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Prevalence and Characterization of Cefotaxime and Ciprofloxacin Co-Resistant *Escherichia coli* Isolates in Retail Chicken Carcasses and Ground Pork, China

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Retail meat products could serve as an important medium for the transfer of multidrug resistant isolates from food-producing animals to the community. In this study, the prevalence and characteristics of cefotaxime and ciprofloxacin co-resistant *Escherichia coli* isolates were investigated in retail chicken and ground pork samples from four provinces of China. The isolates were subjected to phylogenetic group typing and antimicrobial susceptibility testing. All isolates were further characterized by pulsed-field gel electrophoresis to determine the genetic relatedness. These isolates were also screened for beta-lactamase genes, quinolone resistance determinants by PCR, and followed by DNA sequence analysis. Cefotaxime and ciprofloxacin co-resistant *E. coli* isolates with diverse genetic origins were recovered in 31.9% (106/332) of retail meat samples. *E. coli* isolates of phylogenetic group A were dominant (59.4%, 63/106), and all isolates showed multidrug resistant profiles. The dominant resistant profiles were AMP-CAZ-CTX-CIP-CHL-GEN-SXT-TET ($n=43$) and AMP-CAZ-CTX-CIP-CHL-SXT-TET ($n=43$). Point mutations in quinolone resistance determination regions of topoisomerases were identified in all the isolates, and most of the isolates accumulated three ($n=78$) or four ($n=21$) point mutations. Plasmid-mediated quinolone-resistant determinants were identified in 68 isolates, including *oqxAB* ($n=66$), *qnrS1* ($n=7$), *qnrS2* ($n=4$), and *aac(6′)-Ib-cr* ($n=9$). Eight subtypes of $bla_{\text{CTX-M}}$ were identified in 103 *E. coli* isolates, and $bla_{\text{CTX-M-55}}$ ($n=90$) was dominant. This study highlights that retail meat could serve as an important reservoir of cefotaxime and ciprofloxacin co-resistant *E. coli* isolates. It is necessary to evaluate their contribution in the community and hospital infections.

Introduction

Escherichia coli is the most common intestinal bacteria in livestock, and the application of antimicrobials during animal breeding on farms can select and disseminate antimicrobial resistant *E. coli*.[2,8] During the slaughtering process, the prevalence of these resistant isolates could be amplified through cross-contamination of meat products. Studies have shown that retailed meat products are key elements for the transmission of *E. coli* isolates from the breeding farms to the community.[3–7] Resistant *E. coli* isolates can cause intestinal and extra-intestinal infections after ingestion of undercooked or cross-contamination of ready-to-eat food products.[1,18,35]

Fluoroquinolones and third-generation cepholosprins are important antimicrobial classes in clinical treatment for community infections.[14] Recent studies have shown that food-producing animals and retail food products might be important reservoirs of extended-spectrum β-lactamase (ESBL)-resistant *E. coli* isolates.[6,41] *E. coli* isolates resistant to both cefotaxime and ciprofloxacin are known to have greater threat to humans, because they can limit the treatment options.[14] Studies have reported the wide distribution of cefotaxime and ciprofloxacin co-resistant *E. coli* isolates among patients and the emerging of cefotaxime and ciprofloxacin co-resistant *E. coli* isolates in retail meat samples,[12,16] but limited data are available for the distribution and characteristics of

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cefotaxime and ciprofloxacin co-resistant *E. coli* isolates in retail meat of China. In addition, China is one of the main antimicrobial production bases for the world.[26] Of the 210,000 tons of antibiotics made in China, 97,000 tons of antibiotics are used in animal breeding each year.[42] The large volume of antimicrobial use in livestock significantly increases the prevalence of antimicrobial-resistant bacteria in retail meat products, and the poultry farming in large cities usually used a large volume of antimicrobials in disease prevention because of the high density of chicken in limited areas.

The objectives of this study are to investigate the prevalence of cefotaxime and ciprofloxacin co-resistant *E. coli* isolates in retail chicken and ground pork samples. The characteristics of these isolates and the resistant mechanisms of cefotaxime and ciprofloxacin were fully investigated.

Materials and Methods

Sample collection and cefotaxime and ciprofloxacin co-resistant E. coli *isolation*

From April to December in 2011, retailed whole chicken carcasses were collected from 29 supermarkets and 15 farmer' markets (retail market featuring foods sold directly by farmers to consumers) of eight districts or counties in Beijing, Guangdong, Inner Mogolia, and Shanxi province. Ground pork samples were collected from eight supermarkets and from four farmer' markets in Beijing and Shanxi province. No more than three samples were collected from each market for one visit during the study.

Whole chicken carcass and ground meat samples were shipped to the laboratory within 24 hrs on ice. Each whole chicken carcass was thoroughly rinsed with 500 ml buffered peptone water (BPW; BD), and each 25 g ground pork sample was stomached with 225 ml BPW. After incubation at 36 ± 1°C for 20–22 hrs, 100 µl of BPW culture were transferred to 10 ml EC broth (BD) and incubated at 44 ± 1°C with shaking at 100 rpm for 20–22 hr. A loopful of EC broth culture was streaked on to Statens Serum Institut (SSI) enteric agar (Statens Serum Institut, Denmark) supplemented with 4 µg/ml ciprofloxacin and 8 µg/ml cefotaxime, and the plates were incubated at 36 ± 1°C for 22–24 hrs. A single red colony was selected from each plate and confirmed by the API 20E test (BioMérieux). All confirmed *E. coli* isolates were kept in brain heart infusion broth with 50% glycerol in a −70°C freezer for subsequent susceptibility testing and molecular analysis.

Antimicrobial susceptibility testing

Antimicrobial susceptibility of all the *E. coli* isolates was determined by agar dilution method with use of the antibacterial determiner (SAKUMA) to control the inoculum volume, and interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.[5] Susceptibility to 12 antimicrobials was measured for all isolates, including ampicillin (AMP, 1–128 µg/ml), cefotaxime (CTX, 0.015–128 µg/ml), cefotaxime-clavulanic acid, ceftazidime (CAZ, 0.03–64 µg/ml), ceftazidime-clavulanic acid, chloramphenicol (CHL, 1–128 µg/ml), ciprofloxacin (CIP, 0.015–512 µg/ml), gentamicin (GEN, 0.125–64 µg/ml), imipenem (IMP, 0.03–16 µg/ml), tetracycline (TET, 0.25–64 µg/ml), tigecycline (TGC, 0.015–32 µg/ml), and trimethoprim-sulfamethoxazole (SXT, 0.06/1.19–16/304 µg/ml). Isolates showing a ≥3 two-fold concentration decrease in an MIC for either ceftazidime or cefotaxime tested in combination with clavulanic acid versus the MIC of the agent when tested alone were considered as ESBL producing (for example, ceftazidime MIC = 8 µg/ml; ceftazidime-clavulanic acid MIC = 1 µg/ml). All ESBL-producing isolates were counted as resistant to ceftazidime and cefotaxime as recommended by the CLSI standards.[5] *E. coli* ATCC 25922, *E. coli* ATCC 35218, and *Klebsiella. pneumoniae* ATCC 700603 were used as quality control organisms in antimicrobial susceptibility tests. Antimicrobial susceptibility data were analyzed by Whonet 5.4 software (World Health Organization) according to the CLSI interpretive standards. The tigecycline MIC interpretive breakpoints were recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST-2012; www.eucast.org).

PCR amplification and DNA sequence analysis

All the *E. coli* isolates were screened by PCR for transferable quinolone resistance determinants *qnrA*, *qnrB*, *qnrC*, *qnrD*, *qnrS*, *oxqAB*, *aac-(6')-Ib*, and *qepA* as previously described.[24,28,39] All PCR products positive for *aac-(6')-Ib* were digested with BtsCI (New England Biolabs, Beijing, China) to identify *aac-(6')-Ib-cr* that lacked the BtsCI restriction site, while the wild-type *aac-(6')-Ib* PCR product yielded 210-bp and 272-bp fragments after digestion.[24] *Salmonella enterica* isolates carrying *qnrA*, *qnrB6*, *qnrD*, *qnrS2*, and *aac-(6')-Ib-cr* from a previous study[7] and an *E. coli* C600 derivative harboring *qepA* that was confirmed by sequencing were used as control organisms. The quinolone resistance determination regions (QRDRs) of *gyrA*, *gyrB*, *parC*, and *parE* in *E. coli* isolates were amplified by PCR as previously described.[13]

A multiplex PCR was used to screen for $bla_{CTX\text{-}M}$ in all the ESBL-producing isolates,[38] and the sequences of $bla_{CTX\text{-}M}$-like genes were analyzed by primer pairs of M9, M13, and M25 as previously described.[11] Another multiplex PCR method was applied to screen for plasmid-mediated AmpC encoding genes in clavulanic acid-resistant isolates.[25] All the PCR products were either directly sequenced or cloned into pMD18-T plasmid (Takara Biotechnology Cooperation, Dalian, China) for sequence analysis at Takara Biotechnology Cooperation. The sequences obtained were analyzed by Sequencher 4.6 software (Gene Codes Corporation). The *qnrS* allele numbers were designated based on the *qnr* gene nomenclature.[17] The search for homologous sequences was performed by using the BLASTN program at the U.S. National Center for Biotechnology Information Website (www.ncbi.nlm.nih.gov/BLAST/). New $bla_{CTX\text{-}M}$-like sequences identified in this study have been submitted to the National Center for Biotechnology Information Data Libraries(GenBank) and the Lahey Clinic database (www.lahey.org/Studies/).

Phylogenetic analysis

Phylogenetic analysis of recovered isolates was determined through the presence or absence of *chuA*, *yjaA*, and TspE4.C2 as previously described.[4]

Pulsed-field gel electrophoresis

All *E. coli* isolates were analyzed by pulsed-field gel electrophoresis (PFGE) to determine DNA fingerprinting profiles resulting from digestion by restriction enzyme *Xba*I (New

England Biolab, Beijing LTD) according to the procedures developed by the United States Centers for Disease Control and Prevention (U.S. CDC) PulseNet program.[27] The interpretation of the PFGE patterns was performed with BioNumerics software (Applied Maths) using the Dice Similarity coefficient. The tree indicating relative genetic similarity was constructed on the basis of the Unweighted Pair Group Method of Averages (UPGMA), position tolerance of 1%. Clusters were defined as DNA patterns sharing ≥70% similarity (C1, C2, C3,···).

Statistical analysis

The differences of frequencies were analyzed by Chi-square test using Epi Info 6 software (www.cdc.gov/epiinfo/).

Results

Sample collection and E. coli *recovery*

In total, 214 whole chicken carcasses were collected from 29 supermarkets and 15 markets of eight districts or counties in Shanxi ($n=91$), Guangdong ($n=63$), Inner Mogolia ($n=53$), and Beijing ($n=7$). Cefotaxime and ciprofloxacin co-resistant *E. coli* isolates were recovered from 80 (37.4%) chicken samples. This prevalence was found to be significant in the four provinces with the highest prevalence in Beijing (100%, 7/7) and the lowest prevalence in Inner Mongolia (5.7%, 3/53) ($\chi^2=69.524$, $p<0.05$) (Table 1).

Of the 118 ground pork samples collected from eight supermarkets and four farmers' markets in Shanxi province ($n=81$) and Beijing ($n=37$), cefotaxime and ciprofloxacin co-resistant *E. coli* isolates were recovered from 22.0% ($n=26$) samples, which was similar to those (32.7%, 32/98) of chicken samples in the same sampling region ($\chi^2=3.074$, $p>0.05$) (Table 1).

Of the 106 cefotaxime and ciprofloxacin co-resistant *E. coli* isolates, phylogenetic group A was dominant (59.4%, 63/106), followed by group B1 (25.5%, 27/106), D (14.2%, 15/106), and B2 (0.9%, 1/106). No region or sample category-specific distribution pattern was identified for phylogenetic groups (Table 1).

Antimicrobial susceptibility testing

All 106 *E. coli* isolates showed multidrug resistant profiles (at least resistant to three categories of antimicrobials). The dominant resistant profiles were AMP-CAZ-CTX-CIP-CHL-GEN-SXT-TET ($n=43$) and AMP-CAZ-CTX-CIP-CHL-SXT-TET ($n=43$), which were also the dominant resistance profiles of isolates from Beijing (72.2%, 13/18), Guangdong (84.4%, 38/45), and Shanxi provinces (82.5%, 33/40). No correlation was identified for antimicrobial resistance profiles and phylogenetic group or sample categories (Table 2). All the 106 *E. coli* isolates were resistant to ampicillin, cefotaxime, ceftazidime, and ciprofloxacin and susceptible to imipenem and tigecycline. These isolates also showed high resistance prevalence to tetracycline (94.3%, 100/106), trimethoprim-sulfamethoxazole (94.3%, 100/106), chloramphenicol (88.7%, 94/106), and gentamicin (50.9%, 54/106) (Table 3). In total, 103 isolates (97.2%, 103/106) that exhibited at least a ≥3 twofold concentration decrease in an MIC for either ceftazidime or cefotaxime tested in combination with clavulanic acid versus the MIC of the agent when tested alone were confirmed as ESBL producers.

Table 1. Recovery and Phylogenetic Typing of Cefotaxime and Ciprofloxacin Co-Resistant *Escherichia coli* Isolates from Chicken Carcasses and Ground Pork Samples

		Phylogenic groups				
Sampling regions	*Sample categories*	*A*	*B1*	*B2*	*D*	*Total*
Beijing	Chicken ($n=7$)	6	1	0	0	7
	Pork ($n=37$)	5	4	1	1	11
Shanxi	Chicken($n=91$)	15	9	0	1	25
	Pork ($n=81$)	7	5	0	3	15
Guangdong	Chicken ($n=63$)	29	7	0	9	45
Inner Mongolia	Chicken ($n=53$)	1	1	0	1	3
Total	Chicken and pork ($n=332$)	63	27	1	15	106

Identification of quinolone-resistant determinants

Point mutations in QRDRs of GyrA, GyrB, ParC, or ParE were identified in all 106 cefotaxime and ciprofloxacin co-resistant *E. coli* isolates. Three-point mutations in QRDRs were found in 78 isolates with GyrA(S83L, D87N) and ParC(S80I) as the dominant profile (72/106, 67.9%). Four-point mutations were found in 21 isolates (21/106, 19.8%) with profiles GyrA(S83L, D87N), ParC(S80I), and ParE(S458A) as the dominant ($n=9$), followed by GyrA(S83L, D87N) and ParC(S80I, E84G) ($n=4$), GyrA(S83L, D87N) and ParC(S80I, E84A) ($n=3$), and ciprofloxacin MIC of all these isolates was ≥32 μg/ml. Four isolates had single-point mutations in GyrA (S83A or S83L) also acquired one (*qnrS*), two (*qnrS* and *oqxAB*), or three Plasmid-Mediated Quinolone Resistance (PMQR) determinants (*qnrS2, aac(6′)-Ib-cr,* and *oqxAB*). Three isolates accumulated two topoisomerase point mutations in GyrA (S83L), and ParC (S80I or E84V) also acquired two PMQR determinants (*qnrS* and *oqxAB*).

Among the 106 cefotaxime and ciprofloxacin co-resistant *E. coli* isolates, PMQR determinants were identified in 68 isolates (68/106, 64.2%) from both chicken ($n=56$) and ground pork ($n=12$) samples, including *oqxAB* ($n=66$), *qnrS1* ($n=7$), *qnrS2* ($n=4$), and *aac(6′)-Ib-cr* ($n=9$). Compared with the pork isolates (46.2%), chicken isolates (67.5%) showed higher prevalence of *oqxAB*, and isolates of phylogenetic groups A (45/63, 71.4%) and D (11/15, 73.3%) showed higher *oqxAB* prevalence than isolates of phylogenetic group B1 (10/27, 37.0%). The following PMQR determinants, including *qepA*, *qnrA*, *qnrB*, *qnrC*, and *qnrD*, were not detected in all these 106 isolates. Sixteen isolates (16/106, 15.1%) possessed more than one PMQR determinant. Two PMQR determinants were identified in each of the 14 isolates from chicken ($n=10$) and ground pork ($n=4$) samples, including *oqxAB* and *aac(6′)-Ib-cr* ($n=6$), *oqxAB* and *qnrS1*($n=6$), and *oqxAB* and *qnrS2* ($n=2$). Three PMQR determinants (*qnrS2*, *oqxAB*, and *aac(6′)-Ib-cr*) were identified simultaneously in two chicken isolates from Shanxi and Guangdong provinces.

Among the 63 (63/106, 59.4%) ciprofloxacin highly resistant (MIC ≥32 μg/ml) isolates from chicken ($n=48$) and ground pork ($n=15$) samples, a minimum of three topoisomerase point mutations and one PMQR determinant were identified in each of the 50 isolates. Four topoisomerase point

Table 2. Multidrug-Resistant Profiles of *Escherichia coli* Isolates Recovered from Chicken or Pork in Different Phylogenetic Groups

	No. of resistant isolates								
	A (n=63)		*B1* (n=27)		*B2* (n=1)		*D* (n=15)		
Resistant profiles	*Chicken*	*Pork*	*Chicken*	*Pork*	*Chicken*	*Pork*	*Chicken*	*Pork*	*Total*
AMP-CAZ-CTX-CIP-CHL-GEN-SXT-TET	19 (44.2%)[a]	5 (11.6%)	5 (11.6%)	5 (11.6%)	0	0	5 (11.6%)	4 (9.3%)	43
AMP-CAZ-CTX-CIP-CHL-SXT-TET	20 (46.5%)	4 (9.3%)	10 (23.3%)	3 (7%)	0	1 (2.3%)	5 (11.6%)	0	43
AMP-CAZ-CTX-CIP-GEN-SXT-TET	2 (33.3%)	3 (50%)	1 (16.7%)	0	0	0	0	0	6
AMP-CAZ-CTX-CIP-CHL-SXT	3 (75%)	0	1 (25%)	0	0	0	0	0	4
AMP-CAZ-CTX-CIP-CHL-GEN-TET	2 (66.7%)	0	1 (33.3%)	0	0	0	0	0	3
AMP-CAZ-CTX-CIP-SXT-TET	1 (50%)	0	0	0	0	0	1 (50%)	0	2
AMP-CAZ-CTX-CIP-GEN-SXT	1 (100%)	0	0	0	0	0	0	0	1
AMP-CAZ-CTX-CIP-GEN-TET	1 (100%)	0	0	0	0	0	0	0	1
AMP-CAZ-CTX-CIP-CHL-TET	1 (100%)	0	0	0	0	0	0	0	1
AMP-CAZ-CTX-CIP-SXT	1 (100%)	0	0	0	0	0	0	0	1
AMP-CAZ-CTX-CIP-TET	0	0	0	1 (100%)	0	0	0	0	1
Total	51	12	18	9	0	1	11	4	106

[a]The percentage in this type of resistant profiles.
AMP, ampicillin; CAZ, ceftazidime; CTX, cefotaxime; CHL, chloramphenicol; CIP, ciprofloxacin; GEN, gentamicin; SXT, trimethoprim/sulfamethoxazole; TET, tetracycline.

mutations in each of the ten isolates, two topoisomerase point mutations (GyrA[S83L], ParC[S80I]), and two PMQR determinants (*qnrS2* and *oxqAB*) in one isolate were identified. Among the 17 isolates showing ciprofloxacin MIC ≥ 128 μg/ml from chicken ($n=14$) and ground pork ($n=3$) samples, three isolates accumulated four-point mutations in QRDRs; 14 isolates had acquired both point mutations (three [$n=5$] or four [$n=9$] mutations) in QRDRs and a minimum of one PMQR determinant.

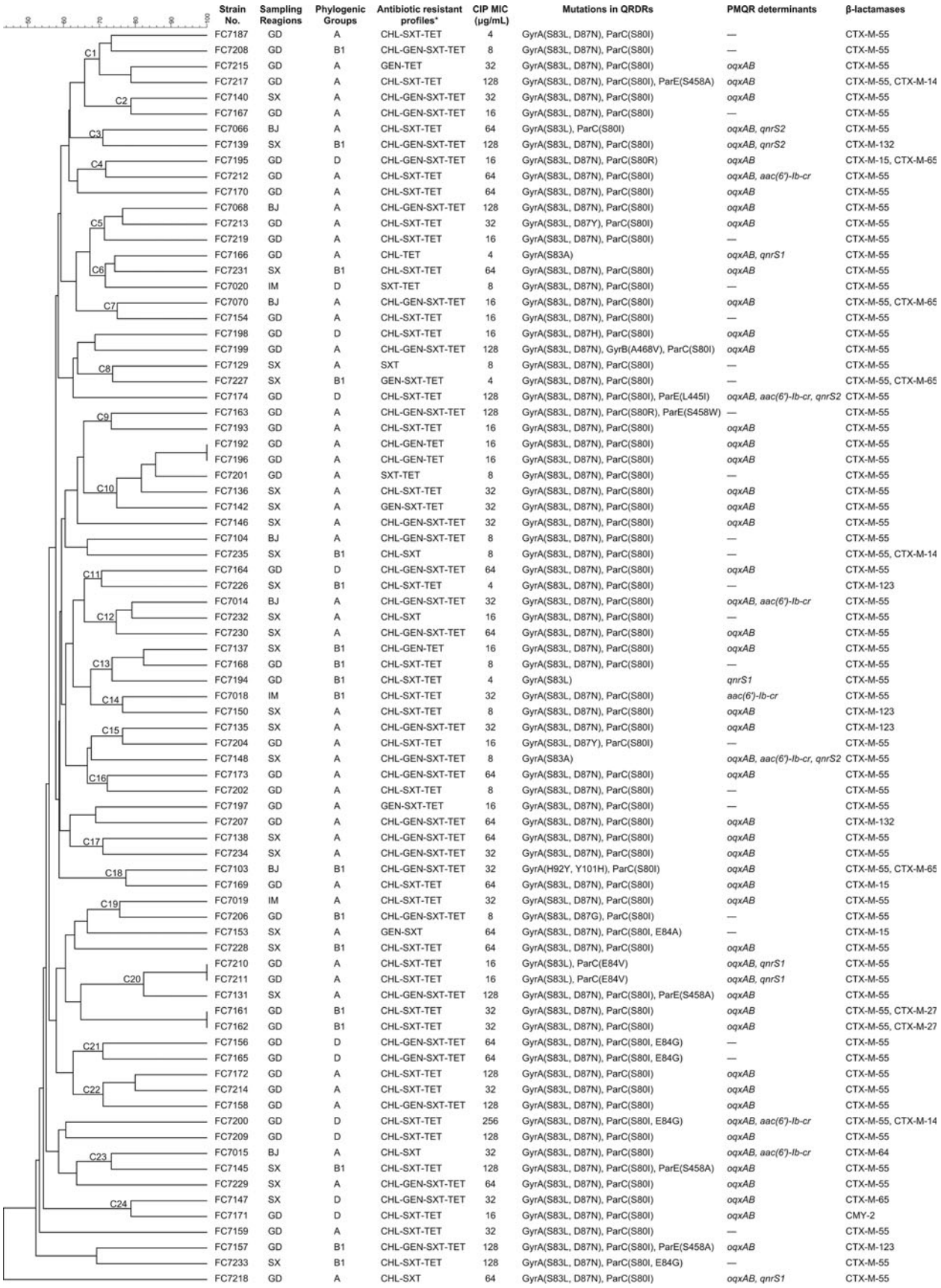
Identification of β-lactam resistance genes

Among the 103 ESBL-producing *E. coli* isolates from chicken and ground pork samples, eight subtypes of $bla_{\text{CTX-M}}$ were identified, including $bla_{\text{CTX-M-55}}$ ($n=90$), $bla_{\text{CTX-M-123}}$ ($n=4$), $bla_{\text{CTX-M-15}}$ ($n=4$), $bla_{\text{CTX-M-64}}$ ($n=2$), $bla_{\text{CTX-M-65}}$ ($n=8$), $bla_{\text{CTX-M-14}}$ ($n=3$), $bla_{\text{CTX-M-27}}$ ($n=2$), and $bla_{\text{CTX-M-132}}$ ($n=2$). The novel $bla_{\text{CTX-M-123}}$ variant with nine-point mutations (S121T, Q195N, H200K, E204D, T205S, L214M, R225Q, T230A, and T233V) was identified in two isolates recovered from chicken samples in Guangdong and Shanxi provinces, respectively (Fig. 1). This new $bla_{\text{CTX-M}}$ gene has been assigned accession numbers in the GenBank and the Lahey Clinic database as JX313020 (CTX-M-132). *E. coli* isolates containing $bla_{\text{CTX-M-55}}$ were recovered from four sampling provinces: Guangdong 40 isolates from chicken, Shanxi 33 isolates from chicken ($n=19$) and ground pork ($n=14$), Beijing 14 isolates from chicken ($n=6$) and ground pork ($n=8$), and Inner Mongolia three isolates from chicken. In each of the 12 (11.3%, 12/106) isolates from chicken ($n=10$) and ground pork ($n=2$) samples, two $bla_{\text{CTX-M}}$ subtypes were detected, including $bla_{\text{CTX-M-55}}$ and $bla_{\text{CTX-M-14}}$ ($n=3$), $bla_{\text{CTX-M-55}}$

Table 3. Resistance Phenotypes of *Escherichia coli* Isolates in Different Sampling Regions

	Break points[a] *(μg/ml)*	*MIC_{10}* *(μg/ml)*[b]	*MIC_{50}* *(μg/ml)*	*MIC_{90}* *(μg/ml)*	*No. of resistant isolates in different provinces*				
					Beijing (n=18)	*Guangdong* (n=45)	*Inner Mongolia* (n=3)	*Shanxi* (n=40)	*Total*
Cefotaxime	≥4	16	32	64	18 (100%)[c]	45 (100%)	3 (100%)	40 (100%)	106 (100%)
Ciprofloxacin	≥4	8	32	128	18 (100%)	45 (100%)	3 (100%)	40 (100%)	106 (100%)
Ampicillin	≥32	>128	>128	>128	18 (100%)	45 (100%)	3 (100%)	40 (100%)	106 (100%)
Ceftazidime	≥16	16	16	16	18 (100%)	45 (100%)	3 (100%)	40 (100%)	106 (100%)
Chloramphenicol	≥32	8	64	128	14 (77.8%)	42 (93.3%)	2 (66.7%)	36 (90%)	94 (88.7%)
Gentamicin	≥16	8	32	64	11 (61.1%)	17 (37.8%)	0 (0%)	26 (65%)	54 (50.9%)
Imipenem	≥4	≤0.25	≤0.25	≤0.25	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Tetracycline	≥16	24	64	64	17 (94.4%)	44 (97.8%)	3 (100%)	36 (90%)	100 (94.3%)
Tigecycline	≥2[a]	0.125	0.25	0.5	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Trimethoprim-sulfamethoxazole	≥4/76	>16/304	>16/304	>16/304	17 (94.4%)	41 (91.1%)	3 (100%)	39 (97.5%)	100 (94.3%)

[a]All antimicrobial breakpoints were recommended by the CLSI-M100-S21, except the tigecycline whose breakpoint was recommended by the EUCAST-2012.
[b]MIC, minimal inhibitory concentration; MIC_{10}, MIC_{50}, MIC_{90}, 10%, 50%, or 90% of isolates were inhibited.
[c]The percentage of the antibiotics in this province.



Strain No.	Sampling Reagions	Phylogenic Groups	Antibiotic resistant profiles*	CIP MIC (μg/mL)	Mutations in QRDRs	PMQR determinants	β-lactamases
FC7187	GD	A	CHL-SXT-TET	4	GyrA(S83L, D87N), ParC(S80I)	—	CTX-M-55
FC7208	GD	B1	CHL-GEN-SXT-TET	8	GyrA(S83L, D87N), ParC(S80I)	—	CTX-M-55
FC7215	GD	A	GEN-TET	32	GyrA(S83L, D87N), ParC(S80I)	*oqxAB*	CTX-M-55
FC7217	GD	A	CHL-SXT-TET	128	GyrA(S83L, D87N), ParC(S80I), ParE(S458A)	*oqxAB*	CTX-M-55, CTX-M-14
FC7140	SX	A	CHL-GEN-SXT-TET	32	GyrA(S83L, D87N), ParC(S80I)	*oqxAB*	CTX-M-55
FC7167	GD	A	CHL-GEN-SXT-TET	16	GyrA(S83L, D87N), ParC(S80I)	—	CTX-M-55
FC7066	BJ	A	CHL-SXT-TET	64	GyrA(S83L), ParC(S80I)	*oqxAB, qnrS2*	CTX-M-55
FC7139	SX	B1	CHL-GEN-SXT-TET	128	GyrA(S83L, D87N), ParC(S80I)	*oqxAB, qnrS2*	CTX-M-132
FC7195	GD	D	CHL-GEN-SXT-TET	16	GyrA(S83L, D87N), ParC(S80R)	*oqxAB*	CTX-M-15, CTX-M-65
FC7212	GD	A	CHL-SXT-TET	64	GyrA(S83L, D87N), ParC(S80I)	*oqxAB, aac(6')-Ib-cr*	CTX-M-55
FC7170	GD	A	CHL-SXT-TET	64	GyrA(S83L, D87N), ParC(S80I)	*oqxAB*	CTX-M-55
FC7068	BJ	A	CHL-GEN-SXT-TET	128	GyrA(S83L, D87N), ParC(S80I)	*oqxAB*	CTX-M-55
FC7213	GD	A	CHL-SXT-TET	32	GyrA(S83L, D87Y), ParC(S80I)	*oqxAB*	CTX-M-55
FC7219	GD	A	CHL-SXT-TET	16	GyrA(S83L, D87N), ParC(S80I)	—	CTX-M-55
FC7166	GD	A	CHL-TET	4	GyrA(S83A)	*oqxAB, qnrS1*	CTX-M-55
FC7231	SX	B1	CHL-SXT-TET	64	GyrA(S83L, D87N), ParC(S80I)	*oqxAB*	CTX-M-55
FC7020	IM	D	SXT-TET	8	GyrA(S83L, D87N), ParC(S80I)	—	CTX-M-55
FC7070	BJ	A	CHL-GEN-SXT-TET	16	GyrA(S83L, D87N), ParC(S80I)	*oqxAB*	CTX-M-55, CTX-M-65
FC7154	GD	A	CHL-SXT-TET	16	GyrA(S83L, D87N), ParC(S80I)	—	CTX-M-55
FC7198	GD	D	CHL-SXT-TET	16	GyrA(S83L, D87H), ParC(S80I)	*oqxAB*	CTX-M-55
FC7199	GD	A	CHL-GEN-SXT-TET	128	GyrA(S83L, D87N), GyrB(A468V), ParC(S80I)	*oqxAB*	CTX-M-55
FC7129	SX	A	SXT	8	GyrA(S83L, D87N), ParC(S80I)	—	CTX-M-55
FC7227	SX	B1	GEN-SXT-TET	4	GyrA(S83L, D87N), ParC(S80I)	—	CTX-M-55, CTX-M-65
FC7174	GD	D	CHL-SXT-TET	128	GyrA(S83L, D87N), ParC(S80I), ParE(L445I)	*oqxAB, aac(6')-Ib-cr, qnrS2*	CTX-M-55
FC7163	GD	A	CHL-GEN-SXT-TET	128	GyrA(S83L, D87N), ParC(S80R), ParE(S458W)	—	CTX-M-55
FC7193	GD	A	CHL-SXT-TET	16	GyrA(S83L, D87N), ParC(S80I)	*oqxAB*	CTX-M-55
FC7192	GD	A	CHL-GEN-TET	16	GyrA(S83L, D87N), ParC(S80I)	*oqxAB*	CTX-M-55
FC7196	GD	A	CHL-GEN-TET	16	GyrA(S83L, D87N), ParC(S80I)	*oqxAB*	CTX-M-55
FC7201	GD	A	SXT-TET	8	GyrA(S83L, D87N), ParC(S80I)	—	CTX-M-55
FC7136	SX	A	CHL-SXT-TET	32	GyrA(S83L, D87N), ParC(S80I)	*oqxAB*	CTX-M-55
FC7142	SX	A	GEN-SXT-TET	32	GyrA(S83L, D87N), ParC(S80I)	*oqxAB*	CTX-M-55
FC7146	SX	A	CHL-GEN-SXT-TET	32	GyrA(S83L, D87N), ParC(S80I)	*oqxAB*	CTX-M-55
FC7104	BJ	A	CHL-GEN-SXT-TET	8	GyrA(S83L, D87N), ParC(S80I)	—	CTX-M-55
FC7235	SX	B1	CHL-SXT	8	GyrA(S83L, D87N), ParC(S80I)	—	CTX-M-55, CTX-M-14
FC7164	GD	D	CHL-GEN-SXT-TET	64	GyrA(S83L, D87N), ParC(S80I)	*oqxAB*	CTX-M-55
FC7226	SX	B1	CHL-SXT-TET	4	GyrA(S83L, D87N), ParC(S80I)	—	CTX-M-123
FC7014	BJ	A	CHL-GEN-SXT-TET	32	GyrA(S83L, D87N), ParC(S80I)	*oqxAB, aac(6')-Ib-cr*	CTX-M-55
FC7232	SX	A	CHL-SXT	16	GyrA(S83L, D87N), ParC(S80I)	—	CTX-M-55
FC7230	SX	A	CHL-GEN-SXT-TET	64	GyrA(S83L, D87N), ParC(S80I)	*oqxAB*	CTX-M-55
FC7137	SX	B1	CHL-GEN-TET	16	GyrA(S83L, D87N), ParC(S80I)	*oqxAB*	CTX-M-55
FC7168	GD	B1	CHL-SXT-TET	8	GyrA(S83L, D87N), ParC(S80I)	—	CTX-M-55
FC7194	GD	B1	CHL-SXT-TET	4	GyrA(S83L)	*qnrS1*	CTX-M-55
FC7018	IM	B1	CHL-SXT-TET	32	GyrA(S83L, D87N), ParC(S80I)	*aac(6')-Ib-cr*	CTX-M-55
FC7150	SX	A	CHL-SXT-TET	8	GyrA(S83L, D87N), ParC(S80I)	*oqxAB*	CTX-M-123
FC7135	SX	A	CHL-GEN-SXT-TET	32	GyrA(S83L, D87N), ParC(S80I)	*oqxAB*	CTX-M-123
FC7204	GD	A	CHL-SXT-TET	16	GyrA(S83L, D87Y), ParC(S80I)	—	CTX-M-55
FC7148	SX	A	CHL-GEN-SXT-TET	8	GyrA(S83A)	*oqxAB, aac(6')-Ib-cr, qnrS2*	CTX-M-55
FC7173	GD	A	CHL-GEN-SXT-TET	64	GyrA(S83L, D87N), ParC(S80I)	*oqxAB*	CTX-M-55
FC7202	GD	A	CHL-SXT-TET	8	GyrA(S83L, D87N), ParC(S80I)	—	CTX-M-55
FC7197	GD	A	GEN-SXT-TET	16	GyrA(S83L, D87N), ParC(S80I)	—	CTX-M-55
FC7207	GD	A	CHL-GEN-SXT-TET	64	GyrA(S83L, D87N), ParC(S80I)	*oqxAB*	CTX-M-132
FC7138	SX	A	CHL-GEN-SXT-TET	64	GyrA(S83L, D87N), ParC(S80I)	*oqxAB*	CTX-M-55
FC7234	SX	A	CHL-GEN-SXT-TET	32	GyrA(S83L, D87N), ParC(S80I)	*oqxAB*	CTX-M-55
FC7103	BJ	B1	CHL-GEN-SXT-TET	32	GyrA(H92Y, Y101H), ParC(S80I)	*oqxAB*	CTX-M-55, CTX-M-65
FC7169	GD	A	CHL-SXT-TET	64	GyrA(S83L, D87N), ParC(S80I)	*oqxAB*	CTX-M-15
FC7019	IM	A	CHL-SXT-TET	32	GyrA(S83L, D87N), ParC(S80I)	*oqxAB*	CTX-M-55
FC7206	GD	B1	CHL-GEN-SXT-TET	8	GyrA(S83L, D87G), ParC(S80I)	—	CTX-M-55
FC7153	SX	A	GEN-SXT	64	GyrA(S83L, D87N), ParC(S80I, E84A)	—	CTX-M-15
FC7228	SX	B1	CHL-SXT-TET	64	GyrA(S83L, D87N), ParC(S80I)	*oqxAB*	CTX-M-55
FC7210	GD	A	CHL-SXT-TET	16	GyrA(S83L), ParC(E84V)	*oqxAB, qnrS1*	CTX-M-55
FC7211	GD	A	CHL-SXT-TET	16	GyrA(S83L), ParC(E84V)	*oqxAB, qnrS1*	CTX-M-55
FC7131	SX	A	CHL-GEN-SXT-TET	128	GyrA(S83L, D87N), ParC(S80I), ParE(S458A)	*oqxAB*	CTX-M-55
FC7161	GD	B1	CHL-SXT-TET	32	GyrA(S83L, D87N), ParC(S80I)	*oqxAB*	CTX-M-55, CTX-M-27
FC7162	GD	B1	CHL-SXT-TET	32	GyrA(S83L, D87N), ParC(S80I)	*oqxAB*	CTX-M-55, CTX-M-27
FC7156	GD	D	CHL-GEN-SXT-TET	64	GyrA(S83L, D87N), ParC(S80I, E84G)	—	CTX-M-55
FC7165	GD	D	CHL-GEN-SXT-TET	64	GyrA(S83L, D87N), ParC(S80I, E84G)	—	CTX-M-55
FC7172	GD	A	CHL-SXT-TET	128	GyrA(S83L, D87N), ParC(S80I)	*oqxAB*	CTX-M-55
FC7214	GD	A	CHL-SXT-TET	32	GyrA(S83L, D87N), ParC(S80I)	*oqxAB*	CTX-M-55
FC7158	GD	A	CHL-GEN-SXT-TET	128	GyrA(S83L, D87N), ParC(S80I)	*oqxAB*	CTX-M-55
FC7200	GD	D	CHL-SXT-TET	256	GyrA(S83L, D87N), ParC(S80I, E84G)	*oqxAB, aac(6')-Ib-cr*	CTX-M-55, CTX-M-14
FC7209	GD	D	CHL-SXT-TET	128	GyrA(S83L, D87N), ParC(S80I)	*oqxAB*	CTX-M-55
FC7015	BJ	A	CHL-SXT	32	GyrA(S83L, D87N), ParC(S80I)	*oqxAB, aac(6')-Ib-cr*	CTX-M-64
FC7145	SX	B1	CHL-SXT-TET	128	GyrA(S83L, D87N), ParC(S80I), ParE(S458A)	*oqxAB*	CTX-M-55
FC7229	SX	A	CHL-GEN-SXT-TET	64	GyrA(S83L, D87N), ParC(S80I)	*oqxAB*	CTX-M-55
FC7147	SX	D	CHL-GEN-SXT-TET	32	GyrA(S83L, D87N), ParC(S80I)	*oqxAB*	CTX-M-65
FC7171	GD	D	CHL-SXT-TET	16	GyrA(S83L, D87N), ParC(S80I)	*oqxAB*	CMY-2
FC7159	GD	A	CHL-SXT-TET	32	GyrA(S83L, D87N), ParC(S80I)	—	CTX-M-55
FC7157	GD	B1	CHL-GEN-SXT-TET	128	GyrA(S83L, D87N), ParC(S80I), ParE(S458A)	*oqxAB*	CTX-M-123
FC7233	SX	B1	CHL-SXT-TET	128	GyrA(S83L, D87N), ParC(S80I, E84G)	—	CTX-M-55
FC7218	GD	A	CHL-SXT	64	GyrA(S83L, D87N), ParC(S80I)	*oqxAB, qnrS1*	CTX-M-55

FIG. 1. Dendrogram of patterns generated by pulsed-field gel electrophoresis (PFGE) of *Escherichia coli* isolates collected from chicken. *Since all *E. coli* isolates were resistant to ampicillin, ceftazidime, ciprofloxacin, and cefotaxime, these four antimicrobials were not shown in the resistant profiles. "–" denotes not detected. CHL, chloramphenicol; GEN, gentamicin; SXT, trimethoprim/sulfamethoxazole; TET, tetracycline; PMQR, plasmid-mediated quinolone resistance; QRDRs, the quinolone resistance-determining regions; BJ, Beijing; GD, Guangdong; SX, Shanxi; IM, Inner Mongolia.

and $bla_{CTX\text{-}M\text{-}27}$ ($n=2$), $bla_{CTX\text{-}M\text{-}55}$ and $bla_{CTX\text{-}M\text{-}65}$ ($n=6$), and $bla_{CTX\text{-}M\text{-}15}$ and $bla_{CTX\text{-}M\text{-}65}$ ($n=1$). $Bla_{CMY\text{-}2}$ was identified in three clavulanic acid-resistant isolates from chicken ($n=1$) and ground pork ($n=2$) samples.

PFGE analysis of E. coli *isolates*

No dominant PFGE cluster or pattern was identified among chicken and ground pork isolates. In total, 77 PFGE patterns and 24 PFGE clusters were identified among 80 chicken isolates and 20 PFGE patterns, and four PFGE clusters were identified among 26 ground pork isolates (Figs. 1 and 2). Three PFGE patterns of chicken isolates and four PFGE patterns of ground pork isolates contained more than one isolate, and all the isolates grouped into the same PFGE pattern were recovered from the same sampling region. Eight chicken isolate clusters (C1, C4, C8, C9, C16, C17, C21, and C22) and two ground pork isolate clusters (C1 and C2) contained isolates from the same sampling region. Of the chicken isolates, 11 clusters (C2, C5, C7, C9, C10, C12, C15, C16, C17, C20, and C22) belonged to phylogenetic group A, two clusters (C21 and C24) belonged to phylogenetic group D, and one cluster (C13) belonged to phylogenetic group B1. Of the ground pork isolates, clusters C2, C3, and C4 contained isolates of phylogentic groups D, A, and B1, respectively.

Discussion

In this study, cefotaxime and ciprofloxacin co-resistant *E. coli* isolates with diverse genetic origins were recovered in 37.4% (80/214) of retail chicken and 22% (26/118) of retail ground pork samples from four sampling provinces in China. In addition, chromosomal and plasmid-mediated quinolone and extended-spectrum cephalosporin-resistant mechanisms were identified in these isolates. These data added to the growing evidence that retail meat could serve as an important medium to transfer multidrug resistant isolates from food-producing animals to the community and highlighted the growing problem of fluoroquinolone resistance and ESBL production in *E. coli* isolates of meat origins.[9,23] To the best of our knowledge, this is the first report focusing on cefotaxime and ciprofloxacin co-resistant *E. coli* isolates from retail meat products in China.

The PFGE profile variation of the 106 isolates from retail chicken and ground pork products demonstrated extensive genetic heterogeneity and diverse origins of these resistant isolates and also provided some information on the high transmission capacity of $bla_{CTX\text{-}M}$ and PMQR determinants detected in these isolates. The diverse genetic heterogeneity of these resistant isolates also indicated a long-term evolutionary process of resistant mechanisms. In addition, the high resistance ratio of these *E. coli* hosts to cephalosprins, trimethoprim-sulfamethoxazole, and gentamicin, which were the common antimicrobials used in clinics, can provide the resistant isolates with selective advantage over other isolates. Besides, several isolates from the same sampling region exhibiting similar or identical PFGE patterns indicate the clonal spread of cefotaxime and ciprofloxacin co-resistant strains.

Our data showed that all 106 cefotaxime and ciprofloxacin co-resistant *E. coli* isolates were multidrug resistant. Isolates were mostly resistant to tetracycline (94.3%), trimethoprim-sulfamethoxazole (94.3%), chloramphenicol (88.7%), and gentamicin (50.9%), which indicated the popular usage of these antimicrobials in the food-producing animals. All the isolates accumulated the same fluoroquinolone and cepholosprin-resistant mechanisms as in isolates from patients, which highlighted the fact that retail meat might be a reservoir of these resistant isolates.[32,40] However, in some

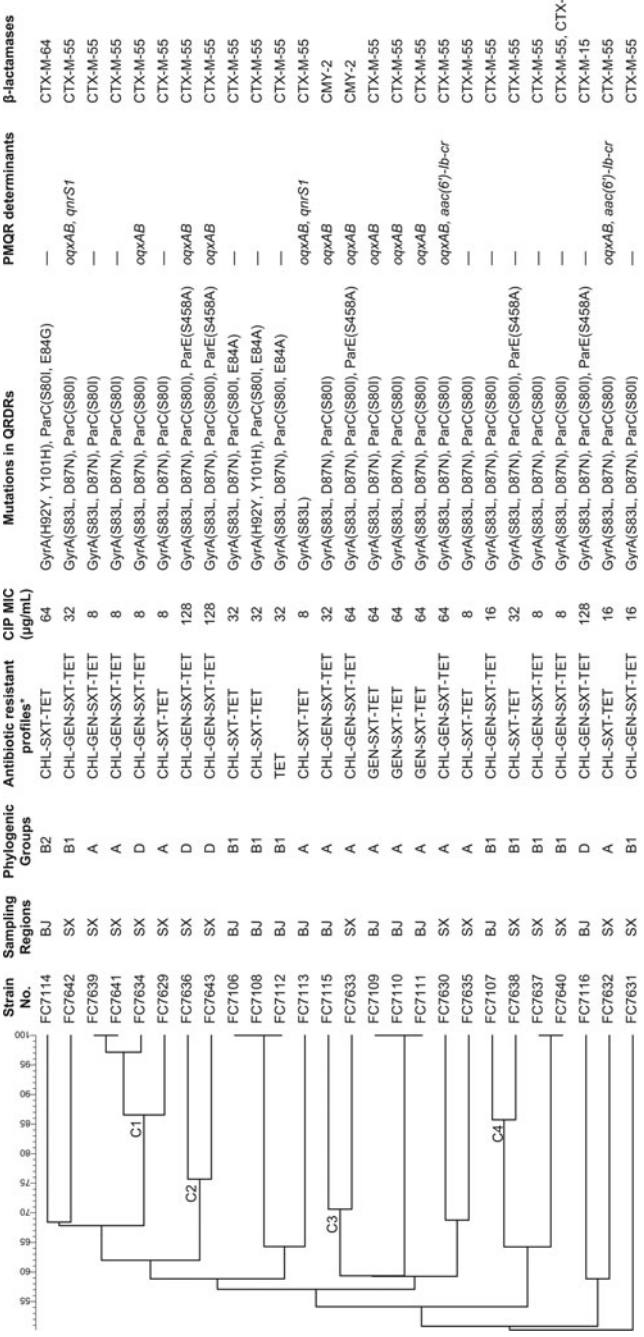


FIG. 2. Dendrogram of patterns generated by PFGE of *Escherichia coli* isolates collected from ground pork samples. *Since all *E. coli* isolates were resistant to ampicillin, ceftazidime, ciprofloxacin, and cefotaxime, these four antimicrobials were not shown in the resistant profiles. "–" denotes not detected.

isolates, both chromosomal and varying plasmid-mediated quinolone-resistant mechanisms were accumulated, and these isolates showed higher ciprofloxacin MIC ($\geq 32\,\mu g/ml$), which further indicated the long-term evolutionary process of these isolates in resistance development (Figs. 1 and 2). Since these isolates were co-resistant to both cefotaxime and ciprofloxacin, the alternative antimicrobial treatment options will be limited once these isolates infect the community population, which might also significantly increase the mortality and financial burdens.[15,34] Furthermore, this study revealed that $bla_{CTX\text{-}M\text{-}55}$ was identified in 90 out of 106 *E. coli* isolates recovered from retail meat products. Since $bla_{CTX\text{-}M\text{-}55}$ has been widely detected in both animal and clinical isolates and $bla_{CTX\text{-}M}$ is usually located on conjugative plasmids,[19,20,30] the contribution of $bla_{CTX\text{-}M\text{-}55}$ in retail meat to the community infections should be studied at the molecular level through DNA sequence analysis of the genetic environment of $bla_{CTX\text{-}M\text{-}55}$.

Our data showed that the prevalence of cefotaxime and ciprofloxacin co-resistant *E. coli* isolates in retail chicken and ground meat was region specific, which might be related to the use of antimicrobials in farms. The lowest prevalence was in Inner Mongolia (5.7%, 3/53), which was a sparsely populated under-developed province. In this province, chicken farming was usually free range and little antimicrobials were used for disease prevention and treatment. However, for large cities as in Beijing, chicken was usually raised in a high density and large volumes and different categories of antimicrobials, such as enrofloxacin and ceftiofur, were widely used for both disease prevention and treatment.[21] Althogh only seven chicken samples were collected in Beijing in this study, cefotaxime and ciprofloxacin co-resistant *E. coli* isolates were recovered from all these samples.

E. coli isolates of phylogenetic B2 and D groups usually carry the greatest number of virulence factors and most commensal isolates belong to group A.[4,10] Among the 106 cefotaxime and ciprofloxacin co-resistant *E. coli* isolates in this study, commensal *E. coli* isolates belonging to group A (59.4%, 63/106) were dominant, which was similar to a recent study in Spain.[12] However, a few isolates belonging to potentially pathogenic groups D (14.2%, 15/106) and B2 (0.9%, 1/106) were also recovered. Studies have shown that *E. coli* in retail meat products could not only serve as a reservoir for antimicrobial-resistant determinants, but also serve as a reservoir for multidrug-resistant isolates, causing both intestinal and extra-intestinal infections.[23,31,35] As the cefotaxime and ciprofloxacin co-resistant *E. coli* isolates became more and more prevalent in patients in China,[36] it is necessary to carry out a rigorous designed study to evaluate the contribution of meat-orginated antimicrobial-resistant isolates in the community infections and provide evidence for the development of new interventions to reduce the risk for transmission.

The value of ciprofloxacin MIC was highly related to the accumulation of resistant mechanisms.[22] In this study, all the 21 isolates with four topoisomerase point mutations showed ciprofloxacin MIC $\geq 32\,\mu g/ml$. Isolates with higher ciprofloxacin MIC in this study usually acquired more complex quinolone-resistant determinants, including PMQR mechanisms. More than 60% of isolates in this study have acquired PMQR determinants, including *oqxAB*, *qnrS*, and *aac(6′)-Ib-cr* (Figs. 1 and 2). Studies showed that transferable quinolone resistance determinants could allow bacteria to survive in the presence of quinonlones and substantially enhance the number of resistant mutants which can be selected from the population.[29,33] However, the accurate relationship of PMQR determinants and point mutations in the QRDRs has not been clearly documented.[3] Since this study revealed a high prevalence of PMQR in the resistant isolates, it might be interesting to carry out a well-designed study to investigate the relationship between these PMQR determinants and point mutations in the QRDR in a defined chromosomal background.

The antimicrobial determinant *oqxAB*-containing isolates showed an unequal phylogenetic group distribution, with a lower percentage of isolates in group B1 (10/27, 37.0%), a higher percentage of phylogenetic groups A (45/63, 71.4%) and D (11/15, 73.3%) isolates. These results suggested that the acquisition of this transferable quinolone resistance determinant might be an uneven event. Since isolates of phylogenetic group D are usually more pathogenic, the virulent factors of those isolates containing transferable quinolone resistance determinants and $bla_{CTX\text{-}M}$ should be further characterized to clarify whether those isolates harbored common virulent factors or not.

In conclusion, our study demonstrated that retail meat products could be an important reservoir for the cefotaxime and ciprofloxacin co-resistant isolates. Since these isolates have accumulated complex resistant mechanisms, it is necessary to study their pathogenicity and evaluate its contribution in the community and hospital infections.

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Disclosure Statement

No competing financial interests exist.

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