Characterization of antimicrobial resistance in chicken-source phylogroup F *Escherichia coli*: similar populations and resistance spectrums between *E. coli* recovered from chicken colibacillosis tissues and retail raw meats in Eastern China

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ABSTRACT The extended-spectrum cephalosporin resistant *E. coli* from food animals transferring to community settings of humans causes a serious threat to public health. Unlike phylogroup B2 *E. coli* strains, the clinical significance of isolates in phylogroup F is not well revealed. Here, we report on a collection ($n = 563$) of phylogroup F *E. coli* isolates recovered from chicken colibacillosis tissues and retail raw chicken meat samples in Eastern China. There was an overlapped distribution of MLST types between chicken colibacillosis-origin and meat-source phylogroup F *E. coli*, including dominant STs (ST648, ST405, ST457, ST393, ST1158, etc). This study further investigated the presence of extended-spectrum β-lactamase (ESBL/pAmpC) producers in these chicken-source phylogroup F *E. coli* strains. The prevalence of extended-spectrum cephalosporin resistant strains in phylogroup F *E. coli* from chicken colibacillosis and raw meat separately accounted for 66.1 and 71.2%. The resistance genotypes and plasmid replicon types of chicken-source phylogroup F *E. coli* isolates were characterized by multiplex PCR. Our results revealed β-lactamase CTX-M, OXA, CMY and TEM genes were widespread in chicken-source phylogroup F *E. coli*, and *bla*CTX-M was the most predominant ESBL gene. Moreover, there was a high prevalence of non-lactamase resistance genes in these β-lactam-resistant isolates. The replicons IncB/O/K/Z, IncI1, IncN, IncFIC, IncQ1, IncX4, IncY, and p0111, associated with antibiotic-resistant large plasmids, were widespread in chicken-source phylogroup F *E. coli*. There was no obvious difference for the populations, resistance spectrums, and resistance genotypes between phylogroup F *E. coli* from chicken colibacillosis tissues and retail meats. This detail assessment of the population and resistance genotype showed chicken-source phylogroup F *E. coli* might hold zoonotic risk and contribute the spread of multidrug-resistant *E. coli* to humans.

Key words: phylogroup F *E. coli*, population, resistance spectrum, ESBL genes, chicken retail meats

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INTRODUCTION

Bacterial antimicrobial resistance (**AMR**), as one of the major public-health concern, causes a great impact on humans, animals, and the environment. Recent reports reveal that bacteria isolated from poultry exhibit clinically relevant AMR and harbor extended-spectrum β-lactamase (**ESBL**) genes, carbapenemase genes, colistin resistance genes, and other plasmid-mediated quinolone genes. Over the last 2 decades, ESBL-producing *E. coli* isolates have been detected with increasing occurrence in human and animal samples (Boswell et al., 2018; Kawamura et al., 2018; Paitan, 2018). ESBLs induce resistance to extended-spectrum (3rd and 4th generation) cephalosporins (e.g., ceftazidime, cefoperazone, cefixime, and cefpirome) and monobactams (Pitout and Laupland, 2008; Magiorakos et al., 2012; Nicolas-Chanoine et al., 2014). In addition, *E. coli* strains harbor plasmid-carrying cephalosporinases (**pAmpCs**) to exhibit a broader spectrum of drug resistance, including the great majority of cephalosporins and cephamycins, and pAmpC property is not repressed by β-lactamase inhibitors, leading to conferring almost

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all therapeutically accessible β-lactam drugs (Pitout, 2012). In veterinary medicine, the β-lactam drugs are undoubtedly the most important and commonly used antimicrobial category to inhibit bacterial infections. The increasing selective pressure of antibiotics could promote the rapid spread of bacterial resistance genes (Collignon and Voss, 2015). Besides the extensive usage of antimicrobial drugs in animal disease treatment, antibiotics consumption for widely subtherapeutic-dose addition in animal feedstuff is a major reason to accelerate the dissemination of antibiotic-resistant bacteria (Liu et al., 2016; Johnson et al., 2017). The occurrence of ESBL-produced *E. coli* from poultry source in China is increasing reported since 2006. Due to *E. coli* as the widespread gram-negative bacteria, the high occurrence of ESBL-produced *E. coli* in animal and food products caused both livestock industry and public-health challenges (Manges and Johnson, 2012; Pitout, 2012; Manges and Johnson, 2015).

E. coli displays wide-ranging phylogenetic substructure. Six phylogroups (A, B1, B2, C, D, and E) are initially delineated by multilocus enzyme electrophoresis with 35 enzyme loci (Selander et al., 1987). In 2000, an *E. coli* strain can be assigned to one of particular phylogroups (A, B1, B2, and D) by a triplex PCR method as pronounced by Clermont et al. (2000). The increasing multilocus sequence data and comparative genomics analysis is helpful for understanding of *E. coli* phylogroup structure, and the Clermont *E. coli* rapid phylotyping method is updated to enhance the specificity and detection of new phylogroups (Clermont et al., 2013). *E. coli* isolates are divided into 8 phylogroups (A, B1, B2, C, D, E, F, and clade I) by the new multiplex PCR method, which is validated to assign over 95% of *E. coli* strains into a different special phylogroups. When *E. coli* core-genome phylogenetic tree is rooted on *Escherichia fergusonii*, the strains assigned to 3 phylogroups (B2, F, and D) are located in the most basal and share closest relationships (Beghain et al., 2018). Phylogroup E then appears, followed by *E. coli* strains in phylogroups C, B1, and A, act as the most recently separated phylogroup (Beghain et al., 2018). Importantly, a historic evolutionary development of the species is associated to the lifestyle of the strains. The most anciently separated phylogroups (B2, F, and D) contain the majority of extraintestinal pathogenic *E. coli* (**ExPEC**) strains (Escobar-Paramo et al., 2004). However, the intestinal pathogenic *E. coli* (**InPEC**) isolates, commensal or environmental strains belong to the most newly diverged phylogroups, such as *E. coli* O157:H7 isolates located in phylogroup E and responsible for the severe intestinal pathologies (Zhu Ge et al., 2014).

Avian pathogenic *E. coli* (**APEC**) contaminated chicken products are associated with infection or colonization of humans (Ewers et al., 2014; Manges and Johnson, 2015). It is noteworthy that ST73, ST95, ST131, and ST141 APEC isolates in phylogroup B2 generally exhibit high virulence and zoonotic risk. In our recent research, chicken-origin *E. coli* within phylogroup F are identified as truly merging APECs and display close relationship with phylogroup B2 APEC strains, holding high virulence and zoonotic potential (Zhuge et al., 2020). Population of phylogroup F APEC isolates is revealed and limited to a few dominant STs (such as ST59, ST354, ST405, and ST648) (Zhuge et al., 2020). Recent reports show the occurrences of ESBL/pAmpC-positive *E. coli* in broiler flocks are existing in China (Li et al., 2010; Tong et al., 2015; Wu et al., 2018; Song et al., 2020). However, there is few report on the ESBL/pAmpC-producing *E. coli* isolated from retail raw chicken meats in China. Furthermore, the systematic assessment of the antibiotic resistance potential among chicken-source phylogroup F *E. coli* strains is described to be substantially lower. In this study, we had characterized the antibiotic resistance of chicken-source *E. coli* isolates in phylogroup F both phenotypically and genotypically. This was a comparison for the genetic background in antibiotic resistant phylogroup F *E. coli* recovered from chicken colibacillosis tissues or retail meats in Eastern China.

MATERIALS AND METHODS

Sample Collection and Bacterial Isolation

In our previously described, *E. coli* isolates were recovered from diseased/dead chicken (diagnosed with typical colibacillosis) in broiler farms among Jiangsu, Zhejiang, Anhui, and Shandong provinces in China, 2012 to 2017 (Zhuge et al., 2020). Phylogroup F *E. coli* strains were detected by the updated Clermont PCR protocols (Clermont et al., 2013), and a total of 289 Phylogroup F *E. coli* were recovered from chicken colibacillosis (Zhuge et al., 2020).

For *E. coli* strains recovered from retail meats, 2,361 chicken samples for retail slaughtered fresh chicken, raw chicken meat portions (including livers, necks, skeletons, etc.), and residual tissues in chicken meat packaging were obtained from 138 different supermarkets and food markets in Eastern China (major cities in the Yangtze River Delta) during the period from 2015 to 2019. These samples of chicken retail meats and packaging were transported quickly under cooling environments to our laboratory and stored at 4°C waiting for the next processes within 24 h. Small pieces (2 g) of chicken meat tissues were incubated overnight at 37°C in 10 mL of Luria-Bertani (**LB**) broth, and plated onto MacConkey agar plates. Then, one bacterial colony per plate of meat sample was isolated and purified in LB broth. Finally, these purified strains were added in peptoneglycerol medium and stocked at −80°C freezer.

Phylogenetic Screening for Phylogroup F E. coli *Isolates*

Phylogroups of chicken-source *E. coli* isolates were identified according to the previously described multiplex PCR (Clermont et al., 2013). *E. coli* isolates were usually distributed in six phylogroups, including A, B1, B2, D, E, and F.

All phylogroup F *E. coli* isolates were further performed MLST typing, according to 7 housekeeping genes (*adk, fumC, gyrB, icd, mdh, purA*, and *recA*) (Maiden et al., 1998). Seven pairs of primers were used to amplify these genes, and PCR amplicons were purified and sequenced for both the forward and reverse strands. DNA sequences for each *E. coli* isolates were matched to EnteroBase, available in *E. coli* MLST database (http://enterobase.warwick.ac.uk/species/ecoli/allele_st_search). Allele numbers corresponding to seven gene sequences of each *E. coli* isolate were obtained, and ST type of each phylogroup F isolate was specially designated by combining 7allelic profiles.

Serogrouping for Phylogroup F E. coli *Isolates*

O-serogroups of phylogroup F *E. coli* were detected by multiplex PCR using special primer pairs as the previously described (Iguchi, et al., 2015). Then, O-serogroups were confirmed by O antigen diagnostic serum (Tianjin Biochip) (Johnson et al., 2008; Zhuge et al., 2020).

Antimicrobial Susceptibility Testing

Based on the characterization standard for MDR, extensively drug-resistant (**XDR**), and PDR (pandrug-resistant) bacteria (Magiorakos et al., 2012), the susceptibility testing was performed by 27 antibiotics, classified into 16 antimicrobial types as follows. Aminoglycosides: amikacin (**AK**), gentamicin (**GEN**), kanamycin (**KAN**), and streptomycin (**STR**). Anti-MRSA cephalosporin: Ceftaroline (**CPT**). Antipseudomonal penicillins + β-lactamase inhibitor: piperacillin/tazobactam (**TZP**). Carbapenem: imipenem (**IPM**). Nonextended spectrum cephalosporins: cefazolin (**CZO**) and cefuroxime (**CXM**). Third and fourth generation cephalosporins: cefotaxime (**CTX**), ceftriaxone (**CRO**), ceftazidime (**CAZ**), and cefepime (**FEP**). Cephamycin: cefoxitin (**FOX**). Fluoroquinolone: ciprofloxacin (**CIP**) and levofloxacin (**LEV**). Folate pathway inhibitor: sulfisoxazole (SMZ) and trimethoprim/sulfamethoxazole (**SXT**). Glycylcycline: tigecycline (**TGC**). Monobactam: aztreonam (**ATM**). Penicillin: ampicillin (**AMP**). Penicillins + β-lactamase inhibitors: amoxicillin/clavulanic acid (**AMC**) and ampicillin-sulbactam (**SAM**). Phenicol: chloramphenicol (**CHL**). Phosphonic acid: fosfomycin (**FOS**). Tetracycline: tetracycline (**TET**). Polymyxins: Colistin (polymyxin E, PE). *E. coli* strains were cultivated at MH agar plates, and the paper disks containing each antibiotic were attached to these plates. The diameter of the inhibition zone for each agent was measured and recorded. *E. coli* ATCC25922 acted as the quality control. The antibiotic susceptibility of phylogroup F *E. coli* strains was determined according to the CLSI standard (CLSI, 2018). These phylogroup F isolates were judged as resistant (**R**), intermediately resistant (**I**), or susceptible (**S**).

Identifying the Types of Lactamase Resistance Genes in E. coli *Isolates*

The presence of ESBL and plasmid-mediated AmpC genes in phylogroup F *E. coli* strains were distinguished by multiplex PCR. ESBL genes (CTX-M-1, -2, -8, and -9 groups), lactamase genes (TEM, OXA, and SHV), and pAmpC genes (CMY, FOX, and DHA) were screened using special primer pairs (Table S1) (Dallenne et al., 2010; Poirel et al., 2011; Johnson et al., 2012a; Kawamura et al., 2018). PCR sequencing method was used to detect the specific types of lactamase resistance genes (ESBL, pAmpC, and other lactamase genes). Full-length nucleic acid sequences were used to decide β-lactamase types by BLAST analysis (http://www.ncbi.nlm.nih.gov/) and β-lactamase classification system (http://www.lahey.org/studies/webt.asp).

Identifying the Types of Non-lactamase Resistance Genes and Plasmid Replicons

Non-lactamase antibiotic resistance genes were detected in phylogroup F *E. coli* isolates by PCR amplification (Dallenne et al., 2010; Poirel et al., 2011; Johnson et al., 2012a; Kawamura et al., 2018), including plasmid-carried fluoroquinolone resistance genes (*aac [6']-Ib-cr, qepA, qnrA, qnrB*, and *qnrS*), sulfonamides resistance genes (*sul1, sul2*, and *sul3*), streptomycin resistance genes (*aadA, strA*, and *strB*), kanamycin resistance genes (*aph [3']-Ia*), tetracycline resistance genes (*tetA, tetB*, and *tetC*), fosfomycin resistance genes (*fosA* and *fosA3*), colistin resistance genes (*mcr-1* to *mcr-3*), etc (Table S1).

The replicon types of plasmid carried by ESBL/pAmpC-producing *E. coli* were detected by multiplex PCR method, as previously described (Carattoli et al., 2005; Johnson et al., 2007). PCR-based replicon typing method could target 19 replicon types, such as FIA, HI1, I1, L/M, A/C, K/B, and FIIA (Carattoli et al., 2005; Johnson et al., 2007).

RESULTS

Comparative Analysis for Population Structure Between Phylogroup F E. coli *Recovered From Chicken Colibacillosis and Retail Raw Chicken Meats*

In our previous study, 289 phylogroup F *E. coli* strains account for 21.7% of all *E. coli* recovered from chicken colibacillosis (Zhuge et al., 2020; Table S2). Almost phylogroup F *E. coli* isolates are recognized as truly APECs and hold ExPEC-associated pathogenic characteristics (Zhuge et al., 2020). In this study, a total of 2178 *E. coli* strains were recovered from the retail raw chicken meats. Overall, a majority of chicken meat-source *E. coli* strains were assigned to eight phylogroups: A (37.2%), B1 (21.7%), B2 (8.7%), C (5.4%), D (7.2%), E (1.4%), F (12.6%), and Clade I (2.2%). Remaining (3.6%) *E. coli* strains were classified as the nonclassified type.

We further assessed the population of chicken meat-source phylogroup F *E. coli* using MLST analysis. MLST assigned these meat-source phylogroup F strains ($n = 274$) to 27 unique STs (Table S3). Similar to the previously described, one ST containing more than 8 strains among the chicken-source *E. coli* was recognized as a dominant ST (Zhuge et al., 2020). These dominant STs harbored 11 STs (ST648, ST405, ST457, ST393, ST362, ST59, ST117, ST135, ST354, ST1158, ST115, and ST501). Our previous results show that the phylogroup F *E. coli* isolates from avian colibacillosis are assigned to 29 STs, including 13 dominant STs (Zhuge et al., 2020). We identified overlapped distribution of MLST types between chicken colibacillosis-origin and meat-source phylogroup F *E. coli.* There were 17 common STs among phylogroup F *E. coli* isolates, including all dominant STs in these chicken meat-source *E. coli.* Moreover, there was an overlapped distribution of O serotypes in same dominant STs among between chicken colibacillosis-origin and meat-source phylogroup F *E. coli.* The dominant ST *E. coli* strains within phylogroup F were summarized in Table 1, which indicated the close association between O serotypes and ST types.

Antimicrobial Susceptibility of Phylogroup F* E. coli *From Chicken Colibacillosis

Surveillance of antimicrobial resistance in chicken-source phylogroup F *E. coli* strains is critical to possibility of controlling avian colibacillosis. For the susceptibility of phylogroup F *E. coli* from chicken colibacillosis, all strains were tested with 27 antibiotics from 16 categories. More than half of phylogroup F *E. coli* from chicken colibacillosis presented the resistance to cephalosporin antibiotics, including CXM (66.1%), CTX (65.7%), CAZ (64.4%), ATM (64.7%), FEP (51.6%), and CPT (56.7%) (Figure 1A). About 32% phylogroup F *E. coli* isolates from chicken colibacillosis were resistant to β-lactamase inhibitors, including CTC, AMC, SAM, CCV, and TZP. Moreover, 34.9% *E. coli* isolates were resistant to FOX. There were high resistance rates of phylogroup F *E. coli* isolates from chicken colibacillosis to non-cephalosporin antibiotics. For 289 colibacillosis-related isolates, 97.2% resistant to AMP, 85.1% were resistant to CIP, and 81.0% resistant to SMZ. And around 60% of colibacillosis-related isolates were resistant to other non-cephalosporin antibiotics, such as GEN, KAN, TET, and STR (Figure 1A). Despite this, there was relatively low resistance to FOS (37.0%) and AK (33.6%). Importantly, 27 (9.3%) colibacillosis-related isolates conferred resistance to colistin with MICs ≥4 mg/L. It was worthy highlighting that all phylogroup F isolates were susceptible to IPM and TGC. The antimicrobial susceptibility tests showed all colibacillosis-related phylogroup F *E. coli* were MDR strains, and 95.5% isolates conferred resistance to more than 5 antimicrobial agents (Table S2). Furthermore, 4 phylogroup F *E. coli* isolates were resistant to 13 drug categories, apart from colistin, carbapenem, and glycylcycline. According to the definition of MDR, XDR, and PDR microbes (Magiorakos et al., 2012), 3 isolates resistant to 14 categories could be considered as XDR strains (Table S2). Based on the resistance spectrum for cephalosporins and β-lactamase inhibitors, more than 66.1% colibacillosis-related phylogroup F *E. coli* might produce ESBLs or pAmpCs. The majority of cephalosporin-resistance isolates were located in the dominant ST117, ST354, ST405, ST457, and ST648.

Antimicrobial Susceptibility of Phylogroup F* E. coli *From Retail Meats

The antimicrobial susceptibility tests were performed to evaluate antimicrobial resistance traits of phylogroup F *E. coli* from retail meats, which is critical to the poultry food safety. Similar to the resistance spectrums of *E. coli* recovered from chicken colibacillosis, isolates from retail meats held the high resistance rates (≥60%) to

Table 1. The association of ST types and O serotypes among chicken-source phylogroup F *E. coli* isolates.

	Colibacillosis-related isolates		Meat-related isolates	
STs[a]	No. of isolates	O serotypes	No. of isolates	O serotypes
ST59	21	O1	18	O1
ST62	8	O7	6	O7
ST115	17	O5, O8, O9, O21, O136	9	O5, O8, O9, O21
ST117	22	O24, O85, O78, O109, O161	17	O24, O78, O109, O111, O161
ST135	12	O2, O50, O83	16	O2, O50, O83
ST354	23	O1, O3, O11, O25, O45, O51	14	O11, O25, O45, O51
ST362	9	O15, O86, O25	19	O7, O15, O86, O25
ST393	16	O11, O15, O25, O86	21	O11, O15, O25, O86, O101
ST405	22	O2, O45, O102	34	O2, O21, O45, O102,
ST457	47	O11, O154, O102	27	O11, O154, O102
ST501	12	O17, O44, O77, O86,	8	O17, O44, O77,
ST648	29	O1, O2, O25, O45, O102	36	O1, O2, O25, O45, O50, O102
ST1158	8	O17, O44, O92, O102	11	O17, O44, O92, O102
Total Percent (%)	246 (85.1%)	-	236 (86.1%)	-

[a] The dominant STs, and each ST harbors more than 8 strains.

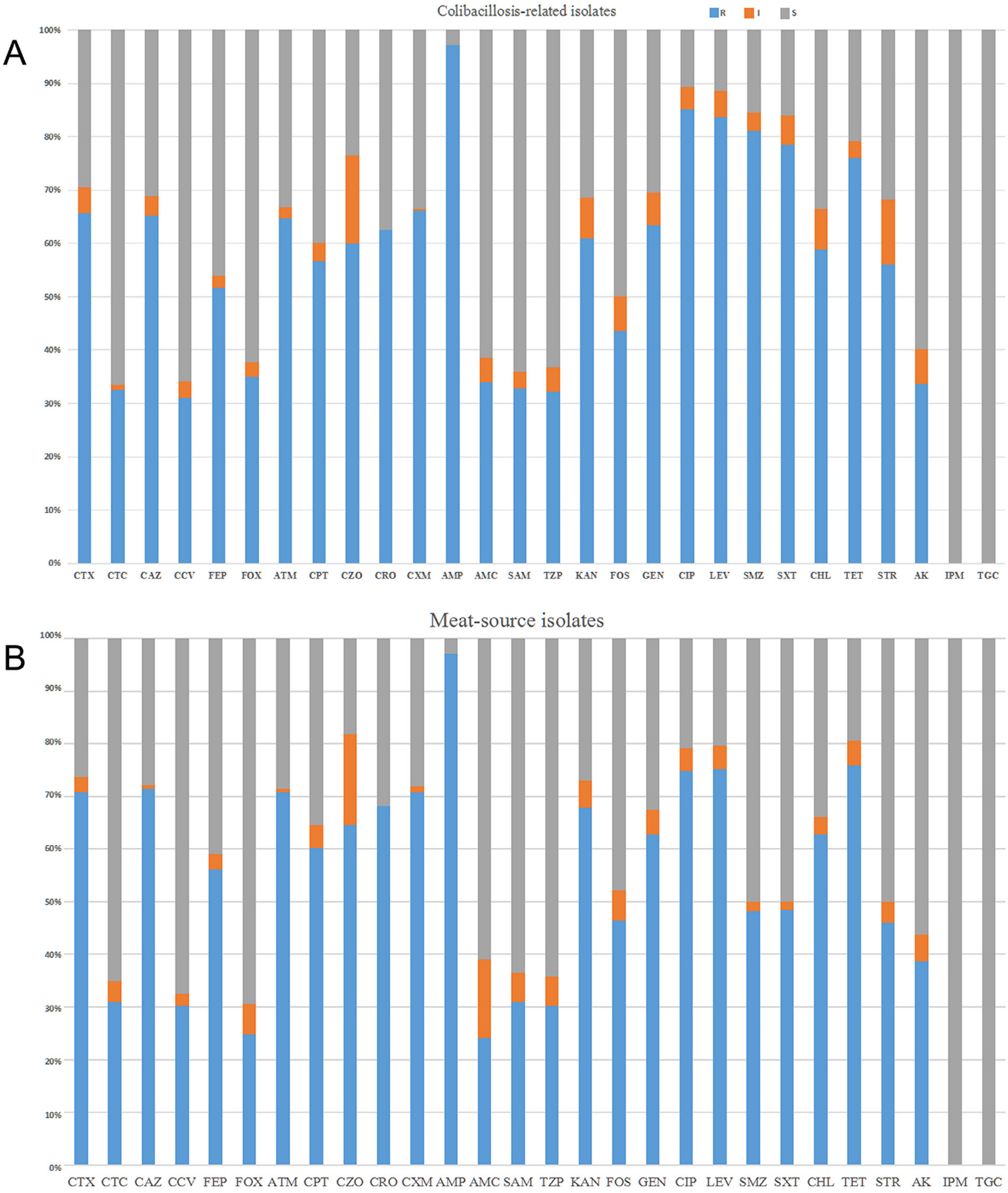

Figure 1. (A) Antimicrobial susceptibility for phylogroup F *E. coli* from chicken colibacillosis. The columns showed the percentages of 289 strains that were resistant (blue), intermediate (orange), or sensitive (gray) to 28 common antibiotics. Abbreviations were indicted in materials and methods. (B) Antimicrobial susceptibility for 274 phylogroup F *E. coli* isolates from chicken retail meats.

cephalosporin antibiotics, including CTX (70.8%), CAZ (71.5%), CRO (68.2%), CPT (60.2%), and others. Moreover, those isolates resistant to β-lactamase inhibitors presented about 24.0 to 30% rates, such as AMC (24.1%) and CTC (31.0%) (Figure 1B). Phylogroup F *E. coli* from chicken meats held low resistance rates to FOS (46.4%) and AK (38.7%). Moreover, 17 (6.2%) isolates were resistant to colistin. Similar to colibacillosis-related isolates, the antimicrobial susceptibility tests showed all meat-related phylogroup F *E. coli* were MDR strains, and 98.9% isolates conferred resistance to more than 5 antimicrobial agents. Furthermore, 5 phylogroup F *E. coli* from retail meats were resistant to 13 drug categories, apart from colistin, carbapenem, and glycylcycline (Table S3). About 71.2% chicken meat-related phylogroup F *E. coli* might produce ESBLs or pAmpCs, and the majority of cephalosporin-resistance isolates also belonged to the dominant ST117, ST354, ST393, ST405, ST457, and ST648.

Wide Distribution of ESBLs and pAmpCs Genes in Chicken-Source Phylogroup F E. coli Isolates

The cephalosporin susceptibility tests suggested that there might be existence of ESBLs and pAmpCs genes among phylogroup F *E. coli* recovered from chicken colibacillosis tissues and retail raw meats. ESBLs/pAmpCs gene profiles in these chicken-source phylogroup F strains were identified by multiplex PCR. For colibacillosis-related phylogroup F *E. coli*, about 89.6% isolates harbored β-lactamase TEM, CTX-M groups, OXA, CMY variants, and others (Table S2). The total 18 types of CTX-M, CMY, and OXA genes for 253 copy were detected in colibacillosis-related phylogroup F *E. coli* isolates (Figure 2A, Table S2). CTX-M genes were detected in 50.2% of colibacillosis-related strains. CTX-M genes presented in these isolates were distributed into 10 types, among which, *bla*$_{\text{CTX-M-15}}$ (37.7%), *bla*$_{\text{CTX-M-27}}$ (18.5%), *bla*$_{\text{CTX-M-55}}$ (13.9%), and *bla*$_{\text{CTX-M-1}}$ (11.9%) were the dominant *bla*$_{\text{CTX-M}}$ types. Plasmid-encoded pAmpC genes (*bla*$_{\text{CMY}}$ and *bla*$_{\text{DHA}}$) were presented in 19.4% colibacillosis-related phylogroup F isolates. The *bla*$_{\text{CMY-2}}$ (69.6%, 39/56) and *bla*$_{\text{CMY-42}}$ (21.4%, 12/56) were the dominant pAmpC types (Figure 2A, Table S2). A part of colibacillosis-related phylogroup F isolates (15.6%) harbored *bla*$_{\text{OXA}}$ genes, among which, (*bla*$_{\text{OXA-1}}$ = 44 and *bla*$_{\text{OXA-10}}$ = 1). Two strains contained the *bla*$_{\text{SHV-15}}$ gene, and one harbored *bla*$_{\text{DHA-1}}$ genes. Moreover, β-lactamase TEM genes were detected in 51.6% colibacillosis-related *E. coli* isolates (Table S2). ESBLs and pAmpC genes were concurrently present in colibacillosis-related *E. coli* isolates, forming a variety of combinations (such as *bla*$_{\text{CTX}}$-*bla*$_{\text{CMY}}$, *bla*$_{\text{CTX-M}}$-*bla*$_{\text{CMY}}$-*bla*$_{\text{OXA}}$, *bla*$_{\text{TEM}}$-*bla*$_{\text{CTX}}$-*bla*$_{\text{CMY}}$, and *bla*$_{\text{TEM}}$-*bla*$_{\text{CTX-M}}$-*bla*$_{\text{OXA}}$).

CTX-M, OXA, CMY, and TEM genes were also widespread in phylogroup F *E.coli* recovered in chicken meats (Figure 2B, Table S3). Similar to colibacillosis-related *E. coli*, CTX-M genes were detected in 53.6% of chicken meat-related isolates. CTX-M genes in these isolates were categorized into 14 types, among which, *bla*$_{\text{CTX-M-15}}$ (36.9%), *bla*$_{\text{CTX-M-14}}$ (17.4%), *bla*$_{\text{CTX-M-1}}$ (12.8%), *bla*$_{\text{CTX-M-27}}$ (10.7%), and *bla*$_{\text{CTX-M-55}}$ (8.1%) were the dominant *bla*$_{\text{CTX-M}}$ types (Figure 2B). Plasmid-encoded pAmpC genes (*bla*$_{\text{CMY}}$ and *bla*$_{\text{DHA}}$) were presented in 17.2% chicken meat-related isolates. The *bla*$_{\text{CMY-2}}$ (84.4%, 38/45) were the dominant pAmpC types (Table S3). The *bla*$_{\text{OXA-1}}$ and *bla*$_{\text{OXA-10}}$ were presented in 16.4% chicken meat-related phylogroup F isolates. β-lactamase TEM genes were detected in 55.8% chicken meat-related *E. coli* isolates (Table S3). Co-existence of ESBLs and pAmpCs genes were also widespread detected in meat-related phylogroup F *E. coli* isolates.

The Presence of Non-lactamase Resistance Genes in Chicken-Source Phylogroup F E. coli Isolates

Besides β-lactamases, many resistance genes located in large plasmids were detected in colibacillosis-related phylogroup F *E. coli*. The plasmid-mediated *strA* and *strB* genes, which conferred streptomycin resistance, were detected together (Moran et al., 2017). The presence of *strA*/*strB* (43.6%) could be detected in streptomycin-resistant phylogroup F isolates, and not distributed in streptomycin-susceptible strains. To date, the transferable fosfomycin-resistant genes (*fosA, fosA3, fosC2*, and *fosK*) were identified in Enterobacteriaceae, and plasmid-encoded *fosA3* mainly conferred the fosfomycin resistance in *E. coli* (Yao et al., 2016). We detected the *fosA* and *fosA3* in colibacillosis-related phylogroup F *E. coli* isolates. The presence of *fosA* (16.6%) and *fosA3* (20.4%) were detected in fosfomycin-resistant phylogroup F isolates, and not presented in fosfomycin-susceptible strains (Table S2). The aminoglycoside-resistance genes *aph(3′)-Ia*, *aac(3)-IId* and *aac(3)-IVa* were closely linked with *E. coli* gentamicin resistance. Our result showed the gentamicin-resistant phylogroup F isolates harbored these genes as the prevalence for 40.5, 29.8, and 20.4%, respectively. The plasmid-encoded *catA1, catB, cmlA*, and *floR* genes linked with chloramphenicol resistance could be detected in chloramphenicol-resistant phylogroup F isolates with the prevalence for 21., 15.9, 19.3, and 22.8% (Table S2). The acquisition of *sul* genes (*sul1, sul2*, and *sul3*) mediated *E. coli* resistance to sulfonamides. The *sul1* (42.6%), *sul2* (56.4%), and *sul3* genes (13.8%) were widespread in sulfonamide-resistant phylogroup F isolates. The plasmid-encoded *dfrA* gene in clinical *E. coli* mainly conferred the resistance to sulfisoxazole and trimethoprim-sulfamethoxazole trimethoprim. The 66.7% prevalence of *dfrA* was detected in colibacillosis-related phylogroup F *E. coli*. The presence of *tet(A), tet(B)*, and *tet (M)* for tetracycline resistance were detected in tetracycline-resistant isolates, among which, *tet(A)* (50.2%), *tet(B)* (29.8%), and *tet(C)* (12.1%) widespread in these phylogroup F strains. In addition to the mutations in *gyrA* and *parC* genes, plasmid-mediated quinolone resistance genes, including *aac(6′)Ib-cr, qnrA, qnrB, qepA, qnrS, oqxA*, and *oqxB* (Gomi et al., 2017; Badi et al., 2018) were closely associated with the resistance to fluoroquinolone. Multiple PCR results indicated that *aac(6′)Ib-cr* (23.2%), *qnrA* (9.7%), *qnrB* (5.9%), *qnrS* (11.7%), *qepA* (15.9%), *oqxA* (12.8%), and *oqxB* (12.5%) were detected in colibacillosis-related phylogroup F *E. coli* (Table S2).

For phylogroup F *E. coli* recovered in chicken meats, the presence of *strA* (46.4%) and *strB* (46.7%) could be detected in streptomycin-resistant meat-related isolates. *fosA* (12.0%) and *fosA3* (20.4%) were presented in fosfomycin-resistant meat-related isolates. The gentamicin-resistance genes *aph(3′)-Ia*, *aac(3)-IId* and *aac(3)-IVa* were detected for 58.4, 37.2, and 20.1%, respectively. The *catA1* (25.9%), *catB* (19.7%), *cmlA* (14.9%), and *floR* (22.3%) could be detected in chloramphenicol-resistant isolates (Table S3). The *sul1* (44.9%), *sul2* (56.6%) genes, and *sul3* (6.2%) were widespread in sulfonamide-resistant phylogroup F isolates. The 63.9% prevalence of *dfrA* was detected in chicken meat-related phylogroup F *E. coli*. The *tet(A)* (62.4%), *tet(B)* (65.3%), and *tet(C)* (28.8%) were also present in these phylogroup F strains. The *aac (6′)Ib-cr* (27.0%), *qnrA* (12.4%), *qnrB*(8.8%), *qnrS* (17.9%), *qepA* (21.9%), *oqxA* (10.6%), and *oqxB* (10.6%) were detected in meat-related phylogroup F *E. coli* (Table S3).

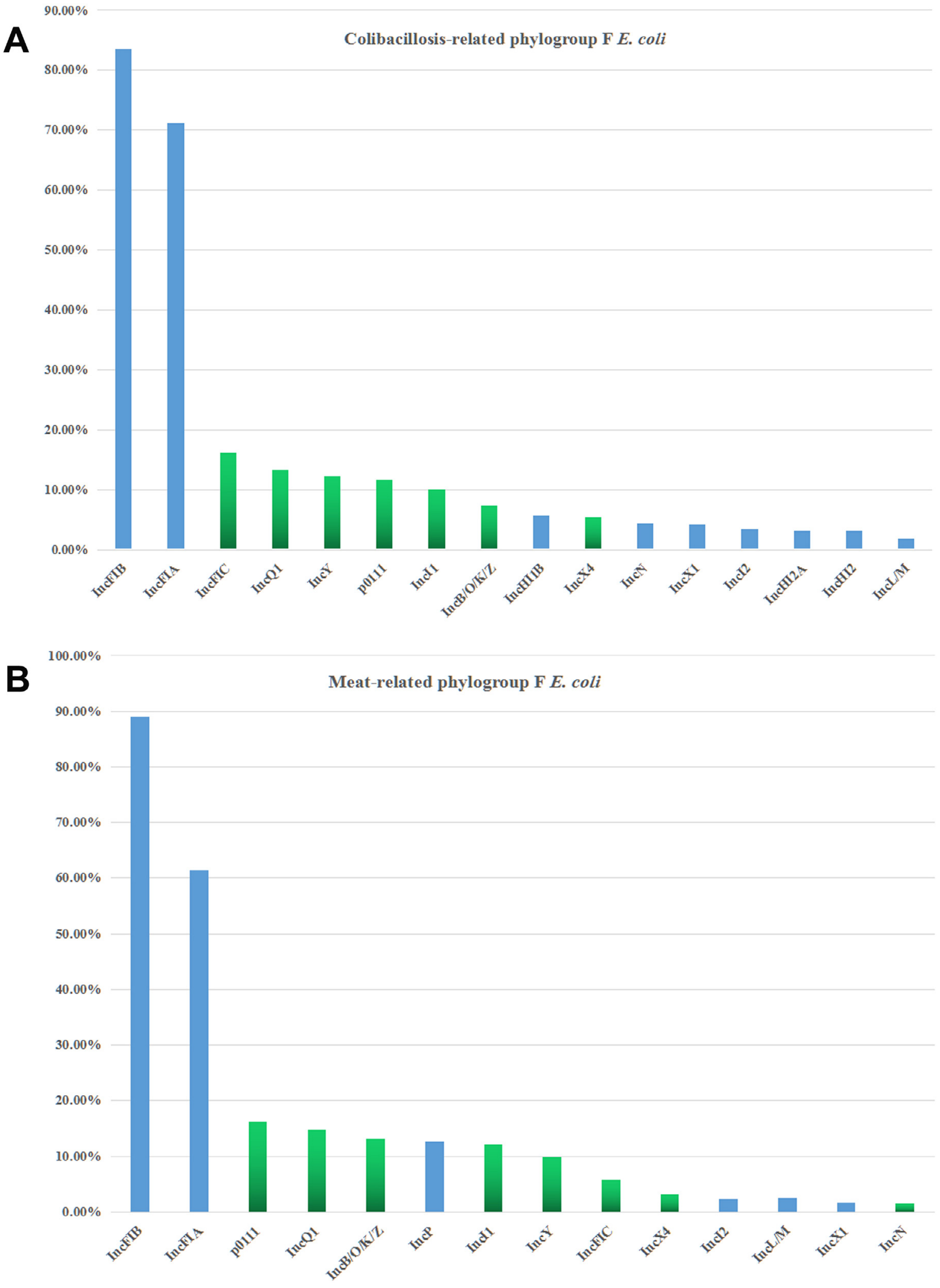

Figure 2. (A) The distribution of total CTX-M, OXA, CMY, and TEM genes in phylogroup F *E. coli* from chicken colibacillosis. (B) The percentages of CTX-M, OXA, CMY, and TEM genes in phylogroup F *E. coli* recovered from chicken retail meats.

Plasmid Replicon Types in Chicken-Source Phylogroup F E. coli *Isolates*

Owing to plasmid-encoded resistance genes widespread in chicken-source phylogroup F *E. coli* isolates, the total 19 replicon types (IncFIA, IncFIB, IncFIC, IncB/O/K/Z, IncP, IncQ1, IncHI2, IncHI2A, IncFII, IncI1, IncI2, IncHI1B, p0111, IncA/C, IncL/M, IncN, IncX1, IncX4, and IncY) were detected in each phylogroup F strain. For colibacillosis-related phylogroup F

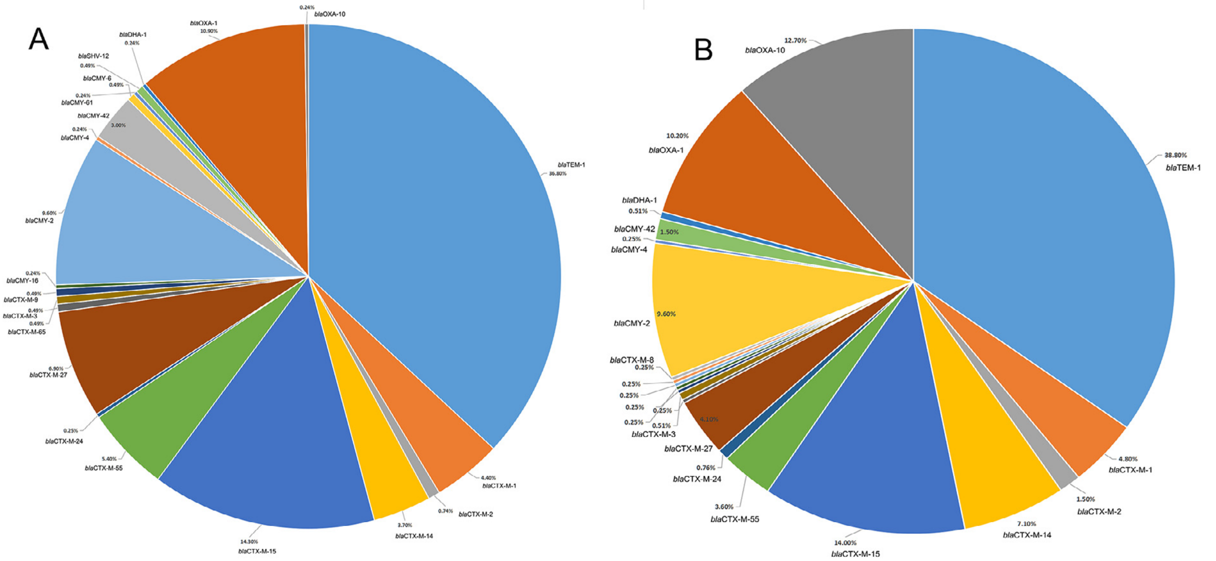

Figure 3. (A) Distribution of plasmid replicon types among the phylogroup F *E. coli* isolates from chicken colibacillosis. (B) Distribution of replicon types among the phylogroup F *E. coli* strains from chicken retail meats.

E. coli, IncFIA, IncFIB, IncFIC, IncFII, IncB/O/K/Z, IncI1, IncN, IncQ1, IncX4, IncY, and p0111 were the most commonly presented in these isolates (Figure 3A). Similar to colibacillosis-related isolates, plasmid replicon types (IncB/O/K/Z, IncFIA, IncFIB, IncFIC, IncFII, IncI1, IncI2, IncL/M, IncQ1, IncX4, IncY, and p0111) were identified in meat-related phylogroup F *E. coli* (Figure 3B). Apart from IncFIA, IncFIB and IncFII replicons, the widespread replicons IncB/O/K/Z, IncI1, IncN, IncFIC, IncQ1, IncX4, IncY, and p0111 were obviously associated with antibiotic-resistant large plasmids (Johnson et al., 2012b; Musicha et al., 2017; Kawamura et al., 2018). And IncB/O/K/Z, IncFIC, IncL/M, IncN, and IncY replicons exhibited the significant relations with resistance genotypes for ESBLs and pAmpCs genes (Johnson et al., 2012b; Musicha et al., 2017).

DISCUSSION

Human ExPECs cause a series of extraintestinal disease syndromes, such as urinary tract infections (**UTI**), bloodstream infections, neonatal meningitis, and wound infections. ExPECs are categorized as several subpathotypes, including uropathogenic *E. coli* (**UPEC**), sepsis-associated *E. coli* (**SEPEC**), and neonatal meningitis *E. coli* (**NMEC**) (Guo, et al., 2015; Mitchell, et al., 2015; Kallonen, et al., 2017). Moreover, the growing body of epidemiological evidences indicate that ExPEC isolates are widespread in nonhuman sources, including poultry, livestock, companion animals, and retail meat products (Bergeron et al., 2012; Manges and Johnson, 2012; Liu et al., 2018). APEC is a typical nonhuman ExPEC subpathotypes. A population assessment of human and avian *E. coli* strains from extraintestinal infections indicates that the isolates reassigned to phylogroup F hold a higher content of ExPEC-related virulence genes and pathogenicity islands, compared to that in the remaining new D and E groups (Logue et al., 2017). ExPEC strains within phylogroup F, are also highly prevalent in companion animals, swine, horses, cattle, and wild birds (Ewers et al., 2014; Abraham et al., 2015; Blyton et al., 2015; Guo, et al., 2015). Moreover, human ExPEC strains in phylogroup F exhibit antibiotic resistance potential and harbor a series of resistance genes (Abraham et al., 2015; Vangchhia et al., 2016; Logue et al., 2017).

Previous research show that majority of ExPEC isolates were assigned to phylogroups B2 and D, determined by triple PCR method (Clermont et al., 2000; Johnson et al., 2003; Rodriguez-Siek et al., 2005; Chapman et al., 2006; Johnson et al., 2008). In recent epidemiology, the revised Clermont multiplex PCR are performed to reclassify the phylogroups of human or nonhuman ExPECs strains. The isolates, originally classified into phylogroup D by old triple PCR, are reassigned to phylogroups D, F, and a minor group E (Logue et al., 2017). In our previous study, phylogroup F chicken-source *E. coli* isolates have been revealed as true APECs and hold virulence (Zhuge et al., 2020). Wu et al. (2018) reports the high prevalence of ESBL genes in chicken-source *E. coli* among the different poultry industries in China (). The findings reveal *bla*CTX-M as the predominant ESBL gene in ESBL-producing isolates (Wu et al., 2018). However, there is few report on the systematic assessment of the antibiotic resistance potential among chicken-source phylogroup F *E. coli* strains in China.

In this study, a total of 563 phylogroup F *E. coli* strains were recovered from chicken colibacillosis tissues and retail raw chicken meat samples in Eastern China. the antimicrobial susceptibilities of these chicken-source isolates were measured by disk diffusion method referring to the CLSI criteria, and about 68.6% prevalence of ESBL/pAmpC-producing isolates in chicken-source phylogroup F *E. coli*. In order to identify their molecular characteristics for antibiotic resistance, resistance genotypes and plasmid replicon types of chicken-source phylogroup F *E. coli* isolates were detected by multiplex PCR. Identification of resistance genotypes and replicon profiling indicated that a majority of chicken-source phylogroup F *E. coli* isolates harbored plasmid-carrying resistance genes to withstand multiple antimicrobial agents. Our results showed co-existence of ESBLs and pAmpCs genes were widespread detected in chicken-source phylogroup F *E. coli* isolates. To date, CTX-M type with an increasing occurrence worldwide becomes the dominant beta-lactamases type in Enterobacteriaceae family (Pitout, 2012; Poirel et al., 2018). Our study showed the $bla_{\text{CTX-M-15}}$ was the most common CTX-M type among phylogroup F *E. coli* recovered from chicken colibacillosis and retail raw meats samples. Besides the cephalosporins, AmpC-type beta-lactamases hold resistance to ESBLs inhibitors and cephamycin (such as cefotetan and cefoxitin) (Poirel et al., 2018). Although plasmid-mediated AmpC including a series of types, CMY-2 type is the most commonly AmpC-type beta-lactamase encountered among chicken-source phylogroup F *E. coli* isolates.

Previous epidemiological research indicates that ST95 *E. coli* isolates presented low frequency of antimicrobial resistance and even pan-susceptibility to antimicrobial agents (Stephens et al., 2017). The ST131 *E. coli* is acknowledged as worldwide high-risk multidrug-resistant clone (Mathers et al., 2015). For improved understanding of the spreading dynamics of ESBL-producing APEC from chicken meats to humans, the ESBL/pAmpC genes and non-lactamase resistance elements and genetic lineages of *E. coli* from chicken meats were analyzed. There was an overlapped distribution of MLST types between chicken colibacillosis-origin and meat-source phylogroup F *E. coli*, including dominant STs (ST648, ST405, ST457, ST393, ST1158, etc). Our results showed these dominant STs for chicken-source phylogroup F *E. coli* isolates were recognized as multidrug-resistant high-risk clones. Moreover, there was similar resistance spectrums and resistance gene contents for phylogroup F *E. coli* isolates from chicken colibacillosis and retail meats in Eastern China. The latest report by Clermont et al. (2019) shows a new phylogroup G, located intermediately between the phylogroups B2 and F. *E. coli* isolates in phylogroup G contains 5 sequence types (ST117, ST174, ST454, ST657, and ST738). There are some STs appearing in this study. We found many phylogroup F chicken-source *E. coli* strains belonged to ST117, ST657, and ST738, based on the identification criteria of phylogroup F in 2013. These strains originally belonging to phylogroup F are generally pathogenic and broadly resistant. This result indicated that this phylogroup G for avian-source *E. coli* was a zoonotic high-risk group, separated from the phygroup F.

These resistance genes are often positioned at transferable large plasmids of APEC isolates (Poirel et al., 2018; Zhuge et al., 2019). The occurrence of multidrug-resistant APECs not only cause difficulties to the avoidance and prevention of APEC infection, but also brings some challenges in the resistance spread of mobile plasmids to other pathogens and commensals. Therefore, as a long-term strategy, it is critical to discover alternative methods to control colibacillosis in poultry industry. Increased consumption of antimicrobial drugs in food-producing animals to enhance production efficiency have contributed to the emergence and spread of multidrug-resistant APEC/ExPEC, which might promote global increase of ExPEC population diversity in human resistant *E. coli* infections (Manges et al., 2007; Liu et al., 2016; Wang et al., 2016; Wang et al., 2017; Mellata et al., 2018; Song et al., 2020). However, there is a missing of direct evidence to disclose the causal association between food-original APEC/ExPEC and human extraintestinal infections, because, when establishing habitation in human gut, ExPEC can persist innocuously as commensal microbes in the intestinal tract for months, even to years until environments approving an extraintestinal infection (Manges and Johnson, 2012; Mellata et al., 2018). The improved surveillance of APEC dissemination among poultry reservoirs and chicken-derived food products, and the zoonotic risk of APEC transmission to human is strictly linked with public health implications.

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DISCLOSURES

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the position presented in, or the review of, the manuscript entitled, "**Characterization of Antimicrobial Resistance in Chicken-source Phylogroup F Escherichia coli: Similar Populations and Resistance Spectrums Between E. coli Recovered from Chicken Colibacillosis Tissues and Retail Raw Meats in Eastern China**"

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.psj.2021.101370.

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