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Short Communication

# Dissemination of the chromosomally encoded CMY-2 cephalosporinase gene in *Escherichia coli* isolated from animals

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ABSTRACT

In this study, 619 individual *Escherichia coli* isolates from food-producing and companion animals were analysed to determine the prevalence of the cephalosporinase gene *bla*$_{CMY-2}$. In total, 18 CMY-2-producers (2.9%) were detected and exhibited multidrug-resistant phenotypes. One of the CMY-2-producers was found to possess a novel *bla*$_{CMY-2}$-like allele, *bla*$_{CMY-130}$. The isolates belonged to distinct pulsotypes, suggesting that the *bla*$_{CMY-2}$ gene was not disseminated by clonal expansion of *bla*$_{CMY-2}$-positive strains. The *bla*$_{CMY-2}$ genes were located on IncA/C-, IncHI2- or IncX-type plasmids in 9 (50%) of the 18 *E. coli* isolates. However, in the other nine isolates I-*Ceu*I-PFGE and hybridisation analyses revealed that the *bla*$_{CMY-2}$ gene was chromosomally located. A CMY gene-containing region composed of five open reading frames (ORFs) (IS*Ecp1*–*bla*$_{CMY-2}$–*blc*–*sugE*–Δ*encR*) was observed in plasmids from eight strains. A CMY gene-containing region composed of ten ORFs was observed in all of the nine chromosomally encoded *bla*$_{CMY-2}$ genes, including a putative IS*66*-like element inserted in this conserved CMY genetic region in three strains. This conserved CMY genetic region was also found to be inserted into the *oriV*$\gamma$ (putative gamma origin), part of the IncX plasmid backbone, by a complete transposition unit flanked by 5-bp DRs (direct repeat sequence) in pS62T. These results demonstrate the high prevalence of the chromosomally encoded *bla*$_{CMY-2}$ gene in *E. coli*. This is the first study reporting a chromosomally encoded *bla*$_{CMY-2}$ gene in *E. coli*. Chromosomally encoded *bla*$_{CMY-2}$ might be a source of some plasmid-mediated *bla*$_{CMY-2}$ genes and this probably facilitates the spread of cephalosporin-resistant strains.



## 1. Introduction

To date, a number of AmpC β-lactamases, especially plasmid-encoded AmpC β-lactamases (pAmpCs), have emerged in Enterobacteriaceae [1]. Among these acquired pAmpCs, *bla*$_{CMY-2}$ is the most prevalent and has been reported in various Gram-negative bacteria globally.

Vectors such as plasmids, insertion sequences (IS), integrative conjugative elements and genomic islands have been involved in the dissemination of *bla*$_{CMY-2}$ genes [2]. Plasmids have been most frequently associated with the mobilisation of *bla*$_{CMY-2}$ genes. Despite the diversity of plasmids encoding the *bla*$_{CMY-2}$ gene, IncA/C or IncI1 plasmids are the most frequently reported *bla*$_{CMY-2}$ carriers [3]. Rapid spread of these plasmids is responsible for the wide distribution of *bla*$_{CMY-2}$ genes among Enterobacteriaceae. However, there is little information regarding chromosomally encoded *bla*$_{CMY-2}$. For example, mobilisation of *bla*$_{CMY-2}$ was via a multidrug resistance genomic island located on the chromosome of *Salmonella enterica* serovar Typhimurium [4].

Although *bla*$_{CMY-40}$, another *bla*$_{CMY-like}$ gene, was confirmed to be located on the chromosome in one *Escherichia coli* isolate [5], there is little information regarding the prevalence of chromosomally encoded *bla*$_{CMY-2-like}$ genes in *E. coli*. In addition, the mechanism by which chromosomally located *bla*$_{CMY-2-like}$ genes become disseminated remains unknown. In this study, *bla*$_{CMY-2}$-producing *E. coli* isolates from diverse animal origins were examined for the following purposes: (i) to investigate the molecular epidemiology of *bla*$_{CMY-2}$ genes in *E. coli* isolates from food-producing and companion animals during 2010–2012; and (ii) to characterise the vectors involved in the dissemination of *bla*$_{CMY-2}$ genes.

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## 2. Materials and methods

### 2.1. Bacterial strains and susceptibility testing

During July 2010 to July 2012, 619 individual *E. coli* strains were obtained from a range of animal species (257 pigs, 175 poultry, 64 cows and 123 dogs) within a defined geographical area in South China. The 496 *E. coli* strains from food-producing animals (pigs, poultry and cows) were isolated from 520 samples submitted from animal farms. The 123 strains from companion animals (dogs) were isolated from 150 samples collected from pet animal hospitals in Guangzhou, China. These pet dogs had no contact with farm animals. All *E. coli* strains were examined for the presence of $bla_{\text{CMY-2-like}}$ genes by PCR amplification/sequencing. The entire $bla_{\text{CMY-2}}$ gene (1146 bp) coding regions were amplified with primers described in a previous study [5]. Antimicrobial susceptibility testing was performed by the agar dilution method following the guidelines of the Clinical and Laboratory Standards Institute (CLSI). The breakpoints for each antimicrobial were as recommended is CLSI documents M100-S18 and VET01-A4/VET01-S2 [6,7]. *E. coli* ATCC 25922 was used as a quality control strain.

### 2.2. Molecular typing

All of the $bla_{\text{CMY-2}}$-positive *E. coli* isolates were classified according to *Xba*I pulsed-field gel electrophoresis (PFGE) typing as previously described [8]. Comparison of PFGE patterns was performed with BioNumerics® v.6.6 (Applied Maths, Ghent, Belgium). Dendrograms were generated with the Dice similarity coefficient (1.5% optimisation and 1.5% tolerance) using the unweighted pair-group method with arithmetic mean (UPGMA), and PFGE types were defined with >90% similarity between clusters.

### 2.3. Transferability of $\text{bla}_{\text{CMY-2}}$ and plasmid replicon typing

Conjugation experiments were performed using *E. coli* C600 (streptomycin-resistant; minimum inhibitory concentration >2000 μg/mL) as a recipient. Transconjugants were selected on MacConkey agar plates (Huankai Co. Ltd., Guangzhou, China) supplemented with streptomycin (500–1000 μg/mL) (Sigma Chemical Co., St Louis, MO) and cefoxitin (16 μg/mL) (Sigma Chemical Co.). For isolates that failed in conjugation experiments, plasmid DNA was extracted using a QIAPrep Plasmid Midi Kit (QIAGEN, Hilden, Germany) and was transformed into electrocompetent *E. coli* DH5α (TaKaRa Biotechnology, Dalian, China) using a Gene Pulser apparatus (Bio-Rad Laboratories, Hercules, CA). Transformants were selected on MacConkey agar plates supplemented with cefoxitin (16 μg/mL). Incompatibility (Inc) groups were assigned by PCR-based replicon typing of the transconjugants/transformants [2].

### 2.4. Gene location

Plasmid analysis was carried out on the transconjugants/transformants or donor strains by DNA linearisation with S1 nuclease (TaKaRa Biotechnology) followed by PFGE [4]. For isolates in which a plasmid was not involved in the mobilisation of $bla_{\text{CMY-2}}$, the entire DNA was digested with I-*Ceu*I (NEB, Ipswich, MA), followed by PFGE [4]. Southern blotting was carried out on S1-PFGE or I-*Ceu*I-PFGE gels with digoxigenin-labelled probes (Roche Diagnostics GmbH, Mannheim, Germany) specific for the $bla_{\text{CMY-2}}$ gene and/or 23S rDNA gene.

### 2.5. Detection of the flanking regions of the $\text{bla}_{\text{CMY-2}}$ gene

The genetic context of the $bla_{\text{CMY-2}}$ gene was explored by PCR mapping and primer walking using the reference regions flanking the $bla_{\text{CMY-2}}$ gene (Supplementary Table S1).

### 2.6. Nucleotide sequence accession number

The partial nucleotide sequence in plasmid pS62 T, strain FS7E9S and the sequence of $bla_{\text{CMY-130}}$ have been submitted to GenBank and assigned accession nos. **KP207590**, **KP207588** and **KP207589**, respectively.

## 3. Results

### 3.1. Antimicrobial susceptibility

In total, 18 (2.9%) of the 619 *E. coli* strains were $bla_{\text{CMY-2}}$-producers, including nine from pigs (9/257; 3.5%), five from poultry (5/175; 2.9%), two from cows (2/64; 3.1%) and two from dogs (2/123; 1.6%). Except for two isolates that were intermediately resistant to cefoxitin, all of the other $bla_{\text{CMY-2}}$-positive *E. coli* isolates were resistant to cefoxitin. Eighteen and 15 *E. coli* isolates were resistant to cefotaxime (18/18; 100%) and ceftiofur (15/18; 83%), respectively. All of the *E. coli* isolates were multidrug-resistant and the most common resistance pattern was ampicillin–cefoxitin–cefotaxime–ceftiofur–chloramphenicol–florfenicol–kanamycin–tetracycline–ciprofloxacin–nalidixic acid–trimethoprim/sulfamethoxazole (13/18; 72%) (Table 1).

### 3.2. Sequences of entire $bla_{CMY}$ gene coding regions

Eighteen $bla_{\text{CMY-2}}$ PCR products (1146 bp), obtained from all of the $bla_{\text{CMY-2}}$-positive isolates, were selected for DNA sequencing. The results are shown in Table 1. A novel AmpC β-lactamase, CMY-130, was found in one strain, and its sequence differs from CMY-2 by one amino acid, with a substitution of leucine to glutamine at position 43 (L43Q).

### 3.3. Molecular typing

Of the 18 *E. coli* strains, 15 were successfully typed. All 15 presented distinct PFGE profiles (data not shown), suggesting that most or all of the strains were epidemiologically unrelated.

### 3.4. Transfer of $\text{bla}_{\text{CMY-2}}$

Six transconjugants and one transformant harbouring $bla_{\text{CMY-2}}$ were successfully obtained from the 18 $bla_{\text{CMY-2}}$-producing *E. coli* isolates. The IncA/C (4/18), IncHI2 (2/18) and IncX (1/18) replicon types were detected in transconjugants/transformants. All of the transconjugants/transformants showed resistance to ampicillin, cefoxitin, ceftiofur and cefotaxime. In addition, the transconjugants also exhibited a chloramphenicol-resistant phenotype.

### 3.5. Location of $\text{bla}_{\text{CMY-2}}$ on plasmids and chromosomes

S1-PFGE and Southern blot analysis showed that the $bla_{\text{CMY-2}}$ probe hybridised to plasmids that were isolated from seven transconjugants/transformants and two donor strains (size ca. 48.5–291 kb). In addition, the chromosomal location of $bla_{\text{CMY-2}}$ was confirmed in the other nine isolates (Table 1).