Preplanned Studies

Prevalence of *Escherichia coli* and Antibiotic Resistance in Animal-Derived Food Samples — Six Districts, Beijing, China, 2020

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Summary

What is already known about this topic?

Escherichia coli is an important hygiene indicator for animal-derived foods such as pork and chicken, and the contamination of retail meat is associated with the spread of antimicrobial resistance (AMR) and public health.

What is added by this report?

The prevalence of *E. coli* in 6 different districts of Beijing was 64.1%. The contamination of chicken was more serious than pork in Beijing. *E. coli* isolates were highly resistant to sulfonamides (87.4%). The *ampC1* and *ampC2* genes were the main antibiotic resistance genotype (94.7% and 99.4%).

What are the implications for public health practice?

This study highlights the need to strengthen the surveillance of antibiotic resistance of *E. coli* in animal-derived foods. A national or regional multicenter study is required to assess the dissemination and evolution of multidrug resistant (MDR) *E. coli* in clinical medicine and animal production for food.

Bacterial resistance has become a global problem, among which the resistance of foodborne pathogens has attracted special attention (*1*). Foodborne pathogens are currently being actively monitored for antimicrobial resistance to analyze the spread and dissemination and support the prevention and control of antimicrobial resistance. Antibiotic-resistant bacteria/genes caused by using antimicrobial drugs in the agricultural industry can spread through the food chain (*2*). In the animal breeding process, China has launched a foodborne pathogenic bacteria antimicrobial resistance monitoring program and obtained a large amount of basic data. However, we still do not fully understand the antimicrobial resistance of food contaminating bacteria in the circulation link. *Escherichia coli* (*E. coli*) bacterial infections have caused a significant increase in morbidity and mortality worldwide, threatening human health (*3–4*). Thus, we are using *E. coli* as a representative to investigate the antibiotic resistance of animal-derived food contaminated bacteria in six districts within Beijing. *E. coli* is also an important hygiene indicator for animal-derived foods such as pork and chicken. Previous studies have shown that retail meat is associated with the spread of *E. coli* (*5–6*). Therefore, strengthening the monitoring of foodborne pathogenic *E. coli* is important to protecting human health. In this study, the epidemiological surveillance and typing study of *E. coli* in retail pork and chicken from six districts (Dongcheng, Xicheng, Haidian, Fengtai, Chaoyang, and Changping) in Beijing, China, was investigated. The results demonstrated that *E. coli* contamination in chicken is more serious than that in pork, and Chaoyang District displayed the highest *E. coli* isolation rate. Antimicrobial susceptibility testing showed that foodborne *E. coli* was highly resistant to sulfonamides with a resistance rate of 87.4%. The prevalence of resistance genes *ampC1* and *ampC2* occurred in most isolates. Therefore, effective supervision of animal-derived food hygiene to control the dissemination of bacterial resistance is essential to safeguard human health.

In this study, a total of 290 raw meat samples (91 pork and 199 chicken) were randomly collected from large and small supermarkets and farmer's markets in the 6 districts of Beijing. *E. coli* were isolated using CHROMagar ECC (CHROMagar TM, Paris, France) colored medium after enrichment. The isolated strains were identified by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS). Detailed materials and methods were provided as supporting information (Supplementary Materials, available in http://weekly.chinacdc.cn/). The gram-negative bacteria drug sensitivity plates Sensititre GNX3F (Thermo Fisher Scientific, Massachusetts, USA) were used for the antibiotic susceptibility test of

the isolated strains according to the recommendations of the Clinical and Laboratory Standards Institute guidelines (CLSI, M100-S30) (*7*) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) standards v10.0 (*8*). The microbroth dilution method was used to determine their susceptibility to 21 antimicrobial agents from 9 classes, including amikacin, gentamicin, tobramycin, doxycycline (DOX), tigecycline, minocycline, ciprofloxacin, levofloxacin, trimethoprim/sulfamethoxazole (SXT), aztreonam, imipenem, meropenem, doripenem, cefepime, ceftazidime, cefotaxime, colistin, polymyxin B, ampicillin/sulbactam, piperacillin/tazobactam, and ticarcillin/clavulanic acid. Whole genome sequencing (WGS) was conducted using an Illumina Hiseq2500 platform (Annoroad Gene Technology, Beijing, China). Multilocus sequence typing (MLST) was identified using the pMLST 2.0 database (mlst Github http://github.com/tseemann/mlst). Antimicrobial resistance genes were identified by searching the Comprehensive Antibiotic Research Database (CARD, https://card.mcmaster.ca/download). All draft genomes were used for constructing a phylogenetic tree using parsnp software (Supplemetary Materials, available in http://weekly.chinacdc.cn/ https://github.com/marbl/) (*9*), and the trees were finally visualized using the online tool iTOL (http://itol.embl.de/).

A total of 186 *E. coli* isolates (186/290, 64.1%) were collected from raw chicken and pork samples from Beijing in 2020. The highest rates (73.9%) of *E. coli* isolates were found in chicken compared to that (42.9%) in pork samples (Supplementary Table S1, available in http://weekly.chinacdc.cn/), which indicated that *E. coli* contamination in chicken was more serious than in pork. For chicken, Chaoyang District displayed the highest *E. coli* isolation rate among the six examined regions of Beijing, while Fengtai had the lowest isolation rate (50.0%) (Supplementary Table S1). While regarding pork, Xicheng displayed the highest *E. coli* isolation rate among the examined regions (66.7%), while Haidian and Chaoyang displayed the lowest isolation rates (33.3%).

The distributions of minimum inhibitory concentrations (MICs) and resistance rates of 186 *E. coli* isolates against 21 antimicrobial agents were shown in Table 1 and Figure 1A. Antimicrobial susceptibility tests showed that *E. coli* isolates were highly resistant to SXT (87.4%), followed by DOX (32.57%) and GEN (29.71%). Carbapenems (IMI, MERO, and DOR) had the lowest resistance rate of 4.0% (Table 1). *E. coli* isolates from six different regions represented high resistance to SXT, and Dongcheng showed 100% resistance to SXT (Figure 1B).

Comprehensive antibiotic resistome analysis using the CARD database in all 166 *E. coli* isolates indicated that diverse antimicrobial genotypes occurred in chicken and pork samples in Beijing. Among all the resistance genes, *ampC1* and *ampC2* were highly observed in all isolates (Figure 2), the detection rates were 94.7% and 99.4%, respectively. Extended-spectrum β -lactamase (ESBL)-producing strains of *E. coli* harboring CTX-M, OXA, CMY, and TEM were detected in this study. The most prevalent ESBL genes were bla_{TEM-1D} (22.7%) and $bla_{CTX-M-9}$ (21.5%), followed by $bla_{CTX-M-1}$ (11.0%) and bla_{OXA-7} (11.0%). Plasmid-mediated transferrable colistin resistant gene *mcr-1* was found in 22 isolates (13.3%). Most *mcr-1* genes were located on plasmid (15/22, 68.2%), and IncI2 (11/15, 73.3%) and IncY (4/15, 26.7%) were the main plasmid types. In addition, sulfonamide-resistant genes *sul1*, *sul2*, and *sul3* were also analyzed, and their detection rates were 16.9% (28 isolates), 32.5% (54 isolates), and 13.9% (23 isolates), respectively. Plasmid typing found that nine plasmid incompatibility (Inc) groups (IncFII, IncFIIp, Inc FIA, IncFIB, IncCol, IncCol156, IncX1, IncI2, and IncY) were common in these isolates. A total of 59 different STs were identified from 166 *E. coli* isolates. The most prevalent one was ST10 (22 isolates, 13.3%), followed by ST399 (11 isolates, 6.6%), and ST1434 (10 isolates, 6.0%). The remaining 56 STs were all lower than 4.2% abundance (Figure 2). Correlation analysis of antimicrobial resistance genotype and phenotype of *E. coli* isolates were shown in Supplementary Figure S1, available in http://weekly.chinacdc.cn/. Antimicrobial phenotype was consistent with resistance genotype for aminoglycosides, tetracyclines, fluoroquinolones, and lipopetides, except sulfonamides, penicillins, and carbapenems. Phylogenomic analysis revealed that all the *E. coli* isolates from animal-derived food and clinic were classified into two lineages, sharing different homologies (Supplementary Figure S2, available in http://weekly.chinacdc.cn/). Typing results showed commonality between human clinical strains, with STs 131 (especially), 297, and 2380 prominent. In addition, there was little crossover between types form

TABLE 1. Antibiotic resistant phenotype of 186 *Escherichia coli* isolates against 21 antimicrobial agents obtained from chicken and pork samples from the 6 districts, Beijing, China, 2020.

Antimicrobial classes	Antimicrobial agents	Chicken			Pork			Overall R%[§]
		S%[*]	I%[†]	R%[§]	S%[*]	I%[†]	R%[§]	
Aminoglycosides	Amikacin (AMI)	94.9	1.5	3.7	100.0	0.0	0.0	2.9
	Gentamicin (GEN)	65.4	1.5	33.1	82.1	0.0	18.0	29.7
	Tobramycin (TOB)	64.7	2.2	33.1	87.2	0.0	12.8	28.6
Tetracyclines	Doxycycline (DOX)	40.4	30.2	29.4	18.0	38.5	43.6	32.6
	Tigecycline (TGC)	98.5	0.0	1.5	94.9	0.0	5.1	2.3
	Minocycline (MIN)	83.8	11.0	5.2	64.1	12.8	23.1	9.1
Fluoroquinolones	Ciprofloxacin (CIP)	69.1	1.5	29.4	79.5	2.6	18.0	26.9
	Levofloxacin (LEVO)	73.5	2.9	23.5	79.5	7.7	12.8	21.1
Sulfonamides	Trimethoprim/sulfamethoxazole (SXT)	14.0	0.0	86.0	7.7	0.0	92.3	87.4
Penicillins	Aztreonam (AZT)	69.1	4.4	26.5	89.7	2.6	7.7	22.3
Carbapenems	Imipenem (IMI)	91.9	3.7	4.4	94.9	2.6	2.6	4.0
	Meropenem (MERO)	94.1	2.9	2.9	97.4	0.0	2.6	2.9
	Doripenem (DOR)	97.8	0.7	1.5	100.0	0.0	0.0	1.1
Cephalosporins	Cefepime (FEP)	80.9	4.4	14.7	97.4	0.0	2.6	12.0
	Ceftazidime (TAZ)	89.7	3.7	6.6	92.3	2.6	5.1	6.3
	Cefotaxime (FOT)	66.2	2.2	31.6	92.3	0.0	7.7	26.3
Lipopeptides	Colistin (COL)	80.9	2.9	16.2	92.3	5.1	2.6	13.1
	Polymixin B (POL)	69.1	15.4	15.4	82.1	15.4	2.6	12.6
β-lactam/β-lactam inhibitors	Ampicillin/sulbactam 2:1 ratio (A/S2)	66.2	11.8	22.1	74.4	20.5	5.1	18.3
	Piperacillin/tazobactam constant 4 (P/T4)	96.3	1.5	2.2	100.0	0.0	0.0	1.7
	Ticarcillin/clavulanic acid constant 2 (TIM2)	67.7	27.2	5.2	76.9	20.5	2.6	4.6

Note: The six districts of Beijing includes Dongcheng, Xicheng, Haidian, Fengtai, Chaoyang, and Changping.
[*] S% means the percentage of isolates that were sensitive to a specific antimicrobial.
[†] I% means the percentage of isolates that were intermediate resistant to a specific antimicrobial.
[§] R% means the percent of isolates that were resistant to a specific antimicrobial.

humans, chicken, and pork, with ST10 dominated among the most common types from animal-derived foods, which is rarely found in humans (Supplementary Figure S2, available in http://weekly. chinacdc.cn/). Thus, we speculated that the risk of *E. coli* isolates passing through the food chain was relatively low.

DISCUSSION

In this study, we determined the prevalence and characteristics of foodborne pathogen *E. coli* in retail meat in six districts of Beijing, China. The prevalence of *E. coli* was 73.9% in chicken samples and 42.86% in pork. Greater contamination of chicken than pork concurs with previous findings (*10*). These results indicated that *E. coli* contamination of raw chicken in Beijing was a serious public health problem. Thus,

better measures should be taken to control *E. coli* contamination in chicken. Antimicrobial susceptibility test in this study demonstrated that all the 186 *E. coli* isolates were highly resistant to at least one tested antibiotic class (sulfonamides, aminoglycosides, tetracyclines, fluoroquinolones, penicillins, carbapenems, cephalosporins, lipopeptides, and β-lactam/β-lactam inhibitors). The highest resistance to trimethoprim/sulfamethoxazole (87.4%) was observed, which might be due to sulfonamides being used widely in animal husbandry in China (*11*). Nowadays, the average resistance rates in *E. coli* to representatives of these antibiotic classes were higher than 40% (*12*). Eating retail chicken and pork without strict hygiene supervision should be avoided. These results highlight the contamination status of antibiotic resistant *E. coli* and provide an important reference value for the risk assessment and control of multidrug-resistant bacteria.
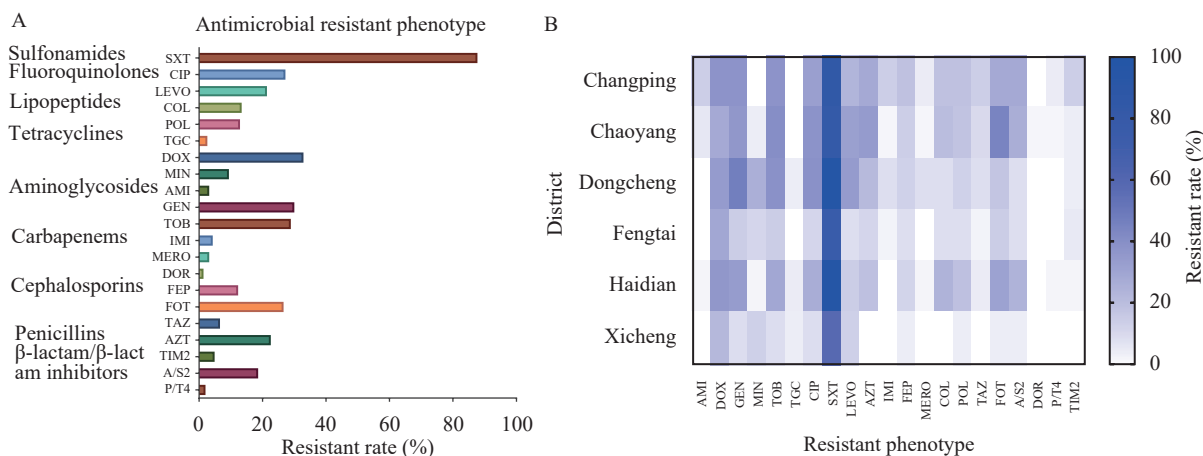
ESBL genes were located on plasmids that can be

FIGURE 1. Phenotypic resistance of *Escherichia coli* isolates in raw meat samples from six districts, Beijing, China, 2020. (A) Resistant rate of all *E. coli* isolates against nine different antibiotic classes. (B) Distribution of *E. coli* resistant phenotypes from different districts in Beijing.
Note: The six districts of Beijing includes Dongcheng, Xicheng, Haidian, Fengtai, Chaoyang, and Changping.
Abbreviations: SXT=trimethoprim/sulfamethoxazole; CIP=ciprofloxacin; LEVO=levofloxacin; COL=colistin; POL=polymyxin B; TGC=tigecycline; DOX=doxycycline; MIN=minocycline; AMI=amikacin; GEN=gentamicin; TOB=tobramycin; IMI=imipenem; MERO=meropenem; DOR=doripenem; FEP=cefepime; FOT=cefotaxime; TAZ=ceftazidime; AZT=aztreonam; TIM2=ticarcillin/clavulanic acid constant 2; A/S2=ampicillin/sulbactam 2:1 ratio; P/T4=piperacillin/tazobactam constant 4.

easily transferred between and within bacterial species. In this study, the resistance gene *ampC1* and *ampC2* were highly observed in all isolates, and detection rates were 94.7%, 99.4%, respectively. However, the detection rate of *E. coli ampC* in retail chicken from 2013–2014 (45.0%) dropped to 13.4% in 2018, UK (*13*). Wu et al. (*14*) reported the ESBL gene and *mcr-1* prevalence of chicken-derived *E. coli* in many provinces in China and found the detection rate of bla_{CTX-M} was 92.7%. The enrichment and changes of *E. coli ampC* resistance genes in raw meat sources in China are higher than those in other countries. With the increasing selection of β-lactam drugs, it will continue to mutate and spread in the food supply chain, causing serious public health problems. Among all the ESBL-producing *E. coli* isolates, the carrying rate of bla_{CTX-M} was 33.1%, $bla_{CTX-M-9}$ and $bla_{CTX-M-1}$ were the dominant subtypes. The carrying rate of $bla_{CTX-M-9}$ was 48.7%. The ST10 is a well-known clonal lineage (mainly harboring CTX-M gene); it is also known that some of the Inc plasmids were implicated in the spread of beta-lactamases genes and other genes encoding resistance to antibiotics. Our WGS results indicated that 76 of the ESBL-producing *E. coli* isolates had 31 distinct STs, and ST10 was the most prevalent (9/76, 11.8%). Colistin serves as the "last line of defense" for the clinical treatment of gram-negative bacterial infections (*15*). With the discovery of *mcr* family, China has banned the use of colistin as a feed additive

to prevent the dissemination of the gene. However, we found high levels of *mcr-1* in chicken-derived *E. coli*, which suggests that we should strengthen strict monitoring of food-borne pathogens in animal-derived foods that carry such drug-resistant genes.

This study has several limitations. The geographical distribution of the samples in this study was mainly concentrated in the central urban area, not representative of the entirety Beijing. The number of isolated strains of *E. coli* in retail pork samples was small, and strengthening the supervision of the strains in pork samples is necessary in future studies.

This study was a typical survey of the prevalence of *E. coli* in animal-derived foods in six districts of Beijing, China. *E. coli* contamination in chicken was more serious than that in pork. The isolates showed multi-drug resistance phenotypes, especially sulfonamides and tetracyclines. The β-lactamase genes *ampC1* and *ampC2* were the main drug resistance genes, and the colistin resistance gene *mcr-1* was found at a high level in chicken-derived *E. coli*. Considering the multidrug resistance of *E. coli* in animal-derived foods, especially ESBL-producing *E. coli*, continuous monitoring the emergence and spread of MDR *E. coli* would facilitate disease control and treatment. Further national or regional multicenter studies are necessary to assess the dissemination and evolution of MDR *E. coli* in both clinical medicine and food animal production in China.

FIGURE 2. Distributions of STs, antimicrobial resistance genes, and plasmid typing among 166 *Escherichia coli* isolates from chicken and pork across the phylogenetic tree.

Note: The color strips indicate areas corresponding to the isolates. Green colored cells represent the presence of genes and white cells represent the absence of the genes. Brown colored cells represent the presence of plasmid typing and white cells represent the absence of the plasmids.

Conflicts of interest: No conflicts of interest.

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SUPPLEMENTARY MATERIALS

METHODS

### Sample Collection, Isolation and Identification

From July to October 2020, a total of 290 animal-derived food samples (91 pork and 199 chicken samples) were collected from 16 large supermarket chains and farmers' markets in 6 districts of Beijing Municipality. The specific collected samples commercially available cuts of pork meat and commercially available chicken cuts. All samples were placed in Labplas TWIRL'EM sterile homogeneous bags (Labplas, Canada) after being collected and brought to the laboratory for testing. A single sample was placed in a sterile sampling bag to prevent cross-contamination during sample collection. The target strains were isolated as previously described (1), and the brief description was shown as following: 10 mL brain heart infusion (BHI) broth (Land Bridge, Beijing, China) was used to wash the sample surface, transfer to a 10 mL EP tube, and incubation at 37 °C for 24 h. 0.5–1 mL of the enrichment culture obtained was cultured on CHROMagar^{TM} ECC medium (CHROMagar, France) and inoculated at 37 °C for 24 h. The blue colonies on the plate were further purified using Luria agar (LA) and identified by matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry analysis.

Antimicrobial Susceptibility Testing

The gram-negative bacteria drug sensitivity plates Sensititre GNX3F were used for the antibiotic susceptibility test of the isolated strains according to the recommendations of the Clinical and Laboratory Standards Institute guidelines (CLSI, M100-S30) (2) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) standard v10.0 (3). E. coli ATCC25922 was used as the quality control strain.

Whole Genome Sequencing

Genomic DNA from E. coli isolates was extracted for whole genome sequencing using the Wizard Genomic DNA Purification Kit (Promega, Madison, WI, US) following the manufacturer's instructions. The DNA libraries were constructed using a KAPA Hyper Prep Kit (Roche, Basel, Switzerland). Sequencing was carried out with Illumina Novaseq 6000 platform (Illumina, San Diego, CA), which generated 150-bp paired-end reads from a library with an average insert size of 350 bp. Raw sequence data of E. coli isolates were assembled using SPAdes v3.13.1 (4) via the
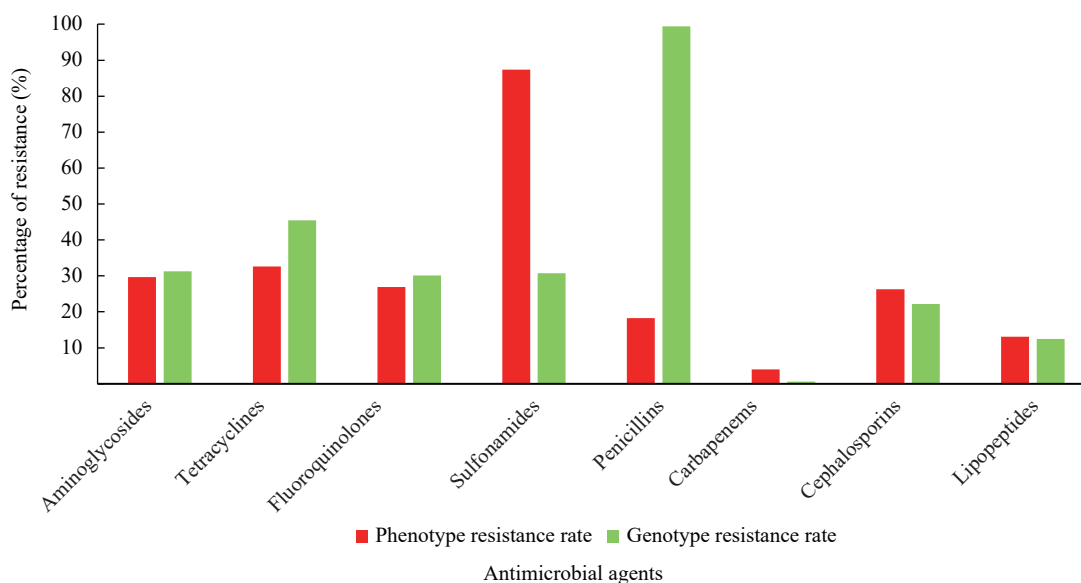
SUPPLEMENTARY FIGURE S1. Correlation analysis of antimicrobial resistance genotype and phenotype of *Escherichia coli* isolates.
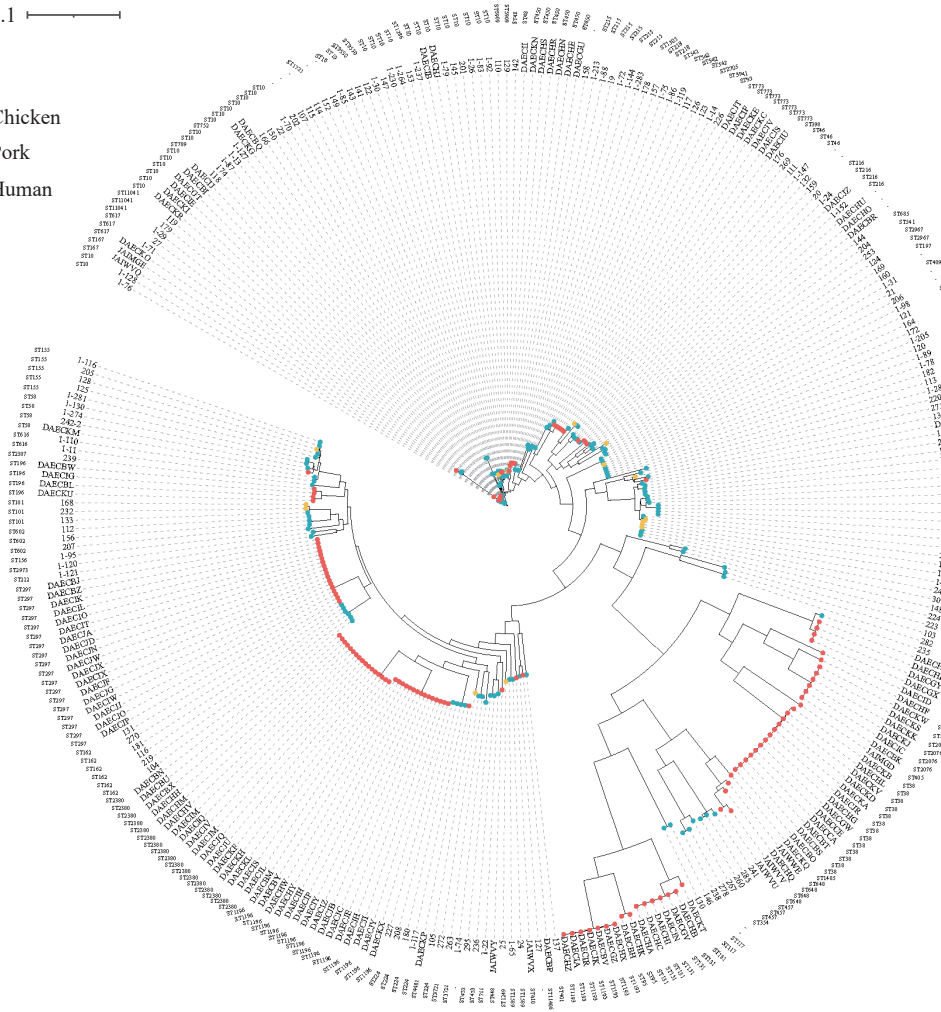
SUPPLEMENTARY FIGURE S2. Phylogenetic analysis of *Escherichia coli* isolates of animal-derived foods (n=166) and human clinic (n=146).
Note: A midpoint-rooted maximum-likelihood phylogenetic tree was constructed using core-genome single-nucleotide polymorphisms (SNPs).

Unicycler v0.4.7 (*5*) assembly pipeline. Additional genomes were downloaded from the National Center for Biotechnology Information (NCBI) Pathogen detection database (https://www.ncbi.nlm.nih.gov/pathogens/isolates#/search/). There were n=806 isolates retrieved with the search criteria "species_taxid:562" and "geo_loc_name: Beijing*" (*E. coli* from Beijing) on November 12th 2021. Among which, 422 clinical isolates collected from *Homo sapiens* were selected. Furthermore, 146 *E. coli* genomes collected between 2018–2021 were downloaded using collection time and WGS accession as filter criteria.

Phylogenetic Analysis

The full set of 312 genomes were used to generate a core-genome SNP alignment and construct a phylogenetic tree, using Parsnp v1.1.2 in the Harvest package (*6*). The mid-point rooted phylogenetic tree was annotated in ITOL (https://itol.embl.de/). To estimate the *E. coli* population structure, we used hierBAPS v6.0 software (*7*) to identified the Bayesian model-based population structures. BAPS groups were assigned based on single-nucleotide polymorphisms (SNPs) were identified with SAMtools v1.3.1 (*8*) using RedDog v1bata.11 (https://github.com/katholt/RedDog) pipeline in the core genome of the *E. coli* strains.

SUPPLEMENTARY TABLE S1. Characteristic and prevalence of *Escherichia coli* isolates from the 6 districts, Beijing, China, 2020.

Region	Chicken		Pork		Total isolating rate (%)
	No. of isolates	Isolating rate (%)	No. of isolates	Isolating rate (%)	
Dongcheng	18	85.7	5	41.7	63.7
Xicheng	14	73.7	8	66.7	70.2
Haidian	32	94.7	5	33.3	60.6
Fengtai	24	50.0	10	45.5	47.7
Chaoyang	43	95.6	6	33.3	65.2
Changping	16	80.0	5	41.7	67.0
Total	147	73.9	39	42.9	64.1

Note: The six districts of Beijing includes Dongcheng, Xicheng, Haidian, Fengtai, Chaoyang, and Changping. The primary objective of the present study was to investigate the isolating rate and prevalence of *E. coli* isolates in Chicken and Pork from the six districts, Beijing, China, in 2020.

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