Genotypic characterization of fluoroquinolone-resistant *Escherichia coli* isolates from edible offal

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**Abstract:** Edible offal is easily contaminated by *Escherichia coli* (*E. coli*) and fluoroquinolone (FQ)-resistant *E. coli* is considered a serious public health problem, thus, this study investigated the genetic characteristics of FQ-resistant *E. coli* from edible offal. A total of 22 FQ-resistant *E. coli* isolates were tested. A double mutation in each *gyrA* and *parC* led the highest MIC. Four (18.2%) isolates carried plasmid-mediated quinolone resistance genes. The *fimH*, *eaeA*, *escV*, *astA*, and *iucC* genes were confirmed. Seventeen isolates (77.3%) were positive for plasmid replicons. The isolates showed high genetic heterogeneity based on pulsed-field gel electrophoresis patterns.

**Keywords:** *Escherichia coli*; edible offal; fluoroquinolones; quinolone resistance-determining region; molecular typing

Fluoroquinolones (FQs) are a group of antibiotics commonly prescribed for a variety of infections in both human and veterinary medicine. FQs are widely used worldwide because of their bactericidal effects against broad range of bacteria. However, due to the indiscriminate use of FQs, the rise of FQ-resistance in bacteria is regarded as a serious public health concern [1].

One of the main risk factors for infections caused by antimicrobial-resistant bacteria is contaminated food. Edible offal, which means non-muscular part of the food-producing animals' carcasses, is a common food product in many countries, but can be easily contaminated by *E. coli* present in the intestinal microflora during slaughter and processing [2]. Therefore, edible offal is a potential source of antimicrobial-resistant *E. coli* that can be transferred to humans via the food chain. Although several studies related to FQ-resistant *E. coli* in livestock and meat products have been conducted [3-5], their significance in edible offal has not been satisfactorily explored. The objective of the current study is to investigate genetic characteristics of FQ-resistant *E. coli* isolates from edible offal.

One hundred-eighteen *E. coli* isolates were collected from edible offal samples (the heart, liver, stomach or gizzard, small intestine, and large intestine) produced at 8 chicken, 9 pig, and 7 cattle slaughterhouses located in the central and southern regions of Korea from January to October 2017. The method for isolating *E. coli* was as follows: 25 g of each sample was incubated in 225 mL of modified EC broth with novobiocin (Merck, Germany) at 37°C for 18-24 h. After incubation, 0.1 mL of each broth was inoculated onto MacConkey agar (BD, USA) and incubated at 37°C for 18-24 h. Fluoroquinolones (FQs) are a group of antibiotics commonly prescribed for a variety of infections in both human and veterinary medicine. FQs are widely used worldwide because of their bactericidal effects against broad range of bacteria. However, due to the indiscriminate use of FQs, the rise of FQ-resistance in bacteria is regarded as a serious public health concern [1].

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Disk diffusion test was performed to characterize the antimicrobial resistance profiles of FQ-resistant *E. coli* isolates according to the Clinical and Laboratory Standards Institute guidelines [7]. The antimicrobial disks (BD) used were nalidixic acid (NA, 30 μg), ciprofloxacin (CIP, 5 μg), ampicillin
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(AM, 10 μg), amoxicillin-clavulanate (AMC, 20/10 μg), cefazolin (CZ, 30 μg), cefepime (FEP, 30 μg), cefotaxime (CTX, 30 μg), cefoxitin (FOX, 30 μg), cefuroxime (CXM, 30 μg), ceftazidime (CAZ, 30 μg), cephalothin (CF, 30 μg), chloramphenicol (C, 30 μg), gentamicin (GM, 10 μg), imipenem (IPM, 10 μg), tetracycline (TE, 30 μg), and trimethoprim-sulfamethoxazole (SXT, 1.25/23.75 μg). If several isolates from one sample had the same resistance patterns, only one isolate was randomly selected. Multidrug resistance (MDR) was defined as resistance to at least one agent in three or more antimicrobial classes [8]. Minimum inhibitory concentrations (MICs) of norfloxacin (NOR), CIP, and enrofloxacin (ENR) for FQ-resistant E. coli isolates were further determined using the agar dilution method. E. coli ATCC 25922 was used as a control strain.

The FQ-resistant E. coli isolates were subjected to the PCR method as described previously for detecting the plasmid-mediated quinolone resistance (PMQR) genes (qnrA, qnrB, qnrD, qnrS, qepA, and aac(6’)-Ib-cr) and the genes causing resistance to β-lactam antimicrobials (blaTEM, blaSHV, and blaOXA).

**Table 1.** Distribution of multidrug resistance pattern among 22 fluoroquinolone-resistant Escherichia coli isolates from edible offal

<table>
<thead>
<tr>
<th>No. of antimicrobial classes shown resistance</th>
<th>Resistance patterns</th>
<th>No. of isolates (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>PCNs-CEPs-FPls-Qs-FQs-AMGs-PHs-TETs</td>
<td>4 (18.2)</td>
</tr>
<tr>
<td>7</td>
<td>PCNs-BL/BLICs-CEPs-Qs-FQs-AMGs-PHs</td>
<td>1 (4.5)</td>
</tr>
<tr>
<td></td>
<td>PCNs-CEPs-FPls-Qs-FQs-AMGs-TETs</td>
<td>1 (4.5)</td>
</tr>
<tr>
<td></td>
<td>PCNs-CEPs-FPls-Qs-FQs-PHs-TETs</td>
<td>1 (4.5)</td>
</tr>
<tr>
<td></td>
<td>PCNs-CEPs-Qs-FQs-AMGs-PHs-TETs</td>
<td>6 (27.3)</td>
</tr>
<tr>
<td></td>
<td>PCNs-CEPs-Qs-FQs-AMGs-PHs-TETs</td>
<td>1 (4.5)</td>
</tr>
<tr>
<td>6</td>
<td>PCNs-CEPs-FPls-Qs-FQs-TETs</td>
<td>4 (18.2)</td>
</tr>
<tr>
<td></td>
<td>PCNs-CEPs-Qs-FQs-PHs-TETs</td>
<td>1 (4.5)</td>
</tr>
<tr>
<td>5</td>
<td>PCNs-CEPs-Qs-FQs-TETs</td>
<td>1 (4.5)</td>
</tr>
<tr>
<td></td>
<td>PCNs-CEPs-Qs-FQs-PHs-TETs</td>
<td>1 (4.5)</td>
</tr>
<tr>
<td>4</td>
<td>Qs-FQs-PHs</td>
<td>0 (0)</td>
</tr>
<tr>
<td>3</td>
<td>Total</td>
<td>22 (100)</td>
</tr>
</tbody>
</table>

PCNs, penicillins; BLICs, β-lactam/β-lactamase inhibitor combinations; CEPs, cephems; FPls, folate pathway inhibitors; Qs, quinolones; FQs, fluoroquinolones; AMGs, aminoglycosides; PHs, phenicols; TETs, tetracyclines.
sulfonamide (sul1 and sul2), tetracycline (tetA and tetB), chloramphenicol (catA1 and cmlA), and aminoglycoside (aac(6')-Ib, aac(3)-II, and ant(2')-I) [6]. Mutations in the quinolone resistance-determining regions (QRDR) of the gyrA and parC genes and other resistance genes were examined by DNA sequencing. The virulence factor genes associated with pathotypes of E. coli (eaeA, excV, ent, bfpB, hly, stx1, stx2, ipaH, invE, aggR, astA, elt, est, fimH, papC, sfa/focDE, and iucC) were also confirmed by PCR as previously described [9,10]. For detecting the 18 major plasmid replicons in Enterobacteriaceae, a PCR-based replicon typing was conducted as described previously [11].

![Fig. 2. Genetic characteristics of 22 fluoroquinolone-resistant Escherichia coli isolates from edible offal. (A) Pulsed-field gel electrophoresis patterns for the E. coli isolates. (B) Antimicrobial resistance, virulence genes, and plasmid profiles of the E. coli isolates. NOR, norfloxacin; CIP, ciprofloxacin; ENR, enrofloxacin.](image)
Pulsed-field gel electrophoresis (PFGE) of the FQ-resistant *E. coli* isolates was performed in accordance with CDC PulseNet protocol [12], using a CHEF-Mapper apparatus (Bio-Rad Laboratories, USA). The dendrogram of PFGE patterns was constructed via Dice coefficients and the unweighted pair group method with arithmetic mean (UPGMA).

The antimicrobial resistance patterns of 22 FQ-resistant *E. coli* isolates are shown in Fig. 1. FQ-resistant *E. coli* isolates showed the highest resistance to AM (95.5%) and TE (90.9%), similar to previous studies on the high prevalence of AM and TE resistance from FQ-resistant *E. coli* from chicken [5] and pigs [3] in Korea. All of the FQ-resistant isolates were also classified as MDR (Table 1), consistent with the report by Hu et al. [3] that 91.5% of the FQ-resistant isolates showed MDR. Although it is unknown whether there is a direct relationship between FQ-resistance and MDR, this result is not surprising because sales of livestock antimicrobials in Korea have been steadily increasing since 2013, with penicillins and tetracyclines sold more than other antibiotics [13].

Comparison of MIC values and genetic characteristics related to the FQ-resistance of the isolates are presented in Fig. 2. The QRDR mutations in FQ target genes, such as *gyrA* and *parC*, play a significant role in the mechanism of FQ resistance in bacteria [1]. Similar to previous study in Korea [3], S83L (18 isolates, 81.8%) and D87N (17 isolates, 77.3%) substitutions in *gyrA* and the S80I substition (20 isolates, 90.9%) in *parC* were found to be widespread. Four isolates carried a double mutation in each *gyrA* and *parC*, and three isolates carried a single mutation in *gyrA* and a double mutation in *parC* showed the highest MIC ranges (≥256 µg/mL for NOR, 64 to 128 µg/mL for CIP, and ≥128 µg/mL for ENR). Four isolates which carried a single mutation in both *gyrA* and *parC* or *gyrA* only showed MICs ≤32 µg/mL for FQs. In consistent with these results, Moon et al. [14] also reported that double mutations in *parC* led to significantly increased MIC values for FQs. The PMQR genes were detected in 18.2% of FQ-resistant isolates, which is similar to that (15.3%) of pig fecal-derived isolates [3] and higher than that (5.56%) of chicken isolates [4] in Korea. Although PMQR genes do not produce high quinolone resistance by themselves, they can facilitate the selection of higher levels of quinolone resistance [5]. The additional antimicrobial resistance genes *blaT_E. coli, blaT_E. coli, sul1, sul2, catA1, cmaA, aac(6')-Ib, aac(3)-II, tetA*, and *tetB* were also detected, which suggests that FQ-resistant *E. coli* may carry resistances against many different antimicrobials as well as deliver FQ-resistance to other bacteria.

A total of five virulence-associated genes (*eaeA*, *escV*, *asta*, *fimH*, and *iucC*) were found in the isolates tested (Fig. 2). All 22 FQ-resistant isolates were found to have the *fimH* gene in their distribution of virulence genes. The *iucC* gene was detected in four isolates, the *asta* gene in three isolates, and the *eaeA* and *escV* genes in one identical isolate. It has been reported that *fimH*, the type 1 fimbrial adhesin gene, and *iucC*, the aerobactin synthase gene, are common in uropathogenic *E. coli* [10]. In addition, the *eaeA*, *escV*, and *asta* genes contribute to the pathogenicity of diarrheagenic *E. coli* [9].

Also, 17 FQ-resistant isolates were positive for any one of the 18 plasmid replicas (Fig. 2). Plasmids are small DNA molecules that are distinct from chromosomes and can provide beneficial effects to bacteria such as antibiotic resistance through horizontal gene transfer [11]. Frep (16 isolates, 94.1%) and FIB (12 isolates, 70.6%), which belong to the IncF group thought to play an important role in the spread of virulence and MDR among Enterobacteriaceae [15], were more frequent than other replicon types.

*XbaI* PFGE analysis identified a total of 20 clusters with ≥85% similarity (Fig. 2). The PFGE patterns of the FQ-resistant isolates revealed generally high genomic diversity (<50%). However, two isolates obtained from chicken (BC3 and BC6) and pig (QP1 and QP7), respectively, showed the same PFGE patterns. The isolates with identical PFGE patterns were harvested from samples from the same slaughterhouses, suggesting the possibility of cross-contamination of clones strains during slaughter and processing.

In conclusion, this study provides evidence for the role of edible offal in the dissemination of FQ-resistance in humans via the food chain and shows importance of enhancing hygiene to reduce the cross-contamination in the production of edible offal.

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**References**


