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SHORT COMMUNICATION

# 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

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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. Among colonies on the agar, those identified as *malB* gene-positive through polymerase chain reaction (PCR) analysis were confirmed as *E. coli* [6]. To find FQ-resistant *E. coli*, The *E. coli* colonies were streaked onto MacConkey agar containing 4 mg/L ciprofloxacin (Sigma-Aldrich, USA) and incubated at 37°C for 18-24 h. Twenty-two FQ-resistant *E. coli* isolates (12 isolates from chicken, 8 isolates from pig, and 2 isolates from cattle) were finally collected.

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

(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), cephalexin (CL, 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 ($bla_{TEM}$, $bla_{SHV}$, and $bla_{OXA}$),

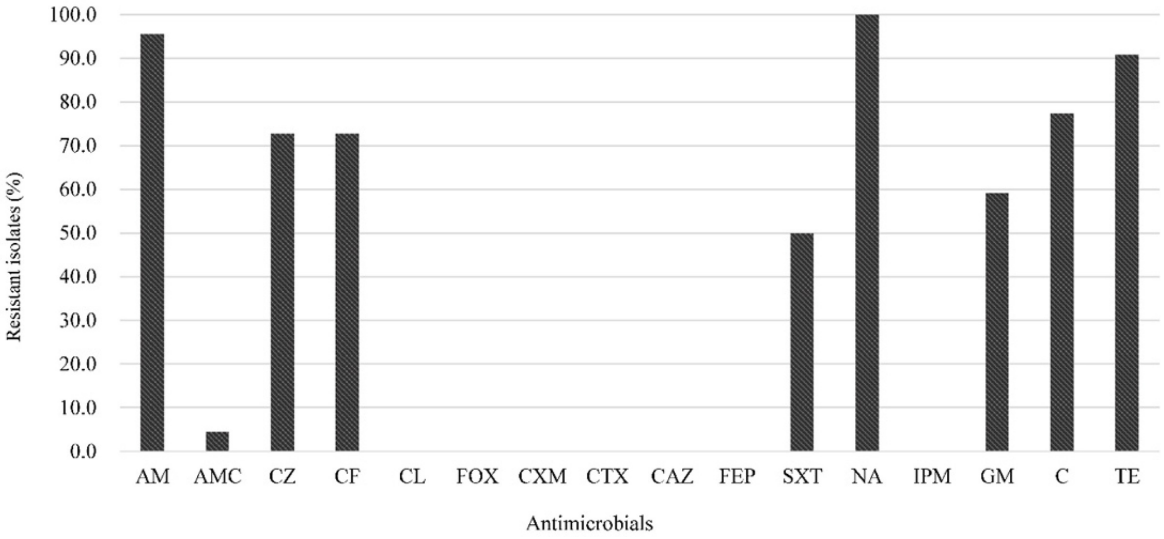


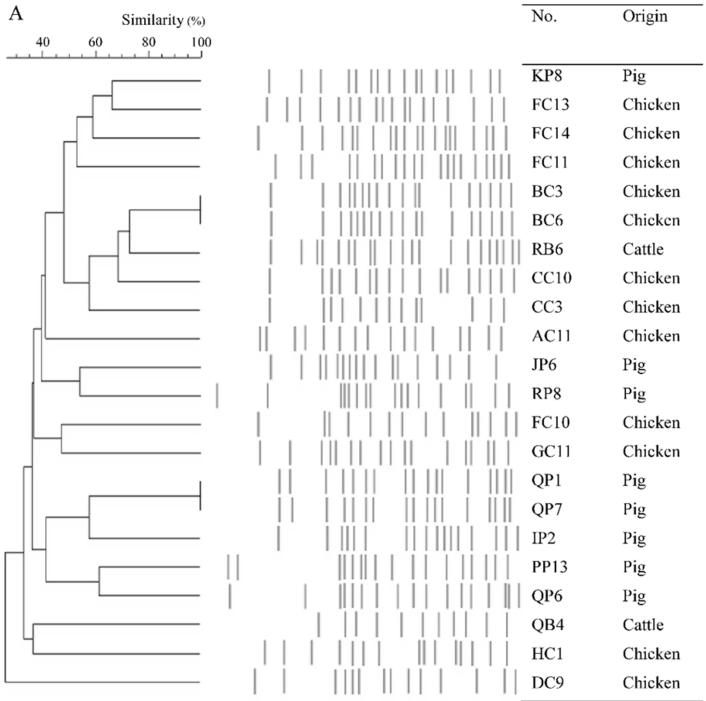

**Fig. 1.** Antimicrobial resistance patterns in 22 fluoroquinolone-resistant *Escherichia coli* isolates from edible offal. AM, ampicillin; AMC, amoxicillin-clavulanic acid; CZ, cefazolin; CF, cephalothin; CL, cephalexin; FOX, cefoxitin; CXM, cefuroxime; CTX, cefotaxime; CAZ, ceftazidime; FEP, cefepime; SXT, trimethoprim-sulfamethoxazole; NA, nalidixic acid; IPM, imipenem; GM, gentamicin; C, chloramphenicol; TE, tetracycline.

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

| No. of antimicrobial classes shown resistance | Resistance patterns | No. of isolates (%) |
|---|---|---|
| 8 | PCNs-CEPs-FPIs-Qs-FQs-AMGs-PHs-TETs | 4 (18.2) |
| 7 | PCNs-BL/BLICs-CEPs-Qs-FQs-AMGs-PHs | 1 (4.5) |
| | PCNs-CEPs-FPIs-Qs-FQs-AMGs-TETs | 1 (4.5) |
| | PCNs-CEPs-FPIs-Qs-FQs-PHs-TETs | 1 (4.5) |
| | PCNs-CEPs-Qs-FQs-AMGs-PHs-TETs | 6 (27.3) |
| | PCNs-FPIs-Qs-FQs-AMGs-PHs-TETs | 1 (4.5) |
| 6 | PCNs-CEPs-FPIs-Qs-FQs-TETs | 4 (18.2) |
| | PCNs-CEPs-Qs-FQs-PHs-TETs | 1 (4.5) |
| 5 | PCNs-CEPs-Qs-FQs-TETs | 1 (4.5) |
| | PCNs-Qs-FQs-PHs-TETs | 1 (4.5) |
| 4 | - | 0 (0) |
| 3 | Qs-FQs-PHs | 1 (4.5) |
| | Total | 22 (100) |

PCNs, penicillins; BLICs, β-lactam/ β-lactamase inhibitor combinations; CEPs, cephems; FPIs, 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, escV, ent, bfpB, hly, stx1, stx2, ipaH, invE, aggR, pic, 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].

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| No. | Origin | PMQR | QRDR mutations | | MIC (μg/ml) | | | Other resistance genes | Virulence genes | Plasmid replicons |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | gyrA | parC | NOR | CIP | ENR | | | |
| KP8 | Pig | - | S83L, D87N | S80I | 64 | 32 | 32 | $bla_{TEM-1}$, *cmlA, tetB* | *fimH* | - |
| FC13 | Chicken | - | S83L, D87N | S80I | 128 | 32 | 32 | $bla_{TEM-1}$, *aac(3)-II, tetA, tetB* | *fimH, iucC* | I1 |
| FC14 | Chicken | - | D87N | S80I, E84G | >512 | 128 | >512 | $bla_{TEM-1}$, *sul1, sul2, catA1, aac(6')-Ib, tetA* | *fimH* | FIB, Frep, I1 |
| FC11 | Chicken | - | S83L, D87N | S80I | 16 | 8 | 4 | $bla_{TEM-1}$, *sul2, tetA* | *eaeA, escV, fimH, iucC* | FIA, FIB, Frep, Y |
| BC3 | Chicken | - | S83L | S80I, E84G | 256 | 64 | 128 | $bla_{TEM-1}$, *cmlA, tetB* | *fimH* | FIB, FIC, Frep |
| BC6 | Chicken | - | S83L | S80I, E84G | 256 | 64 | 128 | $bla_{TEM-1}$, *tetB* | *fimH* | FIB, Frep |
| RB6 | Cattle | *qnrS* | S83L, D87N | S80I, E84A | >512 | 64 | 128 | $bla_{TEM-1}$, *sul1, sul2, aac(3)-II, tetB* | *fimH* | Frep |
| CC10 | Chicken | - | S83L, D87N | S80I, E84G | >512 | 64 | >512 | $bla_{TEM-1}$, *cmlA, tetB* | *fimH* | FIB, I1, Frep |
| CC3 | Chicken | - | S83L, D87N | S80I, E84G | 256 | 128 | >512 | $bla_{TEM-1}$, *cmlA, tetB* | *fimH* | FIB, I1, Frep |
| AC11 | Chicken | - | S84L, D87N | S80I | 32 | 16 | 8 | $bla_{TEM-1}$, *tetB* | *fimH, iucC* | FIB, Frep |
| JP6 | Pig | - | S83L, D87N | S80I, E84G | >512 | 128 | >512 | $bla_{TEM-1}$, *sul1, cmlA, tetB* | *fimH* | FIB, Frep |
| RP8 | Pig | - | S83L, D87N | S80I | 128 | 32 | 128 | - | *fimH* | - |
| FC10 | Chicken | - | D87N | S80I | 16 | 8 | 4 | $bla_{TEM-1}$, *sul2, cmlA, aac(3)-II, tetA* | *fimH* | FIB, Frep |
| GC11 | Chicken | - | S83L, D87Y | S80I | 16 | 8 | 32 | $bla_{TEM-1}$, *tetA* | *astA, fimH* | - |
| QP1 | Pig | *qnrA* | S83L, D87N | S80I | 16 | 8 | 8 | $bla_{TEM-1}$, *sul1, sul2, cmlA, tetB* | *fimH* | Frep |
| QP7 | Pig | *qnrA* | S83L, D87N | S80I | 64 | 8 | 8 | $bla_{TEM-1}$, *sul1, sul2, tetB* | *fimH* | Frep |
| IP2 | Pig | - | S83L | S80I | 16 | 16 | 8 | $bla_{TEM-1}$, *sul1, sul2, cmlA, tetA, tetB* | *fimH* | FIB, FIC, I1, Frep |
| PP13 | Pig | - | S83L, D87N | S80I | 64 | 16 | 8 | $bla_{TEM-1}$, *tetA* | *fimH* | - |
| QP6 | Pig | - | S83L, D87N | S80I | 16 | 8 | 8 | $bla_{TEM-1}$, *aac(3)-II, tetA* | *fimH* | - |
| QB4 | Cattle | *qnrS, aac(6')-Ib-cr* | S83L | - | 8 | 8 | 8 | $bla_{TEM-1}$, $bla_{OXA-2}$, | *fimH, iucC* | FIA,FIB, I1, HI1, N, HI2, Frep |
| HC1 | Chicken | - | D87N | S80I | 64 | 16 | 32 | $bla_{TEM-1}$, *sul1, cmlA, tetA* | *astA, fimH* | FIB, Frep |
| DC9 | Chicken | - | S83L, D87N | S80R | 8 | 16 | 8 | $bla_{TEM-1}$, *sul1, sul2, tetA* | *astA, fimH* | Y,I1, Frep |

**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.

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 *E. coli* 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%) substations in *gyrA* and the S80I substation (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 $bla_{\text{TEM-1}}$, $bla_{OXA-2}$, *sul1, sul2, catA1, cmlA, 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 *iuc* 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 replicons (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.

*Xba*I 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 clonal 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

1. Redgrave LS, Sutton SB, Webber MA, Piddock LJ. Fluoroquinolone resistance: mechanisms, impact on bacteria, and role in evolutionary success. Trends Microbiol 2014;22: 438-445.
2. Saide-Albornoz JJ, Knipe CL, Murano EA, Beran GW. Contamination of pork carcasses during slaughter, fabrication, and chilled storage. J Food Prot 1995;58:993-997.
3. Hu YS, Shin S, Park YH, Park KT. Prevalence and mechanism of fluoroquinolone resistance in *Escherichia coli* isolated from swine feces in Korea. J Food Prot 2017;80: 1145-1151.
4. Koo HJ, Woo GJ. Characterization of antimicrobial resistance of *Escherichia coli* recovered from foods of animal and fish origin in Korea. J Food Prot 2012;75:966-972.
5. Oh JY, Kwon YK, Tamang MD, Jang HK, Jeong OM, Lee HS, Kang MS. Plasmid-mediated quinolone resistance in *Escherichia coli* isolates from wild birds and chickens in South Korea. Microb Drug Resist 2016;22:69-79.
6. Son SH, Seo KW, Kim YB, Jeon HY, Noh EB, Lee YJ.

Molecular characterization of multidrug-resistant *Escherichia coli* isolates from edible offal in Korea. J Food Prot 2019; 82:1183-1190.
7. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing; Twenty-third Informational Supplement. CLSI document M100-S23. Wayne: Clinical and Laboratory Standards Institute; 2013.
8. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Harbarth S, Hindler JF, Kahlmeter G, Olsson-Liljequist B, Paterson DL, Rice LB, Stelling J, Struelens MJ, Vatopoulos A, Weber JT, Monnet DL. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect 2012;18:268-281.
9. Kagambèga A, Martikainen O, Lienemann T, Siitonen A, Traor AS, Barro N, Haukka K. Diarrheagenic *Escherichia coli* detected by 16-plex PCR in raw meat and beef intestines sold at local markets in Ouagadougou, Burkina Faso. Int J Food Microbiol 2012;153:154-158.
10. Tarchouna M, Ferjani A, Ben-Selma W, Boukadida J. Distribution of uropathogenic virulence genes in *Escherichia coli* isolated from patients with urinary tract infection. Int J Infect Dis 2013;17:e450-e453.
11. Johnson TJ, Wannemuehler YM, Johnson SJ, Logue CM, White DG, Doetkott C, Nolan LK. Plasmid replicon typing of commensal and pathogenic *Escherichia coli* isolates. Appl Environ Microbiol 2007;73:1976-1983.
12. Ribot EM, Fair MA, Gautom R, Cameron DN, Hunter SB, Swaminathan B, Barrett TJ. Standardization of pulsed-field gel electrophoresis protocols for the subtyping of *Escherichia coli* O157:H7, *Salmonella*, and *Shigella* for PulseNet. Foodborne Pathog Dis 2006;3:59-67.
13. Animal and Plant Quarantine Agency (APQA). National Antimicrobial Resistance Monitoring Program. Available from: https://ebook.qia.go.kr/20180704_110723.
14. Moon DC, Seol SY, Gurung M, Jin JS, Choi CH, Kim J, Lee YC, Cho DT, Lee JC. Emergence of a new mutation and its accumulation in the topoisomerase IV gene confers high levels of resistance to fluoroquinolones in *Escherichia coli* isolates. Int J Antimicrob Agents 2010;35:76-79.
15. Yang QE, Sun J, Li L, Deng H, Liu BT, Fang LX, Liao XP, Liu YH. IncF plasmid diversity in multi-drug resistant *Escherichia coli* strains from animals in China. Front Microbiol 2015;6:964.