Whole-Genome Sequencing-Based Characteristics in Extended-Spectrum Beta-Lactamase-Producing Escherichia coli Isolated from Retail Meats in Korea

Seokhwan Kim 1,2,*, Hansol Kim 1,*, Yonghoon Kim 1, Migyeong Kim 1, Hyosun Kwak 1, and Sangryeol Ryu 2,3,*

1 Division of Food Microbiology, National Institute of Food and Drug Safety Evaluation, Cheongju 28159, Korea; myksh@korea.kr (S.K.); hskmfds@korea.kr (H.K.); washout71@korea.kr (Y.K.); angelmg@korea.kr (M.K.)
2 Department of Food and Animal Biotechnology, Seoul National University, Seoul 08826, Korea
3 Department of Agricultural Biotechnology, Seoul National University, Seoul 08826, Korea
* Correspondence: hyoskwak@korea.kr (H.K.); sangryu@snu.ac.kr (S.R.)

Received: 20 February 2020; Accepted: 1 April 2020; Published: 2 April 2020

Abstract: The spread of extended-spectrum beta-lactamase-producing Escherichia coli (ESBL-EC) has posed a critical health risk to both humans and animals, because resistance to beta-lactam antibiotics makes treatment for commonly infectious diseases more complicated. In this study, we report the prevalence and genetic characteristics of ESBL-ECs isolated from retail meat samples in Korea. A total of 1205 E. coli strains were isolated from 3234 raw meat samples, purchased from nationwide retail stores between 2015 and 2018. Antimicrobial susceptibility testing was performed for all isolates by a broth microdilution method, and the ESBL phenotype was determined according to the Clinical and Laboratory Standards Institute (CLSI) confirmatory method. All ESBL-EC isolates (n = 29) were subjected to whole-genome sequencing (WGS). The antimicrobial resistance genes, plasmid incompatibility types, E. coli phylogroups, and phylogenetic relations were investigated based on the WGS data. The prevalence of ESBL-ECs in chicken was significantly higher than that in other meat samples. The results in this study demonstrate that clonally diverse ESBL-ECs with a multidrug resistance phenotype were distributed nationwide, although their prevalence from retail meat was 0.9%. The dissemination of ESBL-ECs from retail meat poses a potential risk to consumers and food-handlers, suggesting that the continuous surveillance of ESBL-ECs in retail meat should be conducted at the national level.

Keywords: Escherichia coli; extended-spectrum beta-lactamase; ESBL; retail meat; whole-genome sequencing

1. Introduction

Escherichia coli is a ubiquitous bacterium residing in the intestinal tract of humans and animals, environment, and food. Although most E. coli strains are harmless commensal bacteria, some strains, which harbor virulence factors, can cause various infections, such as diarrhea, hemorrhagic colitis, urinary tract infection, and meningitis [1–3]. E. coli can also serve as an important reservoir of antimicrobial resistance (AMR) genes, that may be transferred to human pathogenic bacteria [4–6]. Therefore, the spread of antimicrobial-resistant E. coli, especially extended-spectrum beta-lactamase (ESBL)-producing E. coli (ESBL-ECs), has become a threat to human as well as animal health worldwide [7].

ESBLs are enzymes that confer resistance to most beta-lactams, such as penicillins and cephalosporins, except for cephamycin or carbapenem, but these enzymes are inhibited by
The resistance to beta-lactams, one of the most widely used antibiotics, makes treatment for common infectious diseases caused by ESBL-ECs more complicated, as it involves hospitalization and intravenous carbapenem administration, instead of taking oral antibiotics at home [9–11]. As some ESBL genes are located on mobile elements such as plasmids and may be easily transferred to various bacterial species [12,13], the prevalence of ESBL-producing isolates from humans, livestock, and even food is rapidly increasing worldwide [14,15]. Some previous studies have suggested that ESBL genes can be disseminated through the food chain [16–18]. Meat contaminated by antimicrobial-resistant bacteria can act as a reservoir of such bacteria, and resistance determinants may be transferred to humans [18,19]. The selection pressure due to the ongoing overuse and misuse of antimicrobial agents possibly accelerates the emergence of antimicrobial-resistant bacteria [20,21].

The Korean government has monitored the antimicrobial susceptibilities of zoonotic bacteria from foods such as retail meat to medically important antimicrobials including beta-lactam antibiotics. Since whole-genome sequencing (WGS) has become affordable and facilitates the acquisition of useful information regarding multiple AMR genes, genomic mutations, and higher-resolved microbial typing from a single assay, some countries, including the USA, have already conducted WGS-based AMR surveillance [22]. A number of studies have reported the prevalence and characteristics of ESBL-EC from humans and food-producing animals in Korea because of the importance of such ESBL-ECs from a public health perspective [23–29]. However, few studies have reported the prevalence and characteristics of ESBL-ECs in retail meat in Korea [30–32]. Therefore, this study aimed to report the prevalence and AMR-related characteristics of ESBL-ECs present in retail meat, including beef, pork, and chicken, collected through the national surveillance program between 2015 and 2018. This information will help to characterize the molecular epidemiology of ESBL-ECs related to retail meat in Korea.

2. Materials and Methods

2.1. Sample Collection and Bacteria Isolation

In total, 3234 meat samples, including beef (n = 1,290), pork (n = 1,126), and chicken (n = 818), were purchased at approximately 100 grocery stores spread across all the provinces of South Korea between 2015 and 2018. Overall, average ~800 raw meat samples were purchased per year. Domestic meat samples were from 43 beef production companies, 32 pork production companies, and 18 chicken production companies; these companies had high market shares. Among the imported meat samples, beef samples were from 5 countries, pork from 14 countries, and chicken from 4 countries. The meat samples were kept on ice during transportation from the grocery stores to the laboratory. Twenty-five grams of each meat sample was homogenized with 225 mL EC broth (Difco, MI, USA) using a stomacher. The homogenized samples were incubated under aerobic conditions at 37 °C for 24 h. An aliquot of each sample was streaked onto selective medium, the Eosin Methylene Blue agar (Oxoid, Cambridge, UK), and incubated at 37 °C for 24 h. Typical E. coli colonies (green metallic sheen) were sub-cultured on nutrient agar (Difco) and confirmed using a Vitek 2 Compact microbial identification system (bioMérieux, France) or Vitek MS (bioMérieux) by following the manufacturer’s instructions. One typical and well-isolated E. coli strain per meat sample was selected. If no typical growth was observed, the sample was treated as a negative sample and was discarded. A total of 1205 E. coli strains were isolated from raw meat samples. All isolates were stored at −80 °C in Tryptic Soy Broth (Difco), mixed with 15% glycerol.

2.2. Antimicrobial Susceptibility Testing and Confirmation of ESBL-ECs

All selected strains of E. coli (n = 1,205) were subjected to antimicrobial susceptibility testing using the following antimicrobials: amoxicillin/clavulanic acid (AmC), ampicillin (AMP), cefoxitin (FOX), ceftiofur (CTF), cefazidime (CAZ), cefepime (FEP), chloramphenicol (CHL), ciprofloxacin (CIP), colistin (COL), gentamicin (GEN), meropenem (MEM), nalidixic acid (NAL), streptomycin (STR), tetracycline
Microorganisms 2020, 8, 508

(TET), and trimethoprim/sulfamethoxazole (SXT). The minimum inhibitory concentrations (MICs) of these antimicrobials were determined using a broth-dilution method that involved a commercially available Sensititre plate KRNV4F (Trek Diagnostic Systems, Cleveland, OH, USA) and by following the manufacturer’s instructions. E. coli ATCC 25922 was used as a reference strain. Susceptibility results in the form of MICs were interpreted by referring to the Clinical and Laboratory Standards Institute (CLSI) guidelines [33], European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines [34], and National Antimicrobial Resistance Monitoring System [35] (Table S1). The strains (n = 120) resistant to the third-generation cephalosporins (ceftiofur or ceftazidime) were tested for the ESBL phenotype, which was determined using the CLSI confirmatory broth microdilution test, that involves cefazidime and cefotaxime with and without clavulanic acid [33].

2.3. Whole-Genome Sequencing and Phylogenetic Analysis

All isolates with an ESBL phenotype (n = 29) were subjected to whole-genome sequencing (WGS). Total bacterial DNA was extracted using an UltraClean microbial DNA isolation kit (MO BIO Laboratories Inc., Carlsbad, CA, USA), by following the manufacturer’s instructions. Sequencing was performed at Senigen Inc. (Seoul, Korea) using the Illumina MiSeq desktop sequencer (Illumina Inc., San Diego, CA, USA), with paired-end reads of length 300 bp. A de novo assembly was performed using SPAdes genome assembler version 3.13.0 [36]. Contigs of less than 200 bp in length and 5× in sequencing depth were removed from analysis. The number of assembled contigs ranged between 41 and 202, with an average sequencing depth of 210×. These assemblies were annotated with Prokka [37] and the output was used for the pan-genome pipeline using Roary [38] to construct the core-genome of 29 ESBL-EC isolates. Roary parameters were set to default (minimum blastp identity 95% and threshold of isolates required to define a core gene 99%). Genes were classified as “core” and “soft core” if they were identified in at least 99% of the isolates and 99%–95% of the isolates, respectively. All genes present in <95% of the isolates were classified as “accessory”. The curve-fitting of the pan-genome growth was performed using a power law regression based on Heap’s law [39–41] as follows: $y = Ax^\gamma + B$. The fitting was conducted using PanGP [42] to fit the power law regression, where y and x are pan-genome size and number of the genomes, respectively. Furthermore, γ is an empirical parameter for estimating whether a pan-genome is open or closed [39,40].

An alignment of polymorphic sites in the core genome alignment were generated using a SNP-Sites tool [43] (https://github.com/sanger-pathogens/snp-sites). This single-nucleotide polymorphism (SNP) alignment was used to construct a maximum likelihood (ML) phylogenetic tree, using RAxML version 8.0.0 [44] under the general time reversible (GTR) substitution model with a Gamma rate of correction heterogeneity. This core-genome SNP alignment was also used to cluster the isolates into unique subpopulations or sequence clusters using the Bayesian analysis of population structure (hierBAPS) [45]. Phylogenetic trees were visualized using FigTree (https://github.com/rambaut/figtree/releases) and Phandango [46].

2.4. Nucleotide Sequence Accession Numbers

The whole-genome sequencing data reported in this study have been deposited at GenBank under the BioProject PRJNA599028 as the following accession numbers: WVUS00000000 (EC2015_85), WVUT00000000 (EC2016_8), WVUU00000000 (EC2016_31), WVUV00000000 (EC2016_174), WVUW00000000 (EC2016_I10), WVUX00000000 (EC2016_I74), WVUY00000000 (EC2016_I77), WVUZ00000000 (EC2017_2), WVVA00000000 (EC2017_136), WVVB00000000 (EC2017_202), WVVC00000000 (EC2017_203), WVVD00000000 (EC2017_240), WVVE00000000 (EC2017_617), WVVF00000000 (EC2017_303), WVVG00000000 (EC2017_575), WVVH00000000 (EC2017_I80), WVVI00000000 (EC2017_I216), WVVI00000000 (EC2017_I306), WVVK00000000 (EC2017_I318), WVVL00000000 (EC2017_I327), WVVM00000000 (EC2018_100), WVVN00000000 (EC2018_I02), WVVO00000000 (EC2018_273), WVVP00000000 (EC2018_311), WVVQ00000000.
2.5. In silico Molecular Typing and Characterization

In silico E. coli phylotyping was performed using ClermonTyping [47], and E. coli isolates were assigned to phylogroups A, B1, B2, C, D, E, and F. In silico plasmid typing was done by searching for plasmid incompatibility groups in PlasmidFinder 2.1 database [48], available on the Center for Genomic Epidemiology (CGE) website (http://www.genomicepidemiology.org). AMR genes were also identified using the ResFinder 3.2 database [49] on the CGE websites. Presence of a gene in an isolate was confirmed if its assembled genome sequence had more than 95% nucleotide identity match with a gene in the database, and a coverage of 100% of the length of the database match.

2.6. Multilocus Sequence Typing

ESBL-EC isolates were subjected to multilocus sequence typing (MLST), using seven housekeeping genes (adk, fumC, gyrB, icd, mdh, purA, recA), as previously described [50]. PCR amplification was performed using a thermal cycler 3500XL (Applied Biosystems, Singapore), under the following condition: 25 cycles of 96 °C for 10 s, 50 °C for 5 s, and 60 °C for 4 min. The internal fragments of all loci were sequenced, and the corresponding sequence types of the isolates were designated according to the E. coli MLST database (http://mlst.warwick.ac.uk/mlst/dbs/Ecoli). We also conducted in silico MLST using MLST 2.0 [51] on the CGE website to cross-check the sequence types (STs).

2.7. Statistical Data Analysis

The 95% confidence intervals (CI) of proportions were calculated with EPi tools (http://epitools.ausvet.com.au) using the binomial exact method. Statistical significance of differences between proportions was evaluated by Chi-square (χ²) test. Means of pairwise SNP differences for identified clusters were compared using a one-way analysis of variance (ANOVA) with sigmaplot 12.5 (Systat Software Inc., San Jose, CA, USA).

3. Results

3.1. Prevalence of ESBL-EC from Retail Raw Meat Samples

Totally, we isolated 1205 E. coli strains from 3234 retail meat samples, purchased from nationwide grocery stores in Korea between 2015 and 2018. These retail meat samples comprised mainly beef cuts, pork cuts, and chicken cuts. E. coli was present in 37.2% of the tested samples. Out of 1205 E. coli strains isolated from meat samples, 120 strains were resistant to third-generation cephalosporins and were tested for the ESBL phenotype. The prevalence of ESBL-ECs recovered from retail meat samples is shown in Table 1. A total of 29 phenotypically positive ESBL-EC isolates were recovered from domestic (n = 18) and imported (n = 11) meat samples. The occurrences of ESBL-ECs in domestic pork and chicken meat were 0.2% (95% CI 0.0–1.0%) and 3.0% (95% CI 1.8–4.8%), respectively. There were no ESBL-EC isolates in the domestic beef samples. Meanwhile, the prevalence of ESBL-ECs in the imported beef, pork, and chicken meat were 0.1% (95% CI 0.0–1.0%), 0.5% (95% CI 0.1–1.6%), and 2.7% (95% CI 1.1–5.5%), respectively. The prevalence of ESBL-ECs in chicken meat was significantly higher than that in other meat samples (p < 0.001). No significant difference in the prevalence of ESBL-ECs was present between domestic and imported meat samples (p > 0.05).

3.2. AMR of ESBL-ECs

The AMR prevalence and profiles of phenotypically positive ESBL-ECs are shown in Table 2 and Table S2. All isolates were resistant to AMP and CTF, whereas they were susceptible to AmC, FOX, and MEM. The most common non-beta-lactam resistance was present against NAL (75.9%, 22/29) and TET (72.4%, 21/29). All isolates showed the multidrug resistance phenotype, which means that
the bacteria were resistant to three or more antimicrobial agents belonging to different categories. Meanwhile, no significant difference in the occurrence of resistance to each antimicrobial agent was present between isolates from domestic and imported meats \((p > 0.05)\).

### Table 1. Prevalence of ESBL-EC (extended-spectrum beta-lactamase-producing Escherichia coli) isolates in retail meat samples.

| Category | ESBL-EC Positive Rate, % (No. of ESBL-EC Confirmed Samples/ No. of Tested Samples) | Domestic | Imported | Total | \(p\)-Value ** |
|----------|------------------------------------------------------------------------------------|----------|----------|-------|---------------|
| Beef     | 0.0 (0/612)                                                                         | 0.1 (1/678) | 0.1 (1/1290) | 1.0000 |
| Pork     | 0.2 (1/565)                                                                         | 0.5 (3/561) | 0.4 (4/1126) | 0.6114 |
| Chicken  | 3.0 (17/560) *                                                                     | 2.7 (7/258) * | 2.9 (24/818) * | 0.9752 |
| Total    | 1.0 (18/1737)                                                                       | 0.7 (11/1497) | 0.9 (29/3234) | 0.4736 |

* \(p < 0.001\), difference between the proportions of each meat category by Chi-squared test. ** \(p\)-value, difference between the proportions of domestic and imported meat by Chi-squared test.

### Table 2. AMR prevalence of the 29 ESBL-ECs isolated from retail meat samples.

| Antibiotics | Resistance Rate, % (No. of Resistant Strains/ No. of Tested ESBL-ECs) | Domestic | Imported | Total | \(p\)-Value * |
|-------------|---------------------------------------------------------------------|----------|----------|-------|---------------|
| Beta-lactams |                                                                     |          |          |       |               |
| AmC         | 0.0 (0/18)                                                          | 0.0 (0/11) | 0.0 (0/29) | NA    |               |
| AMP         | 100.0 (18/18)                                                        | 100.0 (11/11) | 100.0 (29/29) | 1.0000 |
| CAZ         | 22.2 (4/18)                                                          | 0.0 (0/11) | 13.8 (4/29) | 0.2589 |
| CTF         | 100.0 (18/18)                                                        | 100.0 (11/11) | 100.0 (29/29) | 1.0000 |
| FEP         | 16.7 (3/18)                                                          | 9.1 (1/11)  | 13.8 (4/29) | 0.9847 |
| FOX         | 0.0 (0/18)                                                           | 0.0 (0/11)  | 0.0 (0/29) | NA    |               |
| MEM         | 0.0 (0/18)                                                           | 0.0 (0/11)  | 0.0 (0/29) | NA    |               |
| Non-beta-lactams |                                                               |          |          |       |               |
| CHL         | 50.0 (9/18)                                                          | 36.4 (4/11) | 44.8 (13/29) | 0.7401 |
| CIP         | 50.0 (9/18)                                                          | 45.5 (5/11) | 48.4 (14/29) | 1.0000 |
| COL         | 11.1 (2/18)                                                          | 18.2 (2/11) | 13.8 (4/29) | 1.0000 |
| GEN         | 38.9 (7/18)                                                          | 45.5 (5/11) | 41.4 (12/29) | 1.0000 |
| NAL         | 83.3 (15/18)                                                         | 63.6 (7/11) | 75.9 (22/29) | 0.4499 |
| STR         | 55.6 (10/18)                                                         | 63.6 (7/11) | 58.6 (17/29) | 0.9679 |
| SXT         | 38.9 (7/18)                                                          | 54.5 (6/11) | 44.8 (13/29) | 0.6615 |
| TET         | 77.8 (14/18)                                                         | 63.6 (7/11) | 72.4 (21/29) | 0.6902 |
| MDR         | 100.0 (18/18)                                                        | 100.0 (11/11) | 100.0 (29/29) | 1.0000 |

* \(p\)-value, difference between the proportions of domestic and imported meat by Chi-squared test. ** MDR, multidrug resistance; NA, not available.

3.3. Distribution of Beta-Lactamase Genes and Plasmid Incompatibility Groups

The distribution of ESBL genes among 29 ESBL-EC isolates is shown in Figure 1. Out of the 29 ESBL-EC isolates, 28 carried \(bla_{CTX-M}\). One isolate (EC2017_203) not harboring \(bla_{CTX-M}\) had \(bla_{TEM-1b}\) and \(bla_{SHV-12}\). Further, 12 isolates harbored the combination of \(bla_{CTX-M}\) and \(bla_{TEM}\). The CTX-M genotypes in our ESBL-EC isolates were diverse, including \(bla_{CTX-M-1}(n = 2)\), \(bla_{CTX-M-2}(n = 2)\), \(bla_{CTX-M-3}(n = 1)\), \(bla_{CTX-M-4}(n = 3)\), \(bla_{CTX-M-14}(n = 2)\), \(bla_{CTX-M-15}(n = 5)\), \(bla_{CTX-M-27}(n = 1)\), \(bla_{CTX-M-55}(n = 11)\), and \(bla_{CTX-M-65}(n = 2)\). Of these genotypes, the main type was \(bla_{CTX-M-55}\), which was identified in 11 isolates (eight isolates from domestic chicken, two from imported chicken, and one from imported beef). The beta-lactamase genes of CTX-M-2 and CTX-M-8 group were not identified in ESBL-EC isolates from domestic meat. In addition to beta-lactamase genes, all strains carried genes conferring resistance to other classes of antimicrobial agents. Meanwhile, significant difference in the occurrence of each resistance gene was not present between isolates from domestic and imported meat \((p > 0.05)\).
A total of 17 plasmid incompatibility types were identified in our collection of isolates using PlasmidFinder [48]. The most common plasmid replicon groups across all 29 ESBL-EC isolates were IncF\textsubscript{I}$^B$ (n = 23), followed by IncF\textsubscript{I}$^H$ (n = 11) and Inc\textsubscript{I}$^A$ (n = 11). Meanwhile, the difference in the occurrence of plasmid incompatibility groups across ESBL-EC isolates from domestic meat and imported meat was not significant, except for IncF\textsubscript{I}$^B$ group (Figure S1).

### 3.4. STs of ESBL-EC

The results from MLST showed that the ESBL-EC s belonged to 21 different STs (Figure 2). The most frequent clonal types (STs) were ST58 (n = 3) belonging to phylogroup B1 and ST93 (n = 3) belonging to phylogroup A. Other STs were identified in less than two isolates. This implies that the STs of ESBL-EC isolates in this study were highly diverse.

**Table:**

| Isolate | Source |
|---------|--------|
| E. coli | Chicken |

**Figure 1.** Distribution of acquired AMR (antimicrobial resistance) genes and plasmid incompatibility groups in ESBL-EC isolates. The panels are columns that represent presence or absence of AMR genes and plasmid replicons. The grey color indicates the presence of an AMR gene and plasmid replicon based on ResFinder [49] and PlasmidFinder [48], respectively.
A total of 17 plasmid incompatibility types were identified in our collection of isolates using PlasmidFinder [48]. The most common plasmid replicon groups across all 29 ESBL-EC isolates were IncFIB ($n = 23$), followed by IncFII ($n = 11$) and IncI1 ($n = 11$). Meanwhile, the difference in the occurrence of plasmid incompatibility groups across ESBL-EC isolates from domestic meat and imported meat was not significant, except for IncFIB group (Figure S1).

3.4. STs of ESBL-EC

The results from MLST showed that the ESBL-ECs belonged to 21 different STs (Figure 2). The most frequent clonal types (STs) were ST58 ($n = 3$) belonging to phylogroup B1 and ST93 ($n = 3$) belonging to phylogroup A. Other STs were identified in less than two isolates. This implies that the STs of ESBL-EC isolates in this study were highly diverse.

![Figure 2. Maximum-likelihood core-genome SNP (single-nucleotide polymorphism) phylogenetic tree of ESBL-ECs isolated from retail raw meat in Korea with metadata (source and county). Phylogenetic tree was constructed based on 188,735 SNPs in the core-genome of 29 ESBL-EC isolates. MLST (multilocus sequence typing) sequence types (STs), clonal complex (CC), and phylogroups were identified using Clermontyping [47], and sequence cluster was determined with hierBAPS (Bayesian analysis of population structure) [45]. Tree scale in the number of substitutions per site.](image-url)
3.5. Pan-Genome, Population Structure and Phylogeny of ESBL-EC

An average of 4933 genes per isolate was identified by automated annotation. An overall pan-genome consisted of total 14,094 genes, which made up the core gene set (including soft core gene) and the accessory gene set, comprising 3205 genes and 10,889 genes, respectively. The cumulative number of genes in the pan-genome continued to increase as more genomes were added to the collection of analysis (Figure S2). Our estimated pan-genome curve formula was: 

\[ y = 2279.42x^{0.48} + 2666.41 \]

where \( R^2 = 0.9984 \). This result suggested that our ESBL-EC population have an open pan-genome, since a pan-genome is considered open when \( 0 < R^2 < 1 \) [39,40].

A maximum likelihood phylogenetic tree of the 29 ESBL-ECs was constructed based on 188,735 SNPs in the core gene alignment. The phylogeny of core gene SNP revealed diverse clonal-related groups of ESBL-ECs, belonging to four major lineages, which generally correlated with the *E. coli* clonal ST. Meanwhile, three isolates belonging to ST93 (domestic chicken from different companies), two isolates belonging to ST2170 (domestic chicken from different companies), and two isolates belonging to ST602 (domestic chicken from different companies) were closely related, presenting average pairwise SNP differences of 67, 22, and 40, respectively.

A population structure analysis using the hierBAPS sequence clustering approach, based on SNP alignment of core-genomes [45], clustered 29 ESBL-ECs into four lineages: BAPS cluster 1 to BAPS cluster 4 (Figure 2). The BAPS clusters correlated with the *E. coli* phylogroups. All isolates from the phylogroup A (\( n = 6 \)) and phylogroup B2 (\( n = 2 \)) were assigned to the BAPS cluster 2 and BAPS cluster 4, respectively. The phylogroup B1 (\( n = 14 \)) and C (\( n = 1 \)) were included in BAPS cluster 1. The phylogroup F (\( n = 5 \)) and phylogroup D (\( n = 1 \)) isolates were included in BAPS cluster 3. Meanwhile, the BAPS clusters showed significant differences (\( p < 0.001 \)) in the distribution of pairwise SNP differences in each cluster (Figure 3). The BAPS cluster 4 showed the lowest within-BAPS-cluster SNP diversity, characterized by lower average pairwise SNP differences of 2061. In comparison, BAPS cluster 1, 2, and 3 revealed higher average pairwise SNP differences of 16057, 17262, and 50465, respectively. These clusters also comprised a diverse set of STs: 10 STs in the BAPS cluster 1, 4 STs in the BAPS cluster 2, 6 STs in the BAPS cluster 3. Meanwhile there were no clusters of isolates specific to either source or their country groups, except for the BAPS cluster 4, which comprised Korean chicken meat isolates from the same company (phylogroup B2).

![Figure 3](image-url) Distribution of pairwise SNP differences in each of the four BAPS clusters, to demonstrate the variations in sequence diversity in each cluster (\( p < 0.001 \)).

4. Discussion

In our study, we detected 29 ESBL-ECs from raw meat samples purchased at Korean retail stores between 2015 and 2018. Among the 29 ESBL-ECs, 18 isolates were recovered from domestic meat samples and 11 from imported meat samples. The observed 1.0% (18/1737) prevalence of ESBL-ECs...
in domestic meat samples was lower than that reported in a few previous Korean studies [24,26,27]. The observed 0.7% (11/1497) prevalence of ESBL-ECs from imported meat samples, however, was comparable with the previously reported prevalence of 1.1% (20/1771) in imported meat samples in Korea \( (p = 0.328) \) [31]. Meanwhile, the prevalence of ESBL-ECs in chicken was higher than that in pork and beef. This result was consistent with that of the previous studies regarding ESBL-EC prevalence in livestock in Korea [24,26,52], and in Germany, the United Kingdom, Turkey, and Spain [53–56].

All of the ESBL-EC isolates showed susceptibility of FOX (cephamycin) and MEM (carbapenem), as typical characteristics of ESBL-producing Enterobacteriaceae. All of the 1205 E. coli isolates were also susceptible to MEM (data not shown). Carbapenem-resistant E. coli have not yet been reported in food-producing animals and meat products, whereas carbapenem-resistant E. coli have been identified from human samples and companion animals in previous studies in Korea [23,57,58]. All of ESBL-ECs in this study also showed the multidrug resistance phenotype. This result is concordant with that of a previous study, which reported the high prevalence of multidrug resistant ESBL-ECs in raw chicken meat in Korea [30]. Meanwhile, the resistances against TET (tetracycline) and NAL (quinolone) were relatively high, suggesting that the common overuse of tetracyclines and quinolones in livestock farming worldwide has contributed to the acquisition of TET and NAL resistance [59].

In this study, 28 of 29 ESBL-EC isolates had CTX-M genotypes, just as CTX-M ESBLS have increased in occurrence worldwide since the 2000s [60]. Although genotypes of our ESBL-ECs were diverse, CTX-M group 1 \( (bla_{CTX-M-1}, \ bla_{CTX-M-3}, \ bla_{CTX-M-15}, \ bla_{CTX-M-55}) \) and CTX-M group 9 \( (bla_{CTX-M-14}, \ bla_{CTX-M-27}, \ bla_{CTX-M-65}) \) were prevalent types, which was consistent with the findings of previous studies in Korea [25–28,30,32,52,61,62]. The most predominant CTX-M genotype was CTX-M-55, which is a variant of globally emerging CTX-M-15, and it varies by a single amino acid substitution (Ala77 → Val) that contributes to enhancing the enzymatic activity [60,63]. The detection of \( bla_{CTX-M-55} \) has been growing in humans, animals, and food in Asia [29,64–68]. Genotypes of CTX-M-2 and CTX-M-8 were only identified in ESBL-EC isolates from imported meat samples, implying that the meat produced in foreign countries might be contaminated with ESBL-ECs during production in their countries. Most of our ESBL-EC isolates carried IncF plasmids, which have been reported to play a role in the dissemination of CTX-M-related enzyme genes [69]. However, we were unable to identify plasmid types that harbored CTX-M-related genes, because \( bla_{CTX-M} \) could not be assembled as one contig, along with replicon sequences.

The pan-genome analysis showed that our ESBL-EC isolates had an expanding pan-genome and this result is consistent with those of previous studies in pan-genome analyses of E. coli [70,71]. An open pan-genomic trait of E. coli probably contributes to the diversity of species [39]. The 29 ESBL-EC isolates were clustered into four lineages by BAPs, which correlated with E. coli phylogroups. This result was concordant with those of previous studies, which reported a correlation between BAPS clusters and E. coli phylogroups [70,72]. The BAPS cluster of interest in our study is the BAPS cluster 4, which includes the isolates obtained from domestic chicken samples. These isolates belong to ST95. Two isolates of ST95 belong to the B2 phylogroup, which includes most extraintestinal pathogenic E. coli (ExPEC) strains that cause infections [73,74]. ST95 strains have been reported to cause human infection and avian pathologies [74]. ST95 strains have also been reported to frequently appear as causative agents of urinary tract infections and blood sepsis in humans. Moreover, they have been consistently identified in poultry samples, suggesting that there may be a poultry reservoir of human ExPEC ST95 lineage [75]. In our study, two ST95 strains were isolated from cut chicken, processed by the same company on different dates. Further investigation is needed to identify the source of this contamination and to implement measures for constraining the spread of ST95. Other prevalent clonal groups (ST93, ST602, and ST2170) in domestic meat samples were isolated from chicken meat; these groups were isolated from chicken samples procured from different companies. Of these STs, ST93 and ST602 were reported to be infrequent in humans but more frequent in animal samples (specifically chicken) [24,76,77], suggesting that the strains of groups ST93 and ST602 were present in chicken, since
farming stages and chicken act as reservoirs for these clonal groups. ST2170 was previously reported in retail chicken meat and turkey in Japan and Ecuador, respectively [68,78].

The 29 ESBL-EC isolates showed diverse clonal types, representing 21 different STs. The clonal group of ST58, one of the most frequent clonal types in this study, has been reported as a major vector of worldwide dissemination of ESBL-related genes along with ST155 [79,80]. All ST58 strains in our study were isolated from meat samples imported from different countries. ST58 of E. coli was not a common type in Korean human and animal samples [81], supporting the idea that ST58 strains from imported meat samples may be contaminated in the source countries. Although some pairs of isolates with the same clonal type were closely related by no more than 100 SNPs, the core-genome-based phylogeny of ESBL-EC isolates has revealed high diversity, which correlates with the diversity of clonal STs. This result suggests that ESBL-EC isolates have evolved from different ancestors and are not distributed through the clonal spread of predominant types.

A limitation of this study is the sampling design of ESBL-EC isolates. Due to the fact that just one E. coli isolate per meat sample was selected for ESBL phenotype confirmation, the prevalence of ESBL-ECs in our study could be underestimated. Moreover, we were unable to assemble the ESBL-gene-carrying plasmid sequences to further investigate the plasmid sequence diversity and compare plasmid distribution among ESBL-ECs isolated from different sources. Despite these limitations, this study provides a comprehensive overview of ESBL-EC diversity in retail meat in Korea. In addition, it provides information on the meat category that poses a high risk of spreading ESBL-ECs; this information can be used for containing the spread of ESBL-ECs.

In summary, we have described the prevalence and clonal diversity of ESBL-ECs isolated from retail meat samples in Korea. Our data showed that phylogenetically diverse ESBL-ECs with multidrug resistance phenotype were distributed nationwide, although the prevalence of ESBL-ECs in retail meat was 0.9%. The prevalence of ESBL-ECs in chicken (2.9%) was the highest when compared with that of other meat categories. Furthermore, ST95 strains, acting as pathogenic agents, were identified in chicken. Thus, as retail chicken may act as a potential vehicle for the spread of ESBL-ECs, including pathogenic types, it poses a health risk to consumers and food-handlers if contaminated with ESBL-producers. Therefore, close surveillance of ESBL-ECs should be continued, in order to establish a containment strategy for preventing the dissemination of ESBL-producers. Moreover, further studies will need to reduce the contamination of ESBL-producers throughout the food supply chain.

Supplementary Materials: The following are available online at http://www.mdpi.com/2076-2607/8/4/508/s1, Figure S1: Distribution of plasmid replicons in 29 ESBL-EC from retail raw meats in Korea, Figure S2: Pan-genome profile of the 29 ESBL-EC isolates, Table S1: List of antimicrobial agents, their subclasses, MIC range tested concentration, and breakpoints for susceptibility testing, Table S2: AMR profiles of 29 ESBL-ECs recovered from retail meat samples.

Author Contributions: H.K. (Hyosun Kwak) and S.R. designed the study; H.K. (Hansol Kim) and S.K. performed the experiments; S.K., H.K. (Hansol Kim), and Y.K. analyzed the data; S.K., H.K. (Hyosun Kwak), and S.R. wrote the manuscript; S.K., H.K. (Hansol Kim), Y.K., M.K., H.K. (Hyosun Kwak), and S.R. critically reviewed the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by grants (nos. 15161MFDS645 and 20161MFDS009) from the Ministry of Food and Drug Safety. The findings and conclusions of this article are ours and do not necessarily represent the views of the Ministry of Food and Drug Safety.

Acknowledgments: We thank the members of the AMR surveillance working group (J. Kim, S. Seo, and J. Park) for their contribution to collecting some of the isolates of E. coli used in this study.

Conflicts of Interest: The authors declare no conflict of interest.

References
1. Croxen, M.A.; Finlay, B.B. Molecular mechanisms of Escherichia coli pathogenicity. Nat. Rev. Microbiol. 2009, 8, 26–38. [CrossRef] [PubMed]
2. Kaper, J.B.; Nataro, J.P.; Mobley, H.L. Pathogenic Escherichia coli. Nat. Rev. Microbiol. 2004, 2, 123–140. [CrossRef] [PubMed]
3. Pouillot, F.; Chomton, M.; Blois, H.; Courrroux, C.; Noelig, J.; Bidet, P.; Bingen, E.; Bonacorsi, S. Efficacy of bacteriophage therapy in experimental sepsis and meningitis caused by a clone O25b: H4-ST131 Escherichia coli strain producing CTX-M-15. Antimicrob. Agents Chemother. 2012, 56, 3568–3575. [CrossRef] [PubMed]
4. Blake, D.; Hillman, K.; Fenlon, D.; Low, J. Transfer of antibiotic resistance between commensal and pathogenic members of the Enterobacteriaceae under ileal conditions. J. Appl. Microbiol. 2003, 95, 428–436. [CrossRef]
5. Szmolka, A.; Nagy, B. Multidrug resistant commensal Escherichia coli in animals and its impact for public health. Front. Microbiol. 2013, 4, 258. [CrossRef]
6. World Health Organization. Integrated Surveillance of Antimicrobial Resistance in Foodborne Bacteria: Application of a One Health Approach: Guidance from the WHO Advisory Group on Integrated Surveillance of Antimicrobial Resistance (AGISAR); WHO: Geneva, Switzerland, 2017.
7. World Health Organization. Global Priority of Antibiotic-Resistant Bacteria to Guide Research, Discovery, and Development of New Antibiotics; WHO: Geneva, Switzerland, 2017.
8. Paterson, D.L.; Bonomo, R.A. Extended-spectrum β-lactamases: A clinical update. Clin. Microbiol. Rev. 2005, 18, 657–686. [CrossRef] [PubMed]
9. Cho, H.; Uehara, T.; Bernhardt, T.G. Beta-lactam antibiotics induce a lethal malfunctioning of the bacterial cell wall synthesis machinery. Cell 2014, 159, 1300–1311. [CrossRef]
10. Hawkey, P.M.; Warren, R.E.; Livermore, D.M.; McNulty, C.A.; Enoch, D.A.; Otter, J.A.; Wilson, A.P.R. Treatment of infections caused by multidrug-resistant gram-negative bacteria: Report of the British Society for Antimicrobial Chemotherapy/healthcare Infection Society/British Infection Association Joint Working Party. J. Antimicrob. Chemother. 2018, 73, iii2–iii78. [CrossRef]
11. Rodriguez-Baño, J.; Gutiérrez-Gutiérrez, B.; Machuca, I.; Pascual, A. Treatment of infections caused by extended-spectrum-beta-lactamase-, AmpC-, and carbapenemase-producing Enterobacteriaceae. Clin. Microbiol. Rev. 2018, 31. [CrossRef]
12. Livermore, D.M.; Canton, R.; Gniadkowski, M.; Nordmann, P.; Rossolini, G.M.; Arlet, G.; Ayala, J.; Coque, T.M.; Kern-Zdanowicz, I.; Luzzaro, F.; et al. CTX-M: Changing the face of ESBLs in Europe. J. Antimicrob. Chemother. 2006, 59, 165–174. [CrossRef]
13. Dolejska, M.; Papagiannitis, C.C. Plasmid-mediated resistance is going wild. Plasmid 2018, 99, 99–111. [CrossRef] [PubMed]
14. Doi, Y.; Iovleva, A.; Bonomo, R.A. The ecology of extended-spectrum β-lactamases (ESBLs) in the developed world. J. Travel Med. 2017, 24, S44–S51. [CrossRef]
15. Valentin, L.; Sharp, H.; Hille, K.; Seibt, U.; Fischer, J.; Pfeifer, Y.; Michael, G.B.; Nickel, S.; Schmiedel, J.; Falgenhauer, L. Subgrouping of ESBL-producing Escherichia coli from animal and human sources: An approach to quantify the distribution of ESBL types between different reservoirs. Int. J. Med. Microbiol. 2014, 304, 805–816. [CrossRef] [PubMed]
16. De Been, M.; Lanza, V.F.; De Toro, M.; Scharringa, J.; Dohmen, W.; Du, Y.; Hu, J.; Lei, Y.; Li, N.; Tooming-Klunderud, A. Dissemination of cephalexin resistance genes between Escherichia coli strains from farm animals and humans by specific plasmid lineages. PLoS Genet. 2014, 10. [CrossRef] [PubMed]
17. Liebana, E.; Carattoli, A.; Coque, T.M.; Hasman, H.; Magiorakos, A.P.; Mevius, D.; Peixe, L.; Poirel, L.; Schuepbach-Regula, G.; Torneke, K. Public health risks of enterobacterial isolates producing extended-spectrum β-lactamas or AmpC β-lactamases in food and food-producing animals: An EU perspective of epidemiology, analytical methods, risk factors, and control options. Clin. Infect. Dis. 2012, 56, 1030–1037. [CrossRef] [PubMed]
18. Leverstein-van Hall, M.; Dierikx, C.; Cohen Stuart, J.; Voets, G.; Van Den Munckhof, M.; Van Essen-Zandbergen, A.; Platteel, T.; Fluit, A.; Van de Sande-Bruinsma, N.; Scharringa, J. Dutch patients, retail chicken meat and poultry share the same ESBL genes, plasmids and strains. Clin. Microbiol. Infect. 2011, 17, 873–880. [CrossRef] [PubMed]
19. Lazarus, B.; Paterson, D.L.; Mollinger, J.L.; Rogers, B.A. Do human extraintestinal Escherichia coli infections resistant to expanded-spectrum cephalosporins originate from food-producing animals? A systematic review. Clin. Infect. Dis. 2014, 60, 439–452. [CrossRef]
20. Holmes, A.H.; Moore, L.S.; Sundsfjord, A.; Steinbakk, M.; Regmi, S.; Karkey, A.; Guerin, P.J.; Piddock, L.J. Understanding the mechanisms and drivers of antimicrobial resistance. Lancet 2016, 387, 176–187. [CrossRef]
21. Schwarz, S.; Kehrenberg, C.; Walsh, T. Use of antimicrobial agents in veterinary medicine and food animal production. Int. J. Antimicrob. Agents 2001, 17, 431–437. [CrossRef]
22. Oniciuc, E.A.; Likotrafiti, E.; Alvarez-Molina, A.; Prieto, M.; Santos, J.A.; Alvarez-Ordoñez, A. The present and future of whole genome sequencing (WGS) and whole metagenome sequencing (WMS) for surveillance of antimicrobial resistant microorganisms and antimicrobial resistance genes across the food chain. *Genes* 2018, 9, 268. [CrossRef] [PubMed]

23. Hong, J.S.; Song, W.; Park, H.M.; Oh, J.Y.; Chae, J.C.; Han, J.I.; Jeong, S.H. First detection of New Delhi metallo-β-Lactamase-5-producing *Escherichia coli* from companion animals in Korea. *Microb. Drug. Resist.* 2019, 25, 344–349. [CrossRef]

24. Lim, J.S.; Choi, D.S.; Kim, Y.J.; Chon, J.W.; Kim, H.S.; Park, H.J.; Moon, J.S.; Wee, S.H.; Seo, K.H. Characterization of *Escherichia coli*-producing extended-spectrum-β-lactamase (ESBL) isolated from chicken slaughterhouses in South Korea. *Foodborne Pathog. Dis.* 2015, 12, 741–748. [CrossRef] [PubMed]

25. Kim, J.S.; Kim, J.; Kim, S.J.; Jeon, S.E.; Oh, K.H.; Cho, S.H.; Kang, Y.H.; Han, S.Y.; Chung, G.T. Characterization of CTX-M-type extended-spectrum beta-lactamase-producing diarrheagenic *Escherichia coli* isolates in the Republic of Korea during 2008–2011. *J. Microbiol. Biotechnol.* 2014, 24, 421–426. [CrossRef] [PubMed]

26. Tamang, M.D.; Nam, H.M.; Kim, S.R.; Chae, M.H.; Jung, G.C.; Jung, S.C.; Lim, S.K. Prevalence and molecular characterization of CTX-M-β-lactamase–producing *Escherichia coli* isolated from healthy swine and cattle. *Foodborne Pathog. Dis.* 2013, 10, 13–20. [CrossRef] [PubMed]

27. Tamang, M.D.; Nam, H.M.; Gurung, M.; Jang, G.C.; Kim, S.R.; Jung, S.C.; Park, Y.H.; Lim, S.K. Molecular characterization of CTX-M-β-lactamase and associated addiction systems in *Escherichia coli* circulating among cattle, farm workers, and the farm environment. *Appl. Environ. Microbiol.* 2013, 79, 3898–3905. [CrossRef]

28. Kim, J.; Lim, Y.M.; Rheem, I.; Lee, Y.; Lee, J.C.; Seol, S.Y.; Lee, Y.C.; Cho, D.T. CTX-M and SHV-12 β-lactamases are the most common extended-spectrum enzymes in clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae* collected from 3 university hospitals within Korea. *FEMS Microbiol. Lett.* 2005, 245, 93–98. [CrossRef] [PubMed]

29. Kim, Y.A.; Kim, H.; Choi, M.H.; Seo, Y.H.; Lee, H.; Lee, K. Whole-genome analysis of *bla*<sub>CTX-M-55</sub>-carrying *Escherichia coli* among pigs, farm environment, and farm workers. *Ann. Lab. Med.* 2020, 40, 180–183. [CrossRef]

30. Park, H.; Kim, J.; Ryu, S.; Jeon, B. Predominance of *bla*<sub>CTX-M-65</sub> and *bla*<sub>CTX-M-55</sub> in extended-spectrum β-lactamase-producing *Escherichia coli* from raw retail chicken in South Korea. *J. Glob. Antimicrob. Resist.* 2019, 17, 216–220. [CrossRef]

31. Kim, Y.J.; Moon, J.S.; Oh, D.H.; Chon, J.W.; Song, B.R.; Lim, J.S.; Heo, E.J.; Park, H.J.; Wee, S.H.; Sung, K. Genotypic characterization of ESBL-producing *Escherichia coli* from imported meat in South Korea. *Food Res. Int.* 2018, 107, 158–164. [CrossRef]

32. Jo, S.J.; Woo, G.J. Molecular characterization of plasmids encoding CTX-M β-lactamases and their associated addiction systems circulating among *Escherichia coli* from retail chickens, chicken farms, and slaughterhouses in Korea. *J. Microbiol. Biotechnol.* 2016, 26, 270–276. [CrossRef]

33. CLSI. *Performance Standards for Antimicrobial Susceptibility Testing. CLSI Supplement M100*, 29th ed.; Clinical and Laboratory Standards Institute: Wayne, PA, USA, 2019.

34. EUCAST. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint Tables for Interpretation of MICs and Zone Diameters. Version 9.0. Available online: http://www.eucast.org (accessed on 30 January 2019).

35. NARMS. The National Antimicrobial Resistance Monitoring System. Antimicrobial Agents Used for Susceptibility Testing for *Escherichia coli* Isolates. Available online: https://www.cdc.gov/narms/antibiotics-tested.html (accessed on 23 May 2019).

36. Bankevich, A.; Nurk, S.; Antipov, D.; Gurevich, A.A.; Dvorkin, M.; Kulikov, A.S.; Lesin, V.M.; Nikolenko, S.I.; Pham, S.; Prjibelski, A.D. SPAdes: A new genome assembly algorithm and its applications to single-cell sequencing. *J. Comput. Biol.* 2012, 19, 455–477. [CrossRef] [PubMed]

37. Seemann, T. Prokka: Rapid prokaryotic genome annotation. *Bioinformatics* 2014, 30, 2068–2069. [CrossRef] [PubMed]

38. Page, A.J.; Cummins, C.A.; Hunt, M.; Wong, V.K.; Reuter, S.; Holden, M.T.; Fookes, M.; Falush, D.; Keane, J.A.; Parkhill, J. Roary: Rapid large-scale prokaryote pan genome analysis. *Bioinformatics* 2015, 31, 3691–3693. [CrossRef] [PubMed]

39. Tettelin, H.; Riley, D.; Cattuto, C.; Medini, D. Comparative genomics: The bacterial pan-genome. *Curr. Opin. Microbiol.* 2008, 11, 472–477. [CrossRef] [PubMed]
40. Rasko, D.A.; Rosovitz, M.; Myers, G.S.; Mongodin, E.F.; Fricke, W.F.; Gajer, P.; Crabtree, J.; Sebaihia, M.; Thomson, N.R.; Chaudhuri, R. The pan-genome structure of Escherichia coli: Comparative genomic analysis of E. coli commensal and pathogenic isolates. *J. Bacteriol.* 2008, 190, 6881–6893. [CrossRef] [PubMed]

41. Nourdin-Galindo, G.; Sánchez, P.; Molina, C.F.; Espinoza-Rojas, D.A.; Oliver, C.; Ruiz, P.; Vargas-Chacoff, L.; Cárcamo, J.G.; Figueroa, J.E.; Mancilla, M.; et al. Comparative pan-genome analysis of *Piscirickettsia salmonis* reveals genomic divergences within genogroups. *Front. Cell. Infect. Microbiol.* 2017, 7, 459. [CrossRef]

42. Zhao, Y.; Jia, X.; Yang, J.; Zhan, Z.; Yu, J.; Wu, J.; Xiao, J. PanGP: A tool for quickly analyzing bacterial pan-genome profile. *Bioinformatics* 2014, 30, 1297–1299. [CrossRef]

43. Page, A.J.; Taylor, B.; Delaney, A.J.; Soares, J.; Seemann, T.; Keane, J.A.; Harris, S.R. SNP-sites: Rapid extraction of SNPs from multi-FASTA alignments. *Microb. Genom.* 2016, 2. [CrossRef]

44. Stamatakis, A. RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 2014, 30, 1312–1313. [CrossRef]

45. Cheng, L.; Connor, T.R.; Sirén, J.; Aanensen, D.M.; Corander, J. Hierarchical and spatially explicit clustering of DNA sequences with BAPS software. *Mol. Biol. Evol.* 2013, 30, 1224–1228. [CrossRef]

46. Hadfield, J.; Croucher, N.J.; Goater, R.J.; Abudahab, K.; Aanensen, D.M.; Harris, S.R. Phandango: An interactive viewer for bacterial population genomics. *Bioinformatics* 2017, 34, 292–293. [CrossRef]

47. Beghain, J.; Bridier-Nahmias, A.; Le Nagard, H.; Denamur, E.; Clermont, O. ClermonTyping: An easy-to-use and accurate in silico method for *Escherichia* genus strain phylotyping. *Microb. Genom.* 2018, 4. [CrossRef]

48. Carattoli, A.; Zankari, E.; García-Fernández, A.; Voldby Larsen, M.; Lund, O.; Villa, L.; Møller Aarestrup, F.; Hasman, H. In silico detection and typing of plasmids using PlasmidFinder and Plasmid Multilocus Sequence Typing. *Antimicrob. Agents Chemother.* 2014, 58, 3895–3903. [CrossRef] [PubMed]

49. Cheng, L.; Connor, T.R.; Sirén, J.; Aanensen, D.M.; Corander, J. Hierarchical and spatially explicit clustering of *Escherichia* genus strain phylotypes. *Front. Microbiol.* 2018, 9, 1297–1299. [CrossRef] [PubMed]

50. Ojer-Usoz, E.; González, D.; Vitas, A.I.; Leiva, J.; García-Jalón, I.; Febles-Casquero, A.; De la Soledad Escolano, M. Prevalence of extended-spectrum β-lactamase-producing *Escherichia coli* isolates from pig and chicken farms in South Korea. *J. Microbiol. Biotechnol.* 2012, 27, 1716–1723. [CrossRef]

51. Shin, S.W.; Jung, M.; Won, H.G.; Belaynehe, K.M.; Yoon, I.J.; Yoo, H.S. Characteristics of transmissible CTX-M- and CMY-type β-lactamase-producing *Escherichia coli* isolates collected from pig and chicken farms in South Korea. *J. Microbiol. Biotechnol.* 2017, 27, 6881–6893. [CrossRef] [PubMed]

52. Ojer-Usoz, E.; González, D.; Vitas, A.I.; Leiva, J.; García-Jalón, I.; Febles-Casquero, A.; De la Soledad Escolano, M. Prevalence of extended-spectrum β-lactamase-producing *Enterobacteriaceae* in meat products sold in Navarra, Spain. *Meat Sci.* 2013, 93, 316–321. [CrossRef] [PubMed]

53. Yoon, E.J.; Kim, D.; Jeong, S.H. Bloodstream infections and carbapenem-resistant *Enterobacteriaceae* in South Korea. *Lancet Infect. Dis.* 2019, 19, 931–932. [CrossRef]

54. Ahn, J.Y.; Song, J.E.; Kim, M.H.; Choi, H.; Kim, J.K.; Ann, H.W.; Kim, J.H.; Jeon, Y.; Jeong, S.J.; Kim, S.B. Risk factors for the acquisition of carbapenem-resistant *Escherichia coli* at a tertiary care center in South Korea: A matched case-control study. *Am. J. Infect. Control* 2014, 42, 621–625. [CrossRef] [PubMed]
59. Van Boeckel, T.P.; Pires, J.; Silvester, R.; Zhao, C.; Song, J.; Criscuolo, N.G.; Gilbert, M.; Bonhoeffer, S.; Laxminarayan, R. Global trends in antimicrobial resistance in animals in low- and middle-income countries. *Science* 2019, 365, eaaw1944. [CrossRef]  
60. Bevan, E.R.; Jones, A.M.; Hawkey, P.M. Global epidemiology of CTX-M β-lactamas: Temporal and geographical shifts in genotype. *J. Antimicrob. Chemother.* 2017, 72, 2145–2155. [CrossRef]  
61. Hong, J.S.; Song, W.; Park, H.M.; Oh, J.Y.; Chae, J.C.; Shin, S.; Jeong, S.H. Clonal spread of extended-spectrum cephalosporin-resistant *Enterobacteriaceae* between companion animals and humans in South Korea. *Front. Microbiol.* 2019, 10, 1371. [CrossRef]  
62. Na, S.H.; Moon, D.C.; Choi, M.J.; Oh, S.J.; Jung, D.Y.; Sung, E.J.; Kang, H.Y.; Hyun, B.H.; Lim, S.K. Antimicrobial resistance and molecular characterization of extended-spectrum β-lactamase-producing *Escherichia coli* isolated from ducks in South Korea. *Foodborne Pathog. Dis.* 2019, 16, 799–806. [CrossRef]  
63. He, D.; Chiou, J.; Zeng, Z.; Liu, L.; Chen, X.; Zeng, L.; Chan, E.W.C.; Liu, J.H.; Chen, S. Residues distal to the active site contribute to enhanced catalytic activity of variant and hybrid β-lactamas derived from CTX-M-14 and CTX-M-15. *Antimicrob. Agents Chemother.* 2015, 59, 5976–5983. [CrossRef]  
64. Kiratsin, P.; Apisarnthanarak, A.; Saifon, P.; Laesripa, C.; Kitphati, R.; Mundy, L.M. The emergence of a novel ceftazidime-resistant CTX-M extended-spectrum β-lactama, CTX-M-55, in both community-onset and hospital-acquired infections in Thailand. *Diagn. Microbiol. Infect. Dis.* 2007, 58, 349–355. [CrossRef]  
65. Nadimpalli, M.; Fabre, L.; Yith, V.; Sem, N.; Gouali, M.; Delarocque-Astagneau, E.; Breng, N.; Le Hello, S. CTX-M-55-type ESBL-producing *Salmonella enterica* are emerging among retail meats in Phnom Penh, Cambodia. *J. Antimicrob. Chemother.* 2018, 74, 342–348. [CrossRef]  
66. Rao, L.; Lv, L.; Zeng, Z.; Chen, S.; He, D.; Chen, X.; Wu, C.; Wang, Y.; Yang, T.; Wu, P. Increasing prevalence of extended-spectrum cephalosporin-resistant *Escherichia coli* in food animals and the diversity of CTX-M genotypes during 2003–2012. *Vet. Microbiol.* 2014, 172, 534–541. [CrossRef] [PubMed]  
67. Zheng, H.; Zeng, Z.; Chen, S.; Liu, Y.; Yao, Q.; Deng, Y.; Chen, X.; Lv, L.; Zhao, C.; Chen, Z. Prevalence and characterisation of CTX-M β-lactamas amongst *Escherichia coli* isolates from healthy food animals in China. *Int. J. Antimicrob. Agents* 2012, 39, 305–310. [CrossRef] [PubMed]  
68. Hayashi, W.; Ohsaki, Y.; Taniguchi, Y.; Koide, S.; Kawamura, K.; Suzuki, M.; Kimura, K.; Wachino, J.-I.; Nagano, Y.; Arakawa, Y. High prevalence of *bla*<sub>CTX-M-14</sub> among genetically diverse *Escherichia coli* recovered from retail raw chicken meat portions in Japan. *Int. J. Food Microbiol.* 2018, 284, 98–104. [CrossRef]  
69. Mathers, A.J.; Peirano, G.; Pitout, J.D. The role of epidemic resistance plasmids and international high-risk clones in the spread of multidrug-resistant *Enterobacteriaceae*. *Clin. Microbiol. Rev.* 2015, 28, 565–591. [CrossRef] [PubMed]  
70. Musicha, P.; Feasey, N.A.; Cain, A.K.; Kallonon, T.; Chaguusa, C.; Peno, C.; Khonga, M.; Thompson, S.; Gray, K.J.; Mather, A.E. Genomic landscape of extended-spectrum β-lactamase resistance in *Escherichia coli* from an urban African setting. *J. Antimicrob. Chemother.* 2017, 72, 1602–1609. [CrossRef] [PubMed]  
71. Park, S.C.; Lee, K.; Kim, Y.O.; Won, S.; Chun, J. Large-scale genomics reveals the genetic characteristics of seven species and importance of phylogenetic distance for estimating pan-genome size. *Front. Microbiol.* 2019, 10, 834. [CrossRef]  
72. Nguyen, V.T.; Jamroz, D.; Matamoros, S.; Carrique-Mas, J.J.; Ho, H.M.; Thai, Q.H.; Nguyen, T.N.M.; Wagenaar, J.A.; Thwaites, G.; Parkhill, J. Limited contribution of non-intensive chicken farming to ESBL-producing *Escherichia coli* colonization in humans in Vietnam: An epidemiological and genomic analysis. *J. Antimicrob. Chemother.* 2017, 72, 561–570. [CrossRef]  
73. Clermont, O.; Christenson, J.K.; Daubié, A.S.; Gordon, D.M.; Denamur, E. Development of an allele-specific PCR for *Escherichia coli* B2 sub-typing, a rapid and easy to perform substitute of multilocus sequence typing. *J. Microbiol. Methods* 2014, 101, 24–27. [CrossRef] [PubMed]  
74. Mora, A.; Garcia-Peña, F.J.; Alonso, A.S.; Denamur, E.; Pedraza-Diaz, S.; Ortega-Mora, L.M.; Garcia-Parraga, D.; López, C.; Viss, S.; Dahbi, G.; Marzoa, J.; et al. Impact of human-associated *Escherichia coli* clonal groups in antarctic pinnipeds: Presence of ST73, ST95, ST141 and ST131. *Sci. Rep.* 2018, 8, 1–11. [CrossRef] [PubMed]  
75. Manges, A.R. *Escherichia coli* and urinary tract infections: The role of poultry-meat. *Clin. Microbiol. Infect.* 2016, 22, 122–129. [CrossRef] [PubMed]
76. Day, M.J.; Hopkins, K.L.; Wareham, D.W.; Toleman, M.A.; Elviss, N.; Randall, L.; Teale, C.; Cleary, P.; Wiuff, C.; Doumith, M. Extended-spectrum β-lactamase-producing *Escherichia coli* in human-derived and foodchain-derived samples from England, Wales, and Scotland: An epidemiological surveillance and typing study. *Lancet Infect. Dis.* 2019, 19, 1325–1335. [CrossRef]

77. Kim, B.; Kim, J.; Seo, M.R.; Wie, S.-H.; Cho, Y.; Lim, S.K.; Lee, J.; Kwon, K.; Lee, H.; Cheong, H.J. Clinical characteristics of community-acquired acute pyelonephritis caused by ESBL-producing pathogens in South Korea. *Infection* 2013, 41, 603–612. [CrossRef] [PubMed]

78. Villa, M.F.L.; Salinas, L.; Villavivencio, F.; Rafael, T.; Salas, S.; Rivera, R.; Villacis, J.; Satan, C.; Ushiña, L.; Muñoz, O. Diverse *Escherichia coli* lineages, from domestic animals and humans in a household, carry colistin resistance gene *mcr-1* in Ecuador. *bioRxiv* 2018. [CrossRef]

79. Van Hoek, A.H.; Veenman, C.; Florijn, A.; Huijbers, P.M.; Graat, E.A.; De Greeff, S.; Dierikx, C.M.; Van Duijkeren, E. Longitudinal study of ESBL *Escherichia coli* carriage on an organic broiler farm. *J. Antimicrob. Chemother.* 2018, 73, 3298–3304. [CrossRef]

80. Skurnik, D.; Clermont, O.; Guillard, T.; Launay, A.; Danilchanka, O.; Pons, S.; Diancourt, L.; Lebreton, F.; Kadlec, K.; Roux, D. Emergence of antimicrobial-resistant *Escherichia coli* of animal origin spreading in humans. *Mol. Biol. Evol.* 2016, 33, 898–914. [CrossRef]

81. Choi, M.J.; Lim, S.K.; Jung, S.C.; Ko, K.S. Comparisons of CTX-M-producing *Escherichia coli* isolates from humans and animals in South Korea. *J. Bacteriol. Virol.* 2014, 44, 44–51. [CrossRef]