was carried out on both S1-PFGE and I-*Ceu* I-PFGE gels with digoxigenin-labeled probes (Roche Diagnostics GmbH, Mannheim, Germany) specific for bla_{CMY-2} gene and bla_{CMY-2}/*23S rDNA* gene, respectively.

Plasmid DNA from the original strains was extracted by Qiagen Prep Plasmid Midi Kit (Qiagen NV, Venlo, the Netherlands) and electroporated into electrocompetent *E. coli* DH5α (TaKaRa, Dalian, China) and *E. coli* DH10B (stored in our laboratory) by using a Gene Pulser apparatus (Bio-Rad Laboratories Inc., Hercules, California, USA). Electroporants were selected on MacConkey agar plates supplemented with cefoxitin (16 μg/mL). Cells harboring bla_{CMY-2} were confirmed by polymerase chain reaction (PCR) with specific primers as previously described.[31] The minimum inhibitory concentrations (MICs) of cefoxitin (FOX), ceftiofur (CIF), cefotaxime (CTX), ceftazidime (CAZ), kanamycin (KAN), amikacin (AMK), florfenicol (FLF), doxycycline (DOX), and ciprofloxacin (CIP) were determined for the electroporants and the original isolates by the agar dilution method. The results were interpreted according to Clinical and Laboratory Standards Institute (CLSI, 2013; 2015) standards. *E. coli* strain ATCC 25922 was used as the quality control strain. Transfer frequency was calculated as the number of electroporants harboring bla_{CMY-2} divided by the starting number of *E. coli* cells used for electroporation. Incompatibility (Inc) groups were assigned by PCR-based replicon typing (PBRT) of the electroporants as previously described.[32]

Detection of the flanking regions surrounding the bla_{CMY-2} gene

The genetic contexts of bla_{CMY-2} in the original isolates and the complete nucleotide sequences of plasmids harboring bla_{CMY-2} in the electroporants were explored by PCR mapping and a primer walking strategy (Table S1). In addition, one specific set of primers was designed to detect the endogenous ColE1-like plasmid pSC137. The PCR amplification region contained HP4-IS5-RNAII/RNAI. Another five primer pairs were

designed to identify the bla_{CMY-2} loci containing the conserved bla_{CMY-2} region and DNA segments from plasmid pSC137.

Plasmid analysis and second-round electroporation

To further determine the location of bla_{CMY-2} and RNAII (involved in the replication of ColE1-like plasmids), plasmid analysis was carried out in the three electroporants with S1-PFGE followed by Southern blot hybridization with the bla_{CMY-2} and RNAII probes (Table S1) as described above. A second round of electroporation was performed by using plasmids isolated from the first-round electroporants, and transfer frequencies of bla_{CMY-2} were scored as described above. Electroporants from the second round were also tested for antimicrobial susceptibility.

Relative quantification of the mRNA expression of bla_{CMY-2}

E. coli DH5α electroporants as well as the original isolates were evaluated for the expression of bla_{CMY-2} gene. Total RNA was extracted from 1 mL of a 24 h culture in Lysogeny broth (LB) without antibiotics grown at 37°C using an RNAprep pure Cell/Bacteria Kit (Tiangen Biotech, Beijing, China). The total RNA was reverse transcribed by using a PrimeScript RT reagent kit (with DNA Eraser) and random hexamers according to the manufacturer's instructions (TaKaRa). The cDNA samples were used for quantitative real-time PCR (qPCR). Primers used in qPCR are listed in Table S1, and the 16S rRNA gene was used as an internal control for mRNA quantification. Quantification was performed on a Bio-Rad IQ5 instrument (Bio-Rad Laboratories Inc.) by using SYBR Premix Ex Taq TM (TaKaRa) according to the manufacturer's instructions. The thermal conditions were initial denaturation at 95°C for 5 min, followed by 40 cycles of 95°C for 40 s, 63°C for 40 s, and 72°C for 45 s. qPCR assays were performed in duplicate, and each assay sample was tested in triplicate. Product specificity was verified by melting curve analysis by using the software provided with the instrument. 16S rRNA was used to normalize for gene expression levels. Relative quantification was calculated by the $2^{-\Delta\Delta CT}$ method[33] ($\Delta\Delta C_T = (C_{T,Target}–C_{T,Control})_{electroporant} – (C_{T,Target}–C_{T,Control})_{original\ isolate}$).

Nucleotide sequence accession number

The complete nucleotide sequences of plasmids pSC137, pEC4103, pEC5106, and pEC6413 have been assigned GenBank accession numbers KT074362, KY612498, KY612499, and KY612500, respectively.

Results

Location and transfer of bla_{CMY-2}

In our original experiments, we identified three ST641 *E. coli* strains that harbored the bla_{CMY-2} gene, but our attempts at conjugation were unsuccessful.[28] Analysis of genomic DNA from these strains using S1-PFGE identified three or four visible plasmid bands in S1-PFGE gels with sizes ranging from <20 to ~140 kb. Southern blot analyses of I-*Ceu* I and S1-PFGE gels using bla_{CMY-2} probe revealed hybridization only to the chromosome. None of the endogenous plasmids were hybridized to the bla_{CMY-2} probe (Figure S1).

Nonetheless, the three ST641 *E. coli* strains were still able to transfer cefoxitin resistance as well as the bla_{CMY-2} gene to the recipient strains *E. coli* DH5α and DH10B, albeit at low frequencies (10^{-8}–10^{-9}). Interestingly, all the electroporants were ESC resistant with MICs equal to 16–32 μg/mL for CTX, CIF, and CAZ. These values represented 2- to 8-fold increase when compared with their respective original strains (Table 1). No other non-β-lactam resistance was transferred in this process, and no replicon was identified by PBRT in any of the electroporants.

Detection of the flanking regions of the bla_{CMY-2} gene

In the three ST641 *E. coli* isolates, the genetic context of the bla_{CMY-2} gene was identical to that of a clinical isolate *E. coli* TN44889 from France in 2004 (Acc. No. FM246884). This region included a bla_{CMY-2} gene-containing region comprising 11 open reading frames (ORFs) (Figure 1). For the electroporants, we obtained the complete nucleotide sequences of the 14,845 bp circular plasmids (designated pEC6413, pEC4103, and pEC5106) harboring bla_{CMY-2} (Figures 1 and 2). In each plasmid, the 10,179 bp region containing IS*Ecp1*-bla_{CMY-2}-Δ*blc*-Δ*yggR*-Δ*tnp1*-*orf7*-*orf8*-*orf9*-Δ*tnp2*-Δ*hsdR* was identical to that from the chromosome of each original isolate. "Δ*tnp1*" was 97% identical to the last 144 amino acids of IS*200* (Acc. No. 2002282A), and "Δ*tnp2*" was 99% identical to the last 139 amino acids of IS*60* Orf2 in *Shigella flexneri* 2a str. 301 (Acc. No. NP_707701). This region was also identical to that of the IncX4 plasmid pS62T (Acc. No. KP207590) found in our previous study (Figure 1).[31] However, each plasmid had a different arrangement of the bla_{CMY-2}-containing region and a complete transposition unit flanked by 5-bp direct repeats (DRs) that bounded this region in pEC6413 (ACTCA), pEC4103 (TAATA), and pEC5106 (TAAGA). One of the putative 5-bp DR was located immediately adjacent to the IRL (CCTAGATTCTACGT) of IS*Ecp1*. The other was

Table 1 Characteristics of the three CMY-2-ST641 *E. coli* strains and their electroporants

Strains	MIC (μg/mL) of the donors				Gene location of bla_{CMY-2} in the original isolates	MIC (μg/mL) of the electroporants				Size of pColE1-like CMY from electroporants (bp)	Frequency of the first round of electroporation DH5α/DH10B	Frequency of the second round of electroporation DH5α/DH10B
	FOX	CIF	CTX	CAZ		FOX	CIF	CTX	CAZ			
EC5106	128	16	4	16	Chromosome	64	32	16	32	14,845	10^{-8}–10^{-9}	10^{-2}–10^{-3}
EC6413	64	4	2	8	Chromosome	64	32	16	32	14,845	10^{-8}–10^{-9}	10^{-2}–10^{-3}
EC4103	64	4	2	8	Chromosome	64	32	16	32	14,845	10^{-8}–10^{-9}	10^{-2}–10^{-3}
DH5α/DH10B	–	–	–	–	–	2	0.125	0.03	0.03	–	–	–

Note: Electroporants were challenged for the electroporation by using recipients DH5α or DH10B.
Abbreviations: FOX, cefoxitin; CIF, ceftiofur; CTX, cefotaxime; CAZ, ceftazidime; MIC, minimal inhibitory concentration.

located immediately adjacent to the deduced right inverted repeats of IS*Ecp1* (IRR1: GCGCAGTTTTTCGA).

Further analysis revealed that this transposition unit had been inserted into a small plasmid pSC137 (Figure 1). Plasmid pSC137 is a 4,661-bp ColE1-like plasmid containing five ORFs. The region from 355 to 899 bp that contained the RNA I/II region was 94% identical to that in the ColE1-like pNPO1 (Acc. No. KF992024). This region is involved in the initiation and control of ColE1-like plasmid replication (Wang et al[14]). The segments from 120 to 1,077 bp and 686 to 1,796 bp were 91% and 95% identical to that in the ColE1-like plasmids pNPO1 (Acc. No. KF992024; 190-1,170) and pB1022 (Acc. No. JQ319766; 1-1,121), respectively. Furthermore, pSC137 was identical to its counterpart in the CMY-containing ColE1-like plasmids (Figure 2).

PCR confirmed that pSC137-like plasmids existed in the three original strains, while bla_{CMY-2} loci were absent from pSC137-like plasmids. But the bla_{CMY-2} regions appeared on pSC137-like plasmids in the electroporants (Table S2). These results demonstrated that the chromosomal bla_{CMY-2} region could be transferred to the endogenous pSC137-like plasmids, generating larger ColE1-like plasmids containing bla_{CMY-2} by electroporation.

Plasmid analysis and second-round electroporation

Southern blot analysis was performed on the uncut plasmids from both the original isolates and *E. coli* DH5α electroporants to determine the location of the bla_{CMY-2} and RNAII genes. In the original isolates, none of the endogenous plasmids was hybridized with the bla_{CMY-2} gene which indicated that this gene may be located on the chromosome (Figure S1). On the other hand, a small endogenous plasmid was hybridized with the RNAII probe. In the electroporants, both the bla_{CMY-2} and RNAII probes were hybridized to the same plasmids, and they were larger than the small endogenous plasmids that were hybridized with the RNAII probe. These data indicated that the chromosomal bla_{CMY-2} gene had transferred to endogenous plasmid pSC137 that contained the RNAII gene.

Compared to that in the first round of electroporation, the plasmids could be transferred at high frequencies (10^{-2}–10^{-3}) in the second round of electroporation, but the MIC values of FOX, CTX, CIF, and CAZ in these electroporants were the same as that obtained in the first round of electroporation (Table 1).

Relative quantification of the mRNA expression of bla_{CMY-2}

Transfer of bla_{CMY-2} gene resulted in increased ESC resistance in the electroporants over that of the respective original iso-

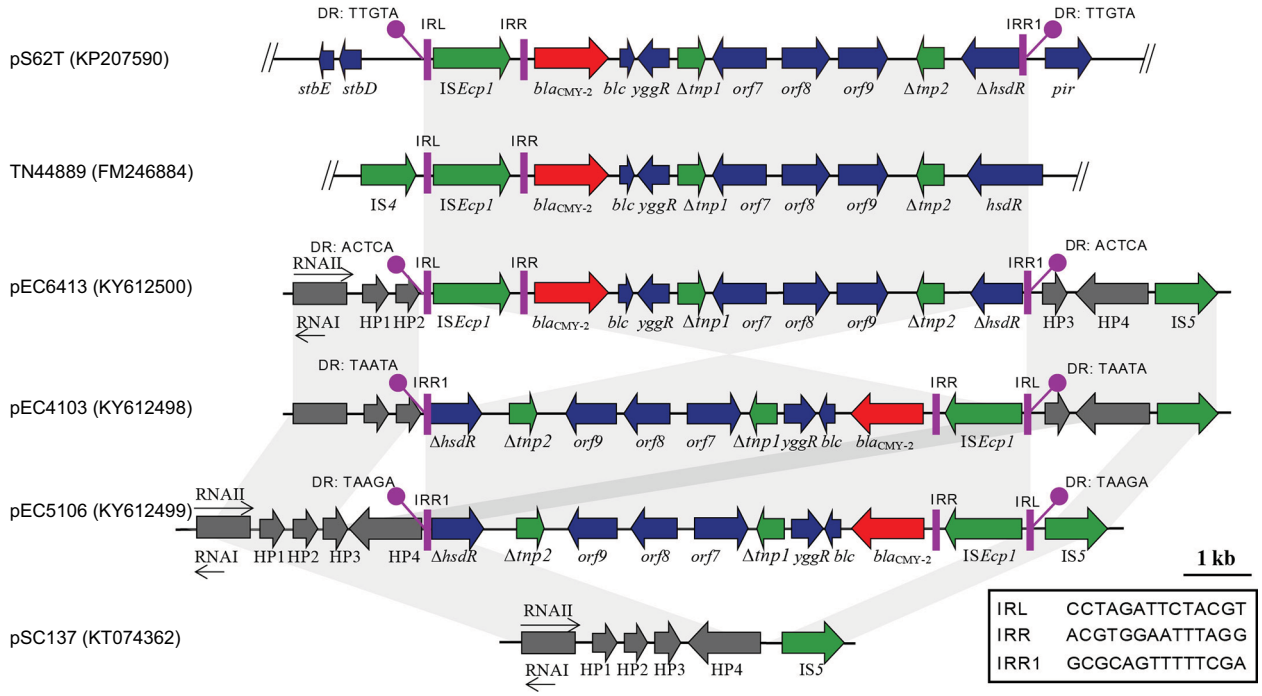

Figure 1 Characteristics of the genetic contexts of bla_{CMY-2}. Structural comparison of plasmids pEC6413, pEC4103, and pEC5106 from the electroporants, ColE1-like plasmid pSC137 from the clinical *Escherichia coli* strain, IncX plasmid pS62T from *E. coli* strain (Acc. No. KP207590), and the clinical *E. coli* strain TN44889 (Acc. No. FM246884). Regions of >99% homology are marked by grey shading. The gray rectangles indicate replication-associated genes of ColE1-like plasmids; gray arrows indicate other genes in the original ColE1-like plasmids. The resistance genes, insertion sequences, and other accessory genes are indicated by red, green, and blue arrows, respectively.
Abbreviations: DR, direct repeat sequences generated by IS*Ecp1*-mediated transposition; IRL and IRR1, left and right inverted repeats of IS*Ecp1*, respectively.

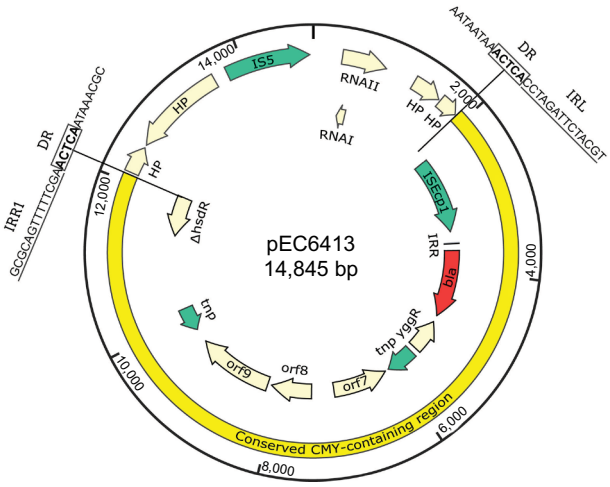

Figure 2 Characteristics of the complete nucleotide sequences of the plasmids pEC6413. The DRs generated by IS*Ecp1*-mediated transposition are highlighted in boldface. The IRL of IS*Ecp1* and the IRR1 of IS*Ecp1* are marked by the underlined letters.
Abbreviations: DR, direct repeat sequences generated by IS*Ecp1*-mediated transposition; IRL and IRR1, left and right inverted repeats of IS*Ecp1*, respectively.

lates. This indicated that gene expression of bla_{CMY-2} was most likely increased. The steady state levels of bla_{CMY-2} mRNA were elevated 14.420-fold (±1.084), 14.455-fold (±1.309), and 7.980-fold (±0.833) in EC6413T, EC4103T, and EC5106T with respect to the original isolates, respectively.

Discussion

In this study, we describe an IS*Ecp1*-mediated transposition of the chromosomally encoded bla_{CMY-2} gene into an endogenous ColE1-like plasmid in three ESC-resistant ST641 *E. coli* strains, which was supported by evidence: 1) the region including 10 ORFs (IS*Ecp1*-bla_{CMY-2}-Δ*blc*-Δ*yggR*-Δ*tnp1*-*orf7*-*orf8*-*orf9*-Δtnp2-Δ*hsdR*) was identical in the ColE1-like plasmids in electroporants and in chromosome of the original isolates; 2) the size of ColE1-like plasmids in electroporants were larger than that in the respective original isolates; 3) a suspected DR exactly emerged neighboring IRL (CCTAGATTCTACGT) and the proposed IRR1 (GCGCAGTTTTTCGA) of IS*Ecp1* (Figures 1 and 2). Thus, we speculated that the fragment carrying bla_{CMY-2} could be introduced into the ColE1-like plasmid during the electroporation experiments.

IS*Ecp1* plays an important role in the mobilization of bla_{CMY-2} gene, and in general, IS*Ecp1* located in front of the antimicrobial resistance gene and moves toward its adjacent region by recognizing its own IRL and supposed IRR, resulting in 5-bp DRs.[34] In our present study, the proposed transposition fragment comprises a typical IS*Ecp1*-mediated unit, in which IS*Ecp1* was 116 bp in front of bla_{CMY-2} and followed by Δ*blc*-Δ*yggR*-Δ*tnp1*-*orf7*-*orf8*-*orf9*-Δ*tnp2*-Δ*hsdR*. All the trans-

position fragments shared 100% identity in the three strains, transposed in identical ColE1-like plasmids. However, the transposition unit in pEC5106 and pEC4103 was completely reversed compared with that in pEC6413, and it was inserted in different locations in ColE1-like plasmids (Figure 1). The transposition generated 5-bp different DRs (ACTCA, TAATA, and TAAGA) adjacent to the IRLs and IRR1s of IS*Ecp1* elements in the three strains. It agreed with previous research where IS*Ecp1*-mediated transposition always resulted in AT-rich DR.[34] In addition to bla_{CMY-2} gene, IS*Ecp1*-mediated transposition was also reported to be related to the spread of bla_{CTX-M},[34,35] bla_{KPC},[36] *qnrB*-like genes,[37] and *rmtC* gene.[38]

Indeed, IS*Ecp1*-mediated transposition might take place in both the original strain and electroporation. However, PCR detection and Southern blotting confirmed the absence of bla_{CMY-2} loci in pSC137 in the original strains, and it was also unsuccessful for our several attempts to obtain the transconjugants harbored bla_{CMY-2} by conjugation (Table S2). Therefore, we speculate that IS*Ecp1*-mediated transposition of bla_{CMY-2} probably occurred in recipient strains rather than original strains. But it could not be excluded that they occurred in the original strains and could not be detected by Southern blot or PCR. Based on the observed structure and detailed sequence analysis, we propose a most probable model for the route of chromosome-borne bla_{CMY-2} into an endogenous ColE1-like plasmid. First, the CMY-containing region was dropped from chromosomal DNA in the process of IS*Ecp1*-mediated transposition. Second, both this region and plasmid DNA were acquired through plasmid extraction and electroporation. Subsequently, the CMY region was integrated into the ColE1-like plasmid under cefoxitin selective pressure, generating a novel ColE1-like plasmid carrying bla_{CMY-2} (Figure 3).

In the present study, the efficiency of bla_{CMY-2} translocation in the second-round was much higher than that in the first-round electroporation, which was consistent with, that ColE1-like plasmid could be transformed into *E. coli* with high efficiency.[39] Interestingly, the transfer of bla_{CMY-2} into the electrocompetent cells contributed to increasing resistance to ESCs even over that of the respective original isolates (Table 1). Positive correlations between β-lactam MICs and β-lactamase gene expression have been previously shown.[40,41] In our study, the relative expression (steady-state mRNA levels) of bla_{CMY-2} was significantly increased in electroporants compared with the original isolates, which might result from the high copy number of ColE1-like plasmids.[42] The chromosome-borne IS*Ecp1*-mediated transposition of bla_{CMY-2} gene into high copy number ColE1-like plasmids would not only increase the resistance levels against cephalosporins but also greatly improve the potential to spread the bla_{CMY-2} gene due to the raised transfer efficiency.

Conclusion

This is the first report of IS*Ecp1*-mediated transposition of the chromosome-borne bla_{CMY-2} gene into a small endogenous plasmid with high copy numbers in *E. coli*. This may increase the levels of cephalosporins resistance, providing an alternative adaptive survival mechanism for bacteria, especially at

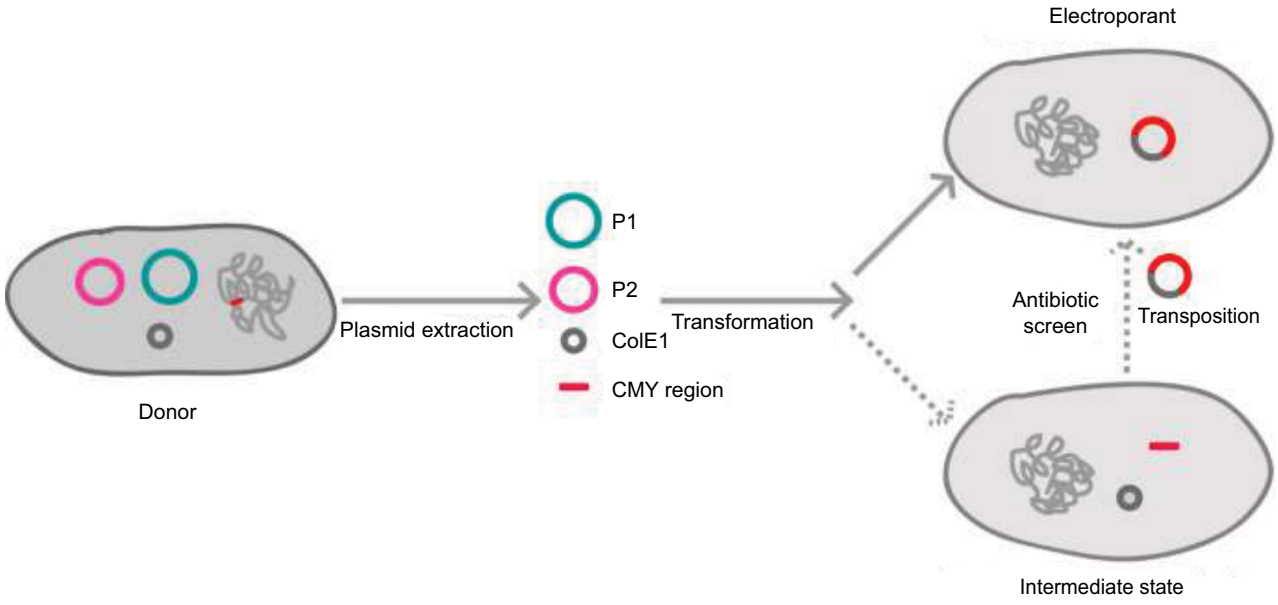

Figure 3 Schematic representation of the transfer of the chromosome-encoded bla_{CMY-2} gene into ColE1-like plasmid. On the left side is the donor strain. On the right side is the electroporant involved in the transposition phenomena by electroporation.

high cephalosporin concentrations, and facilitate the spread of bla_{CMY-2} gene in *E. coli* strains.

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Author contributions

J Sun, Y-H Liu, and P-X Liao designed the experiments and provided reagents and supplies. L-X Fang performed the experiments, analyzed the data, and wrote the manuscript. J Sun, L Li and X-P Li analyzed the data and revised the manuscript. X-P Li, M-Y Chen, C-Y Wu, and L-L Li performed the experiments. All authors contributed toward data analysis, drafting and revising the paper 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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Supplementary materials

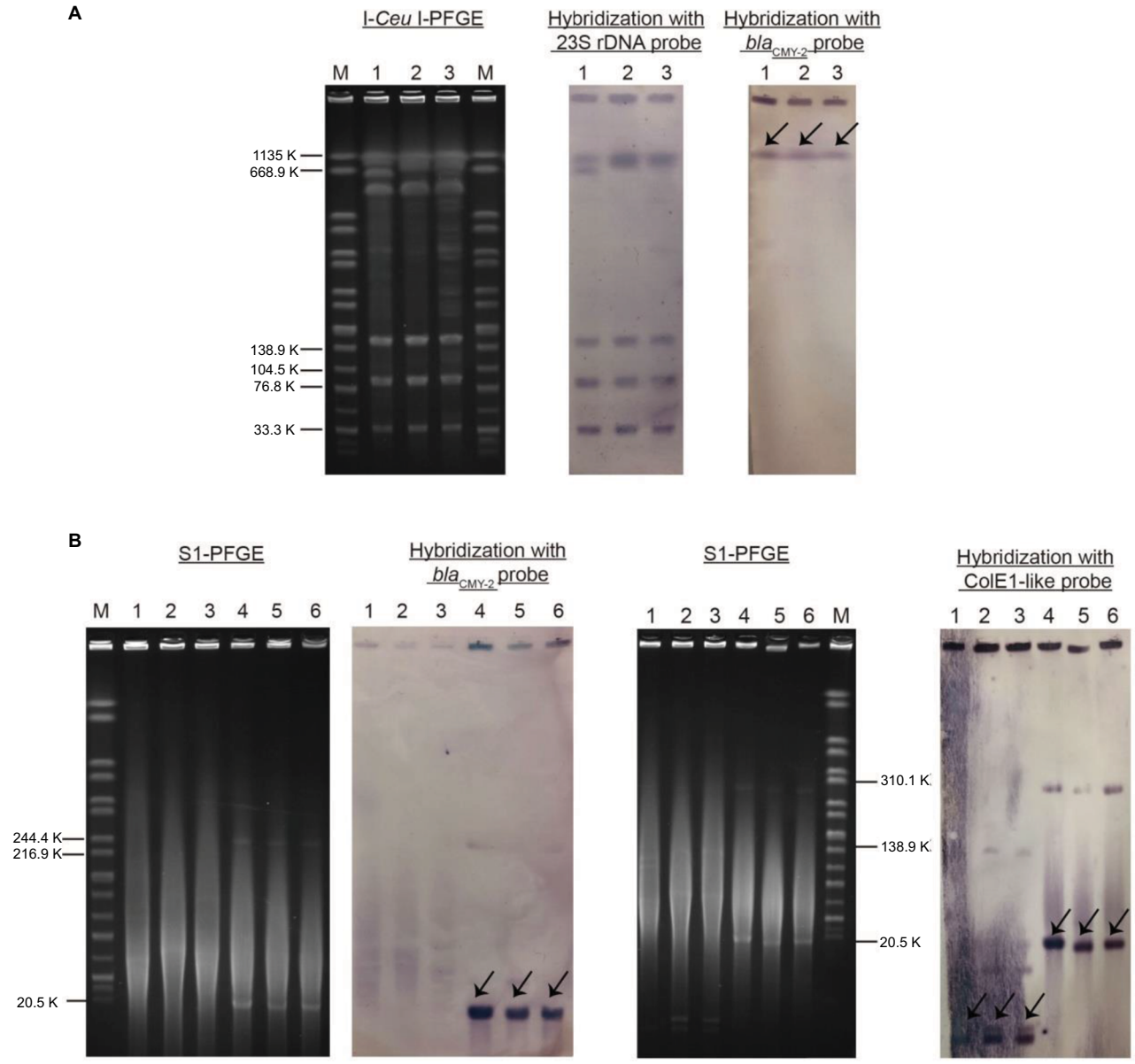

Figure S1 (**A**) I-*Ceu* I-PFGE and Southern blot hybridization with the 23S rDNA and *bla*CMY-2 probes; (**B**) S1 nuclease-PFGE Southern blot hybridization with the *bla*CMY-2 and ColE1-like probe. Line M: H9812 marker; Lines 1–6: EC6413, EC4103, and EC5106, and their corresponding electroporants EC6413T, eC4103T, and EC5106T. Arrows represent the band hybridized with the bla_{CMY-2} or ColE1-like probe.

Abbreviation: PFGE, pulsed-field gel electrophoresis.

Table S1 Primers used for screening for genes and PCR mapping

PCR	Primer	Primer sequence (5′→3′)	Product length (kb)	Target	Reference
A	A^F	GCACTTAGCCACCTATACGGCAG	0.758	bla_{CMY-2} for digoxigenin-labeled probes	1
	A^R	GCTTTTCAAGAATGCGCCAGG			
B	B^F	AATGATGGCCAGGCTGTCTCC	–	23SrRNA for digoxigenin-labeled probes	2
	B^R	CCGCCGTCGATATGAACTCTTG			
C	C^F	GAAGGTTCTCAGAGCTGCAAC	0.3	RNAII for digoxigenin-labeled probes	This study
	C^R	GCCGCGTTTATCTCATTCCAC			
D	D^F	ATCGTCTTTTACCGCCTGTCC	3.1	Screening for the plasmid of pSC137	This study
	D^R	GTTAGCCCTATCCTGCATCGT			
E	E^F	GTCATCGCTGGGAAATCGAAC	3.8	Junction between the *IS4* and yggR	3
	E^R	GCATAACGTCTCGGATCTACACC			
F	F^F	AACTTGACGCCGAAGCCTA	4.8	Junction between the bla_{CMY-2} and *orf9*	3
	F^R	TACGCCTGCAAAATATCACCA			
G	G^F	TTTGTACTGCCAACGTATCCAA	2.2	Junction between the *orf9* and ΔhsdR	3
	G^R	AAAGAACGGGAAATTGCCAAC			
H	H^F	CCATAACAGCGGAATGACACC	3.0	Junction between RNAII and bla_{CMY-2} in the plasmid of pEC6413	This study
	H^R	CAGAGCGCAGCATAACGAT			
I	I^F	ACCGGGTTTTCATCCACGA	1.12	Junction between ΔhsdR and HP3 in the plasmid of pEC6413	This study
	I^R	GTGCGTCTGACCAATATCCAC			
J	J^F	CCATAACAGCGGAATGACACC	3.2/4.8	Junction between RNAII and ΔhsdR in the plasmids of pEC4103 or pEC5106	This study
	J^R	AGGGCTTTACCTGTCAGCTC			
K	K^F	CAGAGCGCAGCATAACGAT	2.3/5.3	Junction between bla_{CMY-2} and HP3 in the plasmid of pEC4103 or pEC5106	This study
	K^R	CGTAATCCGTTGCCAGAGCC			
L	L^F	TATTGTAGCATCGGTTTCCCA	5.4	Junction between the IS*Ecp1*and ΔhsdR including pSC137	This study
	L^R	GCGCGAACATACATATCCAGT			
M	M^F	GCTGCTGACAGCCTCTTT	0.197	qCMY2 for quantitative real-time PCR assays	4
	M^R	GCGTGACTGGGTGGTTAT			
N	N^F	GGCCGCAAGGTTAAAACTCAAATG	0.243	*16S rRNA* for quantitative real-time PCR assays	5
	N^R	AACCGCTGGCAACAAAGGATAAGG			

Notes: F, forward primer; R, reverse primer. "L" represents reverse PCR primers and its 4 amplification region containing IS*Ecp1*-pSC137-Δ*hsdR*.
Abbreviation: PCR, polymerase chain reaction.

Table S2 PCR-typing bla_{CMY-2} gene-containing loci transferred into endogenous plasmid pSC137

PCR typing	Target	Size (kb)	Electroporants			Wild strains		
			EC5106T	EC6413T	EC4103T	EC5106	EC6413	EC4103
H	Junction between RNAII and bla_{CMY-2}	3.0	+	–	–	–	–	–
I	Junction between ΔhsdR and HP3	1.12	+	–	–	–	–	–
J	Junction between RNAII and ΔhsdR	3.2/4.8	–	+	+	–	–	–
K	Junction between bla_{CMY-2} and HP3	2.3/5.3	–	+	+	–	–	–
L	Region containing IS*Ecp1*-pSC137-ΔhsdR	5.4	+	+	+	–	–	–

Note: (+) positive and (–) negative.
Abbreviation: PCR, polymerase chain reaction.

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