Heliyon 10 (2024) e27759

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

# Phenotypic and genotypic characterization of *Escherichia coli* isolated from the chicken liver in relation to slaughterhouse conditions

Mohammad H. Gharaibeh [a,*], Sahba Y. Al Sheyab [a], Ismail M. Malkawi [a], Farah R. Al Qudsi [b]

[a] *Department of Basic Veterinary Medical Science, Faculty of Veterinary Medicine, Jordan University of Science and Technology, P. O. Box 3030, Irbid, 22110, Jordan*

[b] *Department of Nutrition and Food Technology, Jordan University of Science and Technology, Irbid, 21121, Jordan*

ARTICLE INFO

*Keywords:*
*E. coli*
Resistance genes
Formal slaughterhouses
Informal slaughter facilities
Public health
Chicken liver
Colistin resistance
*Mcr*-10

ABSTRACT

Avian pathogenic *Escherichia coli* (APEC) has been identified as a sub-group of extraintestinal pathogenic *E. coli* (ExPEC). Recent studies indicate APEC as a potential foodborne zoonotic pathogen and a source or reservoir of human extraintestinal infections. The slaughtering and processing of poultry in low-income countries such as Jordan occurs in two distinct ways: in informal facilities known as Natafat and in formal slaughterhouses. This study compared *E. coli* phenotypes and genotypes according to slaughtering conditions (formal slaughterhouses vs. informal slaughter facilities). Therefore, liver samples (n = 242) were collected from formal (n = 121) and informal slaughter facilities (n = 121). Results revealed a high prevalence (94.2%) of *E. coli* among all isolates, with 59 (17 formal and 42 informal) isolates considered avian pathogenic *E. coli* (APEC) based on the virulence-associated genes. The prevalence of resistance among isolates was relatively high, reaching up to 99% against penicillin and 97% against nalidixic acid. However, the prevalence of resistance was the lowest (1.3%) against both meropenem and imipenem. Based on the MIC test findings, colistin resistance was 46.9% (107/228). The *mcr* −1 gene prevalence was 51.4% (55/107), of which 17.1 % were from formal plants (6/36) and 68.1% from informal facilities (49/72). Interestingly, only one isolate (0.9%) expressed *mcr*-10. Escherichia coli O157:H7 and associated virulence genes were found more in informal (n = 15 genes) than in formal slaughterhouses (n = 8). Phylogroups B1, C, and A were the most frequent in 228 *E. coli* isolates, while G, B2, and clade were the least frequent. In conclusion, these findings highlight the importance of implementing biosecurity measures in slaughterhouses to reduce antibiotic-resistant *E. coli* spread. Furthermore, this study provides valuable insights into the effects of wet market (Natafat) slaughter conditions on increasing bacterial resistance and virulence.

## 1. Introduction

Foodborne infections have been and remain a serious public health issue. These illnesses are estimated to cause approximately 600

* Corresponding author.
*E-mail address:* mhgharaibeh@just.edu.jo (M.H. Gharaibeh).

https://doi.org/10.1016/j.heliyon.2024.e27759
Received 17 October 2023; Received in revised form 4 March 2024; Accepted 6 March 2024
Available online 12 March 2024

million illnesses and 420,000 deaths yearly. These infections were primarily caused by diarrhea-producing bacteria, such as *E. coli* and non-typhoidal *Salmonella* [1,2]. Therefore, a food-producing animal, such as a chicken, preserves zoonotic pathogens that can produce toxins and pose a significant threat to public health [3], particularly the liver, which has been associated with foodborne pathogens [4] and is an appropriate colonization site for APEC [5]. Additionally, several factors can contribute to the contamination of animal products, including environmental factors (in farms) and human activities related to handling animals (e.g., slaughter, processing, and storage) [6]. Markets for live poultry house large numbers of chickens from various sources and with different treatment histories. Thus, a highly suitable environment is created for bacterial exchange [7]. Moreover, the slaughtering process is conducted near the consumer. The conditions described above allow bacteria to spread rapidly and uncontrollably throughout the food chain [8]. In low-income countries such as Jordan, poultry is slaughtered and processed in two ways: in an informal facility known as Natafat and slaughterhouses. The first type is small-scale butcher shops, also called informal slaughter facilities, where live chickens are kept in cages and slaughtered on-site. The second place is a formal slaughterhouse with various control measures and cold storage facilities [9]. *E. coli* is commonly found in the intestinal tract of warm-blooded animals [10]. Most *E. coli* strains are harmless, but some are classified as extra-intestinal pathogenic *E. coli*. They are divided into four groups: neonatal meningitis *E. coli*, sepsis-associated *E. coli*, uropathogenic *E. coli*, and APEC [11].

APEC is the etiological agent of colibacillosis, which begins as a respiratory infection and then develops into a general infection characterized by fibrinopurulent heart, lung, liver, and brain lesions. During APEC infection, virulence factors, such as adhesins, iron acquisition systems, protectins, toxins, invasins, metabolism, and secretion systems, play roles in colonization and survival [12]. Several recent studies have suggested that APEC may have zoonotic potential and serve as a source of human extra-intestinal infections [13–15]. Nevertheless, APEC, having a high prevalence rate of resistance, functions as a reservoir for resistance genes that pose a risk to human health [12]. Furthermore, *E. coli* O157:H7 is a serious foodborne pathogen that can result in severe disease when it colonizes the intestines and produces toxins that damage the host's cells [16]. The virulence of the *E. coli* strain can be determined using virulence-associated genes (VAGs). Several VAGs, including *iutA*, *hlyF*, *iss*, *iroN*, and *ompT*, have been linked to highly pathogenic avian *E. coli* [17].

Recently, mobile colistin resistance (*mcr*) genes have been identified as major contributors to colistin resistance among pathogenic bacteria [18]. The number of *mcr* variants has increased over the last nine years, from *mcr*-1 in 2015 to *mcr*-10 in 2020. These variants have been detected in bacteria originating from farms, farm workers, and domestic animals. The rapid spread of *mcr* genes among bacteria increases the likelihood of their global spread [19]. Additionally, it is important to recognize that the widespread use of antibiotics in food production has altered the microbiome [20]. The phylotyping of *E. coli* provides insight into its demonstrated function. In accordance with Clermont typing [21], *E. coli* is classified into eight different types (A, B1, B2, C, D, E, F, and G). In general, human pathogenic *E.coli* are mainly associated with groups B2 and D. Both resistant and commensal isolates of *E.coli* belong to groups A, B1, and G [22].

To our knowledge, no previous research has been conducted to assess the prevalence of *E. coli*, along with virulence genes, resistance genes, and phenotypes, in formal and informal slaughter facilities. Therefore, this study aimed to determine the incidence of *E. coli* in chickens in formal and informal poultry slaughterhouses and determine their AMR, resistance genes, virulence genes, and

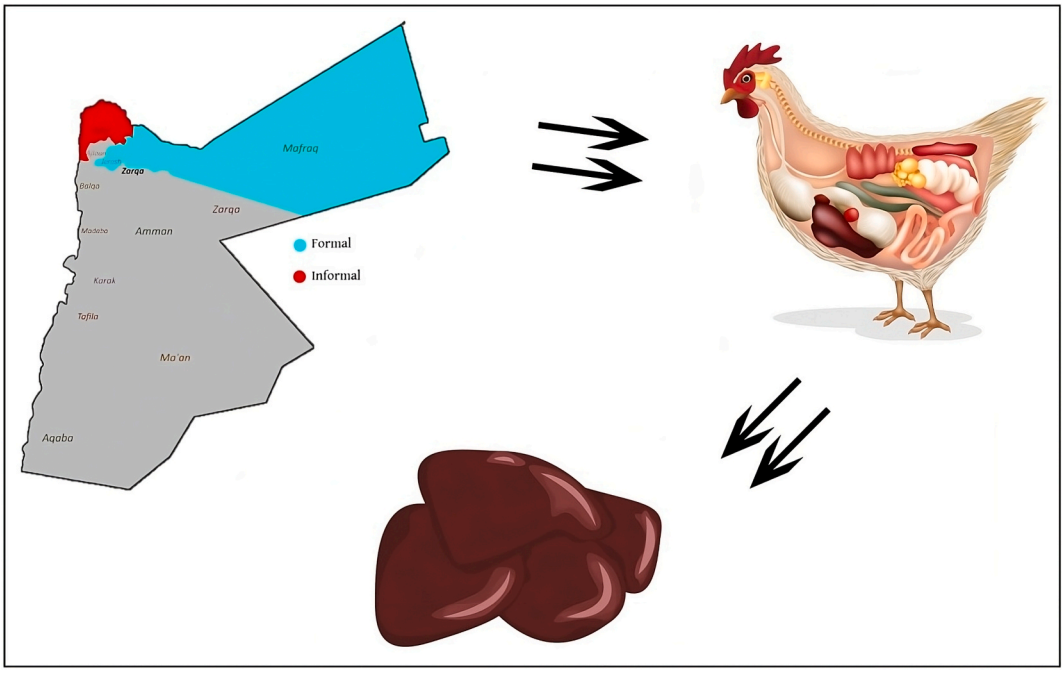


**Fig. 1.** Geographical location of the sample collection area.

detection of *E. coli* O157:H7 and its associated toxins.

## 2. Materials and methods

### *Ethical approval*

This study was reviewed and approved by the Animal Care and Use Committee (ACUC) of the Jordan University of Science and Technology (JUST- ACUC#291/12/4/16).

### *2.1. Samples collection and E. coli identification*

This study was a cross-sectional study conducted between November 2019 and March 2020. A total of 242 liver samples were collected from northern Jordan's formal and informal slaughter facilities (Fig. 1). One hundred twenty-one liver samples were obtained from formal slaughterhouses in Jerash, Al-Mafraq, and Irbid, and 121 samples were gathered from informal slaughter facilities in the countryside of Irbid. Each liver sample was placed in a sterile container and transported to the laboratory on ice. Upon arrival in the laboratory, they were processed immediately. Each liver was mixed with buffered peptone water (Oxoid, UK) at a ratio of 1:10 and homogenized in a stomacher (Seward, UK) at 240 rpm for 60 s. After homogenizing the sample, a loopful was streaked on Hichrome *E. coli* agar (HIMEDIA, India) and incubated at 37 °C for 24 h. Greenish colonies were subcultured on Tryptone Soya Agar (Oxoid, UK) for further analysis. DNA was extracted from the colonies using thermal cell lysis. The isolations were confirmed using PCR of *uspA* gene according to (Anastasi et al., 2010), the PCR mixture contained 4 μl of master mix (Solis BioDyne, Estonia), 4 μl of DNA, 0.6 μl of each primer at a concentration of 10 pmol, and up to 20 μl of nuclease-free water (NFW), the primers, and conditions are listed in (Table S1).

### *2.2. Anti-microbial susceptibility test*

According to the guidelines provided by the European Centre for Disease Prevention and Control [23], broth microdilution was used to determine colistin resistance. The minimum inhibitory concentration (MIC) for colistin was set at 2 mg/ml, and any isolate that grew at a concentration greater than 2 mg/ml was considered resistant to colistin. Disk diffusion was carried out according to the Clinical and Laboratory Standards Institute (CLSI) guidelines to assess resistance to 20 different antibiotics [24]. Resistance was examined by measuring and comparing the inhibition zone to established standards.

### *2.3. Resistance genes detection*

Colistin-resistant isolates were screened for *mcr* genes (1−10) using multiplex PCR, according to Refs. [25–27]. The PCR mixture contained 4 μl master mix (Solis BioDyne, Estonia), 4 μl DNA, 0.6 μl of each primer at a concentration of 10 pmol and up to 20 μl NFW (Table S2). Detection of Expanded spectrum β–lactamase (ESβL) genes and Ampicillin class C β-lactamase (*AmpC*) was done according to El-Shazly et al. [28]. The primers and PCR conditions are listed in (Tables S1–2).

### *2.4. Virulence genes detection*

According to Ibrahim et al., the isolated *E. coli* strains were subjected to two multiplex PCR assays to identify 16 virulence-associated genes [29]. The first multiplex PCR targeted 9 virulence genes, and the second targeted 7 virulence genes. Each PCR assay contained 4 μl master mix (Solis BioDyne, Estonia), 4 μl DNA, 1.2 μl of each primer pair at a concentration of 10 pmol, and up to 20 μl NFW. Initial denaturation at 94 °C for 3 min, 25 cycles of denaturation at 94 °C for 30 s, annealing at 63 °C for the first reaction and 58 °C for the second reaction for 30 s, extension at 68 °C for 3 min, followed by a final extension at 72 °C for 10 min. Based on Subedi et al. [20], an isolate is considered APEC if it has five or more virulence genes. A list of primers and conditions is provided in (Table S3).

### *2.5. E. coli O157:H7 and virulence genes detection*

Multiplex PCR was used with specific primers for the *rfb* (O157) and *flic* (H7) genes to confirm the presence of *E. coli* O157:H7. Additionally, the same multiplex PCR was employed to detect virulence genes for the *stx*1, *stx*2, *eaeA*, and *hly* genes, according to Ref. [30]. The primers and conditions are presented in (Table S4).

### *2.6. Identification of phylogenetic groups*

The Phylogenetic groups of *E. coli* isolates was done according to the methods described by Clermont et al. [21,31]. All primers and reaction conditions are presented in (Table S5).

### 2.7. Statistical analysis

The Chi-squared test was used to compare *E. coli* isolates and antibiotic resistance between formal and informal slaughter facilities using SPSS® version 25 (IBM, USA). The significance level for an association was set when $p < 0.05$.

## 3. Results

### 3.1. Bacterial isolates and anti-microbial susceptibility testing

The overall prevalence of *E. coli* in chicken livers was 228/242 (94.2%), 113/121 (93.4%) from formal slaughterhouses, and 115/121 (95%) from informal slaughter facilities. *Escherichia coli* isolates with multi-drug resistance (MDR, i.e., resisted three classes or more) constituted 98.24% (224/228). The prevalence of MDR was 100% among *E. coli* isolated from informal slaughter facilities, while 96.46% among those isolated from formal slaughterhouses. In our analysis of the MDR isolates, we identified 151 MDR patterns. The lowest incidence of resistance was observed for meropenem (1.31%) and imipenem (1.31%) followed by cefoxitin (12.71%), cefepime (17.98%), aztreonam (20.61%), tigecycline (34.64%), fosfomycin (36.40%), amoxicillin-clavulanic acid (42.98%), gentamicin (45.61%) and colistin (46.92%) (Fig. S1, Table S6). The occurrence of AMR was significantly higher in informal isolates than in formal isolates ($p \leq 00.05$), particularly against doxycycline, kanamycin, gentamicin, fosfomycin, chloramphenicol, florfenicol, cefepime, aztreonam, ciprofloxacin, sulphamethoxazole-trimethoprim and colistin (Fig. 2, Table S6).

### 3.2. Detection of virulence genes

The overall incidence of an APEC containing five or more VAGs was 59/228 (25.8%). However, there was a significant difference between the number of APECs from informal slaughter facilities: 42/115 (36.5%) and 17/113 (15%) (*p-value*< 0.05). In total, 228 isolates were screened for acquired virulence-associated genes, which resulted in the identification of 72 different virulence patterns (Table S7). In terms of prevalence, *sitA* (144, 63.2%) was the most prevalent gene, followed by Irp2 (113, 49.6%), *astA* (80, 35.1%), *iucC* (78, 34.2%), *iss* (77, 33.8%), *iucD* (61, 26.8%), *kpsII* (21, 9.2%), *cva/cvi* (19, 8.3%), *SFA* (15, 6.6%), *vat* (10, 4.4%), *kpsM* (6, 2.6%) and *tsh* (2, 0.9%) (Table 1, Fig. S2, Fig. S3).

### 3.3. Detection of MCR, ESβL, and AMPC genes

Based on the analysis of the *mcr* genes (1−10), *mcr-1* was detected in (55, 51.4%) of the isolates resistant to colistin (n = 107), whereas interestingly, only one isolate (0.9%) expressed *mcr-10*. No *mcr-2* to *mcr-9* genes were detected (Table S8). The *mcr-1* prevalence was significantly higher in informal slaughter facilities (49, 68.1%) than in formal slaughterhouses (6, 17.1%) (*p-value* 00.00001) (Table S3). In terms of ESβL genes, $bla_{TEM}$ (157, 69%) was the most dominant gene, followed by $bla_{CTX\text{-}M}$ (117, 51%), $bla_{SHV}$ (53, 23%), $bla_{CTX\text{-}M\text{-}1}$ (29, 13%), and $bla_{CTX\text{-}M\text{-}9}$ (3, 1%). The occurrence of $bla_{TEM}$ was notably higher in informal slaughter facilities (101, 87.8%) compared to formal slaughterhouses (56, 49.5%) (Table 2). The results of the present study indicated the presence of three AMPC genes: *moxM* (1, 0.4%), *citM* (7, 3%), and *ebcM* (1, 0.4%) (Table 2).

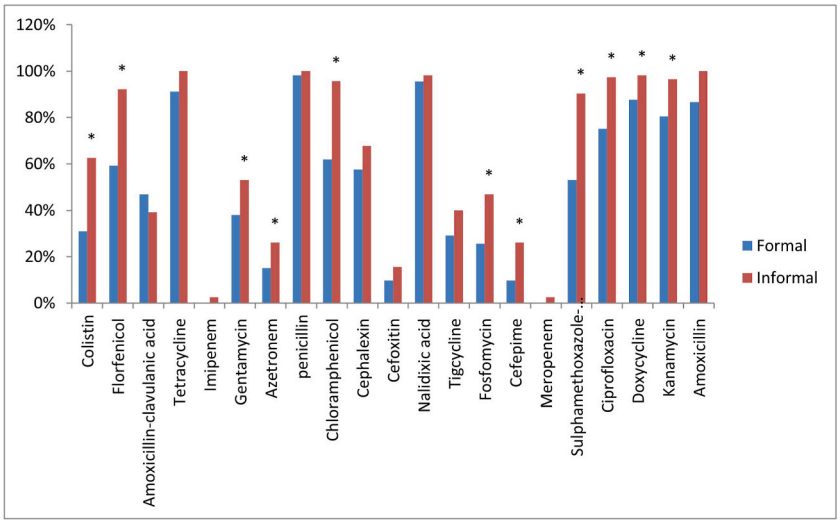


**Fig. 2.** Percentages of resistance and susceptibility among all isolates from the formal and informal slaughter facilities.

**Table 1**
The number and percentages of *E. coli* isolates harboring virulence-associated genes according to source (Formal vs. Informal).

| Gene | All Isolates (n = 228) | Formal (n = 113) | Informal (n = 115) | *Chi-square (P- value)* | Odds Ratio | CI 95 | |
|---|---|---|---|---|---|---|---|
| *iss* | 77 (33.77%) | 18 (15.93%) | 59 (51.3%) | <0.00001* | 5.561 | 2.98 | 10.36 |
| *Asta* | 80 (35.09%) | 19 (16.81%) | 61 (53.04%) | <0.00001* | 5.59 | 3.04 | 10.32 |
| *hlyD* | 0 (0%) | 0 (0%) | 0 (0%) | *NA* | *NA* | *NA* | *NA* |
| *KpsM* | 6 (2.63%) | 1 (0.88%) | 5 (4.35%) | 0.2126 | 5.09 | 0.585 | 44.28 |
| *KpsII* | 21 (9.21%) | 5 (4.42%) | 16 (13.91%) | 0.013245 | 3.49 | 1.233 | 9.882 |
| *KpsIII* | 0 (0%) | 0 (0%) | 0 (0%) | NA | NA | NA | NA |
| *cva/cvi* | 19 (8.33%) | 12 (10.62%) | 7 (6.09%) | *0.215691* | 0.546 | 0.207 | 1.44 |
| *papC* | 0 (0%) | 0 (0%) | 0 (0%) | *NA* | *NA* | *NA* | *NA* |
| *sfa* | 15 (6.58%) | 7 (6.19%) | 8 (6.96%) | *0.816542* | 1.132 | 0.396 | 3.233 |
| *IbeA* | 0 (0%) | 0 (0%) | 0 (0%) | *NA* | *NA* | *NA* | *NA* |
| *Irp2* | 113 (49.56%) | 46 (40.71%) | 67 (58.26%) | *0.008039** | 2.033 | 1.2 | 3.44 |
| *IucC* | 78 (34.21%) | 26 (23.01%) | 52 (45.22%) | *0.000409** | 2.762 | 1.55 | 4.89 |
| *SitA* | 144 (63.16%) | 63 (55.75%) | 81 (70.43%) | *0.021566** | 1.89 | 1.095 | 3.264 |
| *Tsh* | 2 (0.88%) | 1 (0.88%) | 1 (0.87%) | *1* | 0.982 | 0.061 | 15.9 |
| *IucD* | 61 (26.75%) | 22 (19.47%) | 39 (33.91%) | *0.013766** | 2.123 | 1.159 | 3.887 |
| *Vat* | 10 (4.39%) | 5 (4.42%) | 5 (4.35%) | *0.977367* | 0.982 | 0.276 | 3.488 |

*indicates significance (*p-value*< 0.05).

**Table 2**
The number and percentages of *E. coli* isolates harboring resistance genes (ESBL and AMPC) according to source (Formal vs. Informal).

| Genes | | All Isolates (n = 228) | Formal (n = 113) | Informal (n = 115) | *Chi-square (P- value)* | Odds Ratio | CI 95 | |
|---|---|---|---|---|---|---|---|---|
| ESβL | *TEM* | 157 (69%) | 56 (49.5%) | 101 (87.8%) | <0.00001* | 7.343 | 3.759 | 14.345 |
| | *SHV* | 53 (23%) | 30 (26.5%) | 23 (20%) | 0.241821 | 0.692 | 0.372 | 1.284 |
| | *CTXM* | 117 (51%) | 58 (51.3%) | 59 (51.3%) | 0.997218 | 0.999 | 0.594 | 1.679 |
| | *CTXM1* | 29 (13%) | 10 (8.8%) | 19 (16.5%) | 0.082141 | 2.038542 | 0.902663 | 4.603768 |
| | *CTXM2* | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | NA | NA | NA | NA |
| | *CTXM8* | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | NA | NA | NA | NA |
| | *CTXM9* | 3 (1%) | 1 (0.8%) | 2 (1.7%) | 1 | 1.982301 | 0.177217 | 22.173489 |
| AMPC | *MOXM* | 1 (0.4%) | 1 (0.8%) | 0 (0.0%) | NA | NA | NA | NA |
| | *CITM* | 7 (3%) | 3 (2.6%) | 4 (3.4%) | 1 | 1.321321 | 0.28899 | 6.041341 |
| | *DHAM* | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | NA | NA | NA | NA |
| | *ACCM* | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | NA | NA | NA | NA |
| | *EBCM* | 1 (0.4%) | 1 (0.8%) | 0 (0.0%) | NA | NA | NA | NA |
| | *FOXM* | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | NA | NA | NA | NA |

*indicates significance (*p-value*< 0.05).

### 3.4. Detection of Escherichia coli O157, H7, and associated genes

Of the 228 positive isolates, only 5 (2.19%) contained the O157 gene (4 formal and 1 informal). These isolates, however, did not comprise the *fliCH7* gene and were therefore classified as *E. coli* O157. On the other hand, five isolates (2.19%) had *fliCH7* gene (2 formal and 3 informal). Among the major virulence factors (*stx*1, *stx*2, *hlyA*, *eaeA*), 11 genes were observed in informal slaughter facilities compared to only 2 genes in formal slaughterhouses. In general, the findings of the six genes indicated an increased number of genes in informal slaughter facilities (n = 15) than in formal slaughterhouses (n = 8) (Table 3).

### 3.5. Phylogenetic groups of E. coli

Among the 228 *E. coli* isolates, the most prevalent phylotype was B1 (79, 34.6%), followed by C (59, 25.9%), A (38, 16.7%), E (19, 8.3%), F (13, 5.7%), D (7, 3.1%), G (2, 0.9% and B2 (1, 0.4%) and clade (1, 0.4%). Only nine (3.9%) of the isolates were not assigned to any phylotype. In all phylotypes, there were no significant variances between formal and informal slaughterhouses, except phylotype

**Table 3**
The number and percentages of isolates harboring O157 and H7 genes according to source (Formal vs. Informal); *p-value* < 0.05 indicates significance.

| Toxins | | Formal (n = 113) | Informal (n = 118) | *p-value* |
|---|---|---|---|---|
| Shiga Toxin | *stx1* | 0 | 1 | NA |
| | *stx2* | 0 | 2 | NA |
| Enteropathogenic Toxin | *eaeA* | 0 | 4 | NA |
| Haemolysin | *hly* | 2 | 4 | 0.6838 |
| H gene | *flich7* | 2 | 3 | 1 |
| O gene | *rfbE* | 4 | 1 | 0.2049 |
| Total | | 8 | 15 | 0.152968. |

E, which showed an increase (p-value = 0.015) in informal slaughterhouses (15, 13%) compared to formal slaughterhouses (4, 3.5%) (Table 4).

## 4. Discussion

Chicken liver is a nutrient-dense food that contains essential minerals such as iron, zinc, and folate. This diet is a rich source of high-quality protein, necessary for tissue growth and repair. In addition, chicken liver is one of the best sources of vitamin A. Pate, prepared at low temperatures and for short durations, is gaining popularity and demand due to its superior nutritional and textural characteristics [32]. However, several outbreaks of foodborne illness have been caused by the consumption of undercooked chicken liver. For example, between 2000 and 2016, 28 foodborne outbreaks were caused by undercooked chicken liver, pate, or both made from chicken liver in the United States [4]. In Jordan, livers can be obtained from informal slaughter facilities (Natafats) and formal slaughterhouses [33]. Informal slaughter facilities lack the necessary equipment and infrastructure to handle and process meat, which increases the risk of contamination and spoilage. Informal slaughter facilities may also heighten contamination risk due to inadequate sanitation measures. In contrast, formal slaughterhouses follow strict food safety protocols, including sanitation, temperature control, and quality control procedures, which minimize the likelihood of foodborne diseases. Due to these factors, it is necessary to recommend that health oversight bodies close informal slaughterhouses and encourage formal slaughterhouses to provide hygienic and safe meat handling practices. As this goal is difficult to achieve in the short term, especially in countries with low incomes such as Jordan, health oversight agencies need to increase public awareness of sound slaughter practices and encourage informal slaughterhouses to improve slaughter practices, including improving sanitation and hygiene standards, as well as providing adequate animal care. Nevertheless, de-feathering, evisceration, and chicken washing in both slaughterhouses pose a high possibility of contamination. When evisceration of the animal is not appropriately performed, and the equipment is not cleaned and sanitized correctly, intestinal bacteria can contaminate the meat [34].

In this study, 95.6% and 93% of the isolates were resistant to tetracycline and doxycycline, respectively. This investigation's findings share similar reports from Jordan [29] and Egypt [35]. Even though tetracyclines were heavily used as growth promoters in the poultry industry [36], 34% of poultry birds resisted tigecycline. This concern is alarming since tigecycline has been widely recognized as a last resort treatment for multidrug-resistant gram-negative infections in humans [37]. As a result of tetracycline misuse, *tet* (X4), a newly mobile gene unaffected by tigecycline, can emerge [38]. According to this research, 46.9% of *E. coli* isolates were resistant to colistin, whereas resistance rates were lower in the United States (13.2%) and Nepal (28.5%) [39]. In many countries, such as Jordan, colistin is believed to be used as a prophylactic and growth promoter [18]. Consequently, a high prevalence of resistance may develop. Also, the presence of avian-origin *mcr*-1 positive strains of *E. coli* is considered a significant public health apprehension as they can cause human infections. Colistin use in animal husbandry is a major driver of *mcr-1* emergence [40], raising fears about the antibiotic's effectiveness as a last-resort medication for MDR human infections. Additionally, infections caused by *mcr*-positive *E. coli* have been reported in humans worldwide, particularly concerning high-virulent strains found in poultry. In this study, the *mcr*-10 gene was detected in an *E. coli* isolate from formal slaughterhouses. Similarly, the *mcr*-10 gene was found in *E. coli* from slaughterhouse workers in China in 2022 [41]. *Mcr*-10 is common among Enterobacter isolates [42,43]; however, some research indicates that it is also prevalent among *Klebsiella* isolates [41]. In the Middle East, *mcr*-10 was discovered in Egypt [44]. To our knowledge, this is the first report of *mcr*-10 emergence in Jordan. These findings may alert researchers to the possibility that *mcr*-10 will spread throughout the region in the future.

Transmission of antibiotic resistance from animals to humans through the food chain or direct contact is a potential risk, highlighting the need for improved prevention strategies [45]. Moreover, the presence of ColV plasmids in ExPEC strains is strongly associated with avian-source strains responsible for causing human infections [46], providing insight into the epidemiology of avian-source ExPEC infections and aiding in developing control strategies [47]. Meanwhile, resistance against imipenem and meropenem was generally low (1.31%), consistent with [39], who reported a resistance rate of 1.5% against imipenem. Imipenem resistance is often linked with *K. pneumonia* [48], and resistance genes will also be derived from *K. pneumonia* [49]. This low resistance is attributed to the restricted use of imipenem and meropenem in the Jordanian veterinary sector. AMR threats to human health can result in ineffective treatment. Besides, it yields to increased illnesses and deaths, heightening healthcare costs.

**Table 4**
The detected phylotypes of *E. coli* according to source (Formal vs. Informal).

| Group | All Isolates (n = 228) | Formal (n = 113) | Informal (n = 115) | *Chi-square (P- value)* | Odds Ratio | CI 95 | |
|---|---|---|---|---|---|---|---|
| A | 38 (16.7%) | 21 (18.6%) | 17 (14.8%) | 0.441 | 0.76 | 0.377 | 1.53 |
| B1 | 79 (34.6%) | 42 (37.2%) | 37 (32.2%) | 0.428 | 0.802 | 0.464 | 1.385 |
| B2 | 1 (0.4%) | 1 (0.9%) | 0 (0%) | NA | NA | NA | NA |
| C | 59 (25.9%) | 35 (31%) | 24 (20.9%) | 0.082 | 0.588 | 0.322 | 1.072 |
| Clade | 1 (0.4%) | 1 (0.9%) | 0 (0%) | NA | NA | NA | NA |
| D | 7 (3.1%) | 2 (1.8%) | 5 (4.3%) | 0.446 | 2.523 | 0.479 | 13.28 |
| E | 19 (8.3%) | 4 (3.5%) | 15 (13%) | 0.015* | 4.088 | 1.313 | 12.728 |
| F | 13 (5.7%) | 3 (2.7%) | 10 (8.7%) | 0.083 | 3.492 | 0.935 | 13.041 |
| G | 2 (0.9%) | 0 (0%) | 2 (1.7%) | NA | NA | NA | NA |
| ND | 9 (3.9%) | 4 (3.5%) | 5 (4.3%) | 1 | 1.239 | 0.324 | 4.736 |

*$p$-value $< 0.05$ indicates significance. ND: not determined.

Managing and preventing AMR infections is more difficult since they spread rapidly and easily [50,51]. Most have become antibiotic-resistant, including urinary tract infections, pneumonia, and sepsis. Therefore, patients suffering from these infections may require more intensive and prolonged medication and may potentially need hospitalization. In some cases, ineffective treatment can result in severe health conditions or even death [50]. Hence, healthcare providers, policymakers, and individuals must work together to prevent the spread of AMR. Several actions can be taken to achieve this objective, including raising consumer awareness and reducing antibiotic use in food-producing animals [52,53].

Formal slaughterhouses are government-approved facilities that adhere to strict animal welfare, hygiene, and food safety standards. Using these systems ensures humane treatment of animals and minimizes contamination risks [54]. Many low-income countries, including Jordan, have informal slaughter facilities known as backyard or unregulated slaughterhouses. These places may lack equipment, training, or hygiene standards, which can increase the risk of disease transmission. The prevalence of *E. coli* in chicken visceral products has been studied in several studies. The Brazilian research by Ref. [55] revealed that 8% of *E. coli* isolates were derived from chicken livers, with 36% coming from consumption-oriented livers. An analysis by Ref. [56] reported a 51.7% survival rate of multidrug-resistant *E. coli* from origin. In addition, $bla_{SHV}$ was detected in 12% of all *E. coli* isolated from meat. In Algeria [57], found an 86.6% prevalence of *E. coli* in 180 chicken viscera, with 50 isolates belonging to serogroups O1, O2, and O78. Additionally, 66% of the isolates resisted at least seven antibiotics [57]. APEC is sub-grouped in the ExPEC; it is informed that APEC is a foodborne zoonotic pathogen and a source or reservoir of extra-intestinal infections in humans [15]. Most research has demonstrated similarities between *E. coli* causing urinary tract infections in humans and *E. coli* causing colibacillosis in chickens, providing APEC's zoonotic potential [58]. Moreover, *E. coli* Sequence Type 131 (ST131) has been well established in poultry populations worldwide and is an infective agent to humans. However, additional investigation are required to unravel the total fraction of human extra-intestinal infections attributable to food animal *E. coli* strains [59].

In the context of APEC [60], reported that 79 *E. coli* isolates were recovered from liver swabs associated with APEC. The most common serogroup was O78, followed by O2. Also, in this study, all five virulence-associated genes (*hlyF*, *iroN*, *iss*, *iutA*, and *ompT*) were detected in 62 of the isolates, including three ESBL genes ($bla_{TEM-1}$, $bla_{CTX-M-1}$, and $bla_{CTX-M-15}$). In Jordan [29], found that APEC was present in 53.4% of bird viscera samples, and the most frequently identified serotypes were O1, O2, and O78, with five virulence-associated genes discovered in 69.2%. In Korea, 73% of the cases were caused by Enterotoxigenic *E. coli* (ETEC) isolated from children who had consumed infected kimchi [61]. Furthermore, 54 people have died in Germany from eating sprouts contaminated with *E. coli* [62]. In a study by Ref. [63], ExPEC was noticed in 21% of chicken and 4.7% of egg samples. These percentages are associated with a potential risk to humans. In this study, most isolates belonged to phylogroup B1, which is understandable since *E. coli* from groups B1 and A occur most frequently in animals, and they are usually commensal and resistant to antimicrobial agents [64]. In contrast, Escherichia coli isolates belonging to groups E and D are considered more pathogenic to broilers than those belonging to groups B or A [65].

## 5. Conclusion

To the best of our knowledge, this is the first study to compare the prevalence of *E. coli* in chickens from formal and informal poultry slaughterhouses. Based on our findings, informal slaughter facilities have higher levels of antibiotic resistance, resistance genes, and virulence genes than formal slaughterhouses. Additionally, this study has demonstrated that poor slaughtering conditions in informal slaughter facilities were significantly associated with high contamination levels, along with an increased prevalence of resistant and virulent isolates. Therefore, control measures must be implemented to minimize contamination risks while ensuring the humane handling of the animals.

## Data availability

All relevant data are within the paper and its supporting information files.

## CRediT authorship contribution statement

**Mohammad H. Gharaibeh:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Funding acquisition, Formal analysis, Conceptualization. **Sahba Y. Al Sheyab:** Writing – original draft, Methodology, Data curation. **Ismail M. Malkawi:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Farah R. Al Qudsi:** Writing – review & editing, Writing – original draft, Conceptualization.

## Declaration of competing interest

The authors declare no conflict of interest.

## Acknowledgments

This work was supported by the Deanship of Research, Jordan University of Science and Technology, Irbid, Jordan (Research Grant No:328/2022).

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e27759.

## References


[1] A.A. Al-Nabulsi, Z.W. Jaradat, F.R. Al Qudsi, L. Elsalem, T.M. Osaili, A.N. Olaimat, G. Esposito, S.-Q. Liu, M.M. Ayyash, Characterization and Bioactive Properties of Exopolysaccharides Produced by *Streptococcus Thermophilus* and Lactobacillus Bulgaricus Isolated from Labaneh, 2022, https://doi.org/10.1016/j.lwt.2022.113817.

[2] Who, WHO Estimates of the Global Burden of Foodborne Diseases: Executive Summary, 2015. www.who.int. (Accessed 6 February 2022).

[3] N. Heredia, S. García, Animals as sources of food-borne pathogens: a review, Anim. Nutr. 4 (2018) 250–255, https://doi.org/10.1016/J.ANINU.2018.04.006.

[4] W.A. Lanier, K.R. Hale, A.L. Geissler, D. Dewey-Mattia, Chicken liver-associated outbreaks of campylobacteriosis and salmonellosis, United States, 2000-2016: identifying opportunities for prevention, Foodborne Pathog. Dis. 15 (2018) 726–733, https://doi.org/10.1089/fpd.2018.2489.

[5] S.M. Lutful Kabir, Avian colibacillosis and salmonellosis: a closer look at epidemiology, pathogenesis, diagnosis, control and public health concerns, Int. J. Environ. Res. Public Health. 7 (2010) 89–114, https://doi.org/10.3390/IJERPH7010089.

[6] J.N. Sofos, Challenges to meat safety in the 21st century, Meat Sci. 78 (2008) 3–13, https://doi.org/10.1016/J.MEATSCI.2007.07.027.

[7] G. Dantas, M.O.A. Sommer, R.D. Oluwasegun, G.M. Church, Bacteria subsisting on antibiotics, Science 320 (2008) 100–103, https://doi.org/10.1126/SCIENCE.1155157.

[8] M.S. Sarker, M.S. Mannan, M.Y. Ali, M. Bayzid, A. Ahad, Z.B. Bupasha, Antibiotic resistance of Escherichia coli isolated from broilers sold at live bird markets in Chattogram, Bangladesh, J. Adv. Vet. Anim. Res. 6 (2019) 272–277, https://doi.org/10.5455/JAVAR.2019.F344.

[9] A.R. Alaboudi, I.M. Malkawi, T.M. Osaili, E.A. Abu-Basha, J. Guitian, Prevalence, antibiotic resistance and genotypes of Campylobacter jejuni and Campylobacter coli isolated from chickens in Irbid governorate, Jordan, Int. J. Food Microbiol. 327 (2020), https://doi.org/10.1016/J.IJFOODMICRO.2020.108656.

[10] O. Tenaillon, D. Skurnik, B. Picard, E. Denamur, The population genetics of commensal Escherichia coli, Nat. Rev. Microbiol. 8 (2010) 207–217, https://doi.org/10.1038/nrmicro2298.

[11] Z.R. Stromberg, J.R. Johnson, J.M. Fairbrother, J. Kilbourne, A. Van Goor, R. Curtiss, M. Mellata, Evaluation of Escherichia coli isolates from healthy chickens to determine their potential risk to poultry and human health, PLoS One 12 (2017) 1–18, https://doi.org/10.1371/journal.pone.0180599.

[12] J. Hu, D.J.A. Afayibo, B. Zhang, H. Zhu, L. Yao, W. Guo, X. Wang, Z. Wang, D. Wang, H. Peng, M. Tian, J. Qi, S. Wang, Characteristics, pathogenic mechanism, zoonotic potential, drug resistance, and prevention of avian pathogenic Escherichia coli (APEC), Front. Microbiol. 13 (2022) 1049391, https://doi.org/10.3389/FMICB.2022.1049391/BIBTEX.

[13] K.E. Rodriguez-Siek, C.W. Giddings, C. Doetkott, T.J. Johnson, M.K. Fakhr, L.K. Nolan, Comparison of Escherichia coli isolates implicated in human urinary tract infection and avian colibacillosis, Microbiology 151 (2005) 2097–2110, https://doi.org/10.1099/MIC.0.27499-0.

[14] A.R. Manges, Escherichia coli and urinary tract infections: the role of poultry-meat, Clin. Microbiol. Infect. 22 (2016) 122–129, https://doi.org/10.1016/J.CMI.2015.11.010.

[15] D. Kathayat, D. Lokesh, S. Ranjit, G. Rajashekara, Avian Pathogenic Escherichia coli (APEC): an Overview of Virulence and Pathogenesis Factors, Zoonotic Potential, and Control Strategies, vol. 10, Pathog., Basel, Switzerland, 2021, https://doi.org/10.3390/PATHOGENS10040467.

[16] L.M. Lim, N. Ly, D. Anderson, J.C. Yang, L. Macander, A. Jarkowski, A. Forrest, J.B. Bulitta, B.T. Tsuji, Resurgence of colistin: a review of resistance, toxicity, pharmacodynamics, and dosing, Pharmacotherapy 30 (2010) 1279–1291, https://doi.org/10.1592/phco.30.12.1279.

[17] T.J. Johnson, Y. Wannemuehler, C. Doetkott, S.J. Johnson, S.C. Rosenberger, L.K. Nolan, Identification of minimal predictors of avian pathogenic Escherichia coli virulence for use as a rapid diagnostic tool, J. Clin. Microbiol. 46 (2008) 3987–3996, https://doi.org/10.1128/JCM.00816-08.

[18] M.H. Gharaibeh, S.Q. Shatnawi, An overview of colistin resistance , mobilized colistin resistance genes dissemination , global responses , and the alternatives to colistin : a review, Vet. World 12 (2019) 1735–1746.

[19] S. Yan, A. Hussein, T. Cavender, C.M. Logue, N. Lima Barbieri, L.K. Nolan, Y. Wannemuehler, D.W. Nielsen, mcr-1 identified in Avian Pathogenic Escherichia coli (APEC), PLoS One 12 (2017) e0172997, https://doi.org/10.1371/journal.pone.0172997.

[20] M. Subedi, H. Luitel, B. Devkota, R.K. Bhattarai, S. Phuyal, P. Panthi, A. Shrestha, D.K. Chaudhary, Antibiotic resistance pattern and virulence genes content in avian pathogenic Escherichia coli (APEC) from broiler chickens in Chitwan, Nepal, BMC Vet. Res. 14 (2018), https://doi.org/10.1186/s12917-018-1442-z.

[21] O. Clermont, O.V.A. Dixit, B. Vangchhia, B. Condamine, S. Dion, A. Bridier-Nahmias, E. Denamur, D. Gordon, Characterization and rapid identification of phylogroup G in Escherichia coli, a lineage with high virulence and antibiotic resistance potential, Environ. Microbiol. 21 (2019) 3107–3117, https://doi.org/10.1111/1462-2920.14713.

[22] K. Mendoza, B. Ortiz, L. Rivera, T. Peña, M. Chirinos-Escobar, L. Enríquez, V. Maldonado, G. Fontecha, Monitoring of microbial contamination of groundwater in the upper choluteca river basin, Honduras, Water (Switzerland) 15 (2023) 1–14, https://doi.org/10.3390/w15112116.

[23] E.C. for D.P. and Control, Laboratory Manual for Carbapenem and Colistin Resistance Detection and Characterisation for the Survey of Carbapenem- And/or Colistin-Resistant Enterobacteriaceae, vol. 2, ECDC, Stockholm, 2019. https://www.ecdc.europa.eu/en/publications-data/laboratory-manual-carbapenem-and-colistin-resistance-detection-and#no-link. (Accessed 12 November 2020).

[24] CLSI, Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Seventh Informational Supplement, 2017. CLSI Document M100-S27, www.clsi.org.

[25] A.R. Rebelo, V. Bortolaia, J.S. Kjeldgaard, S.K. Pedersen, P. Leekitcharoenphon, I.M. Hansen, B. Guerra, B. Malorny, M. Borowiak, J.A. Hammerl, A. Battisti, A. Franco, P. Alba, A. Perrin-guyomard, S.A. Granier, C. De Frutos, S. Malhotra-kumar, L. Villa, A. Carattoli, R.S. Hendriksen, Multiplex PCR for detection of plasmid-mediated mcr-4 and mcr-5 for surveillance purposes, Euro Surveill. 23 (2018), https://doi.org/10.2807/1560-7917.ES.2018.23.6.17-00672 pii=17-00672.

[26] M. Borowiak, B. Baumann, J. Fischer, K. Thomas, C. Deneke, J.A. Hammerl, I. Szabo, B. Malorny, Development of a novel mcr-6 to mcr-9 multiplex PCR and assessment of mcr-1 to mcr-9 occurrence in colistin-resistant Salmonella enterica isolates from environment, feed, animals and food (2011–2018) in Germany, Front. Microbiol. 11 (2020) 497098, https://doi.org/10.3389/FMICB.2020.00080/BIBTEX.

[27] T. Xu, C. Zhang, Y. Ji, J. Song, Y. Liu, Y. Guo, K. Zhou, Identification of mcr-10 carried by self-transmissible plasmids and chromosome in Enterobacter roggenkampii strains isolated from hospital sewage water, Environ. Pollut. 268 (2021) 115706, https://doi.org/10.1016/j.envpol.2020.115706.

[28] D.A. El-Shazly, S.A. Nasef, F.F. Mahmoud, D. Jonas, Expanded spectrum β–lactamase producing Escherichia coli isolated from chickens with colibacillosis in Egypt, Poult. Sci. 96 (2017) 2375–2384, https://doi.org/10.3382/ps/pew493.

[29] R.A. Ibrahim, T.L. Cryer, S.Q. Lafi, E.A. Basha, L. Good, Y.H. Tarazi, Identification of Escherichia coli from broiler chickens in Jordan, their antimicrobial resistance, gene characterization and the associated risk factors, BMC Vet. Res. 15 (2019) 1–16, https://doi.org/10.1186/s12917-019-1901-1.

[30] P.B. Shridhar, L.W. Noll, X. Shi, N. Cernicchiaro, D.G. Renter, J. Bai, T.G. Nagaraja, Escherichia coli O104 in feedlot cattle feces: prevalence, isolation and characterization, PLoS One 11 (2016) 1–17, https://doi.org/10.1371/journal.pone.0152101.

[31] O. Clermont, J.K. Christenson, E. Denamur, D.M. Gordon, The Clermont Escherichia coli phylo-typing method revisited: improvement of specificity and detection of new phylo-groups, Environ. Microbiol. Rep. 5 (2013) 58–65, https://doi.org/10.1111/1758-2229.12019.

[32] A.C.S. Porto-Fett, B.A. Shoyer, L.E. Shane, M. Osoria, E. Henry, Y. Jung, J.B. Luchansky, Thermal inactivation of salmonella in pâté made from chicken liver, J. Food Prot. 82 (2019) 980–987, https://doi.org/10.4315/0362-028X.JFP-18-423.

[33] M. Ibrahem Abu-Iteleh, Moh A.M.Rahahleh, Sulaiman Alrkaibat, M. Nabeel Daood, Poultry Sector Country Review This Review Is Based on the Following Report: Poultry Sector Analysis: Structure & Market and Importance of Commercial Poultry Production in Jordan, for a Better Understanding of Avian Influenza Challenges, 2007. http://www.fao.org/3/a-ai322e.pdf.

[34] M. Paul, V. Baritaux, S. Wongnarkpet, C. Poolkhet, W. Thanapongtharm, F. Roger, P. Bonnet, C. Ducrot, Practices associated with Highly Pathogenic Avian Influenza spread in traditional poultry marketing chains: social and economic perspectives, Acta Trop. 126 (2013) 43–53, https://doi.org/10.1016/j.actatropica.2013.01.008.

[35] A.M. Ahmed, T. Shimamoto, T. Shimamoto, Molecular characterization of multidrug-resistant avian pathogenic Escherichia coli isolated from septicemic broilers, Int. J. Med. Microbiol. 303 (2013) 475–483, https://doi.org/10.1016/j.ijmm.2013.06.009.

[36] S. Bista, U.T. Shrestha, B. Dhungel, P. Koirala, T.R. Gompo, N. Shrestha, N. Adhikari, D.R. Joshi, M.R. Banjara, B. Adhikari, K.R. Rijal, P. Ghimire, Detection of plasmid-mediated colistin resistant mcr-1 gene in escherichia coli isolated from infected chicken livers in Nepal, Animals 10 (2020) 1–13, https://doi.org/10.3390/ani10112060.

[37] S. Yaghoubi, A.O. Zekiy, M. Krutova, M. Gholami, E. Kouhsari, M. Sholeh, Z. Ghafouri, F. Maleki, Tigecycline antibacterial activity, clinical effectiveness, and mechanisms and epidemiology of resistance: narrative review, Eur. J. Clin. Microbiol. Infect. Dis. 41 (2022) 1003–1022, https://doi.org/10.1007/s10096-020-04121-1.

[38] J. Sun, C. Chen, C. Cui, Y. Zhang, X. Liu, Z. Cui, Y. Feng, L. Fang, X. Lian, R. Zhang, Y. Tang, K. Zhang, H. Liu, Z. Zhuang, S. Zhou, N. Lv, H. Du, B. Huang, F. Yu, B. Mathema, B.N. Kreiswirth, X. Liao, L. Chen, Y. Liu, C. Unit, C. Unit, H. Municipal, C. Hospital, S.P. Hospital, HHS Public Access 4 (2019) 1457–1464, https://doi.org/10.1038/s41564-019-0496-4.Plasmid-encoded.

[39] M.J. Khong, A.M. Snyder, A.K. Magnaterra, M.M. Young, N.L. Barbieri, S.L. Weimer, Antimicrobial resistance profile of Escherichia coli isolated from poultry litter, Poult. Sci. 102 (2023) 102305, https://doi.org/10.1016/j.psj.2022.102305.

[40] Y. Shen, R. Zhang, S. Schwarz, C. Wu, J. Shen, T.R. Walsh, Y. Wang, Farm animals and aquaculture: significant reservoirs of mobile colistin resistance genes, Environ. Microbiol. 22 (2020) 2469–2484, https://doi.org/10.1111/1462-2920.14961.

[41] L. Xu, F. Wan, H. Fu, B. Tang, Z. Ruan, Y. Xiao, Q. Luo, Emergence of colistin resistance gene mcr - 10 in enterobacterales isolates recovered from fecal samples of chickens, slaughterhouse workers, and a nearby resident, Microbiol. Spectr. 10 (2022) 1–10, https://doi.org/10.1128/spectrum.00418-22.

[42] C. Wang, Y. Feng, L. Liu, L. Wei, M. Kang, Z. Zong, Identification of Novel Mobile Colistin Resistance Gene Mcr-10, 2020, p. 1751, https://doi.org/10.1080/22221751.2020.1732231.

[43] W. Liao, Y. Cui, J. Quan, D. Zhao, X. Han, Q. Shi, Q. Wang, Y. Jiang, X. Du, X. Li, Y. Yu, High prevalence of colistin resistance and mcr-9/10 genes in Enterobacter spp. in a tertiary hospital over a decade, Int. J. Antimicrob. Agents 59 (2022), https://doi.org/10.1016/j.ijantimicag.2022.106573.

[44] Y.H. Tartor, N.K. Abd El-Aziz, R.M.A. Gharieb, H.M. El Damaty, S. Enany, E.A. Soliman, S.S. Abdellatif, A.S.A. Attia, M.M. Bahnass, Y.A. El-Shazly, M. Elbediwi, H. Ramadan, Whole-genome sequencing of gram-negative bacteria isolated from bovine mastitis and raw milk: the first emergence of colistin mcr-10 and fosfomycin fosA5 resistance genes in Klebsiella pneumoniae in Middle East, Front. Microbiol. 12 (2021) 3596, https://doi.org/10.3389/FMICB.2021.770813/BIBTEX.

[45] F. Ma, S. Xu, Z. Tang, Z. Li, L. Zhang, Use of antimicrobials in food animals and impact of transmission of antimicrobial resistance on humans, Biosaf. Heal. 3 (2021) 32–38, https://doi.org/10.1016/j.bsheal.2020.09.004.

[46] C.J. Reid, M.L. Cummins, S. Börjesson, M.S.M. Brouwer, H. Hasman, A.M. Hammerum, L. Roer, S. Hess, T. Berendonk, K. Nešporová, M. Haenni, J.Y. Madec, A. Bethe, G.B. Michael, A.K. Schink, S. Schwarz, M. Dolejska, S.P. Djordjevic, A role for ColV plasmids in the evolution of pathogenic Escherichia coli ST58, Nat. Commun. 13 (2022) 1–15, https://doi.org/10.1038/s41467-022-28342-4.

[47] X. Zhuge, M. Jiang, F. Tang, Y. Sun, Y. Ji, F. Xue, J. Ren, W. Zhu, J. Dai, Avian-source mcr-1-positive Escherichia coli is phylogenetically diverse and shares virulence characteristics with *E. coli* causing human extra-intestinal infections, Vet. Microbiol. 239 (2019) 108483, https://doi.org/10.1016/J.VETMIC.2019.108483.

[48] M. Karlsson, J.D. Lutgring, U. Ansari, A. Lawsin, V. Albrecht, G. McAllister, J. Daniels, D. Lonsway, S. McKay, Z. Beldavs, C. Bower, G. Dumyati, A. Gross, J. Jacob, S. Janelle, M.A. Kainer, R. Lynfield, E.C. Phipps, K. Schutz, L. Wilson, M.L. Witwer, S.N. Bulens, M.S. Walters, N. Duffy, A.J. Kallen, C.A. Elkins, J. K. Rasheed, Molecular characterization of carbapenem-resistant enterobacterales collected in the United States, microb, Drug Resist 28 (2022) 389–397, https://doi.org/10.1089/MDR.2021.0106/ASSET/IMAGES/LARGE/MDR.2021.0106_FIGURE1.JPEG.

[49] P. Nordmann, L. Poirel, Epidemiology and diagnostics of carbapenem resistance in gram-negative bacteria, Clin. Infect. Dis. 69 (2019) S521–S528, https://doi.org/10.1093/CID/CIZ824.

[50] I. Ahmad Samreen, H.A. Malak, H.H. Abulreesh, Environmental antimicrobial resistance and its drivers: a potential threat to public health, J. Glob. Antimicrob. Resist. 27 (2021) 101–111, https://doi.org/10.1016/j.jgar.2021.08.001.

[51] Y. Jung, A.C.S. Porto-Fett, B.A. Shoyer, E. Henry, L.E. Shane, M. Osoria, J.B. Luchansky, Prevalence, levels, and viability of Salmonella in and on raw chicken livers, J. Food Prot. 82 (2019) 834–843, https://doi.org/10.4315/0362-028X.JFP-18-430.

[52] Y. Jung, A.C.S. Porto-Fett, B.A. Shoyer, E. Henry, L.E. Shane, M. Osoria, J.B. Luchansky, Prevalence, levels, and viability of Salmonella in and on raw chicken livers, J. Food Prot. 82 (2019) 834–843, https://doi.org/10.4315/0362-028X.JFP-18-430.

[53] G.D. Ritter, G.R. Acuff, G. Bergeron, M.W. Bourassa, B.J. Chapman, J.S. Dickson, K. Opengart, M.J. Salois, R.S. Singer, C. Storrs, Antimicrobial-resistant bacterial infections from foods of animal origin: understanding and effectively communicating to consumers, Ann. N. Y. Acad. Sci. 1441 (2019) 40–49, https://doi.org/10.1111/nyas.14091.

[54] S. Nacer, F.Z. El Ftouhy, S. Derqaoui, M. Khayli, S. Nassik, M. Lkhider, Prevalence and antibiotic resistance of Salmonella spp. and Staphylococcus aureus isolated from broiler chicken meat in modern and traditional slaughterhouses of Morocco, World's Vet. J. (WVJ) 12 (2022) 430–439, https://doi.org/10.54203/scil.2022.wvj53.

[55] F.M. Coura, S.A. Diniz, M.X. Silva, T.L.M. Arcebismo, S. Minharro, A.C.F. Feitosa, A.P. Lage, T. Knöbl, J.M.S. Mussi, M.B. Heinemann, Phylogenetic group of Escherichia coli isolates from broilers in Brazilian poultry slaughterhouse, Sci. World J. 2017 (2017), https://doi.org/10.1155/2017/5898701.

[56] M.M. Rahman, A. Husna, H.A. Elshabrawy, J. Alam, N.Y. Runa, A.T.M. Badruzzaman, N.A. Banu, M. Al Mamun, B. Paul, S. Das, M.M. Rahman, A.T.M. Mahbub-E-Elahi, A.S. Khairalla, H.M. Ashour, Isolation and molecular characterization of multidrug-resistant Escherichia coli from chicken meat, Sci. Rep. 10 (2020) 1–11, https://doi.org/10.1038/s41598-020-78367-2.

[57] Z. Halfaoui, N.M. Menoueri, L.M. Bendali, Serogrouping and antibiotic resistance of Escherichia coli isolated from broiler chicken with colibacillosis in center of Algeria, Vet. World 10 (2017) 830–835, https://doi.org/10.14202/vetworld.2017.830-835.

[58] S. Najafi, M. Rahimi, Z. Nikousefat, Extra-intestinal pathogenic Escherichia coli from human and avian origin: detection of the most common virulence-encoding genes, Vet. Res. Forum 10 (2019) 43, https://doi.org/10.30466/VRF.2019.34307.

[59] H. St-, F. Uropathogen, P. Keim, J.R. Johnson, B. Price, crossm, (n.d.). https://journals.asm.org/doi/10.1128/mbio.00470-18#:~:text=ST131-H22isjustone,attributabletofoodanimalE.

[60] Y. Bin Kim, M.Y. Yoon, J.S. Ha, K.W. Seo, E.B. Noh, S.H. Son, Y.J. Lee, Molecular characterization of avian pathogenic Escherichia coli from broiler chickens with colibacillosis, Poult. Sci. 99 (2020) 1088–1095, https://doi.org/10.1016/j.psj.2019.10.047.

[61] S.H. Cho, J. Kim, K.H. Oh, J.K. Hu, J. Seo, S.S. Oh, M.J. Hur, Y.H. Choi, S.K. Youn, G.T. Chung, Y.J. Choe, Outbreak of enterotoxigenic Escherichia coli O169 enteritis in schoolchildren associated with consumption of kimchi, Republic of Korea, 2012, Epidemiol. Infect. 142 (2014) 616–623, https://doi.org/10.1017/S0950268813001477.

[62] F.M. Aarestrup, L. Domínguez, H. Hasman, J.F. Fernández-Garayzábal, M. Concepción Porrero, A.I. Vela, Clonal diversity of Staphylococcus aureus originating from the small ruminants goats and sheep, Vet. Microbiol. 156 (2011) 157–161, https://doi.org/10.1016/j.vetmic.2011.10.015.

[63] N.M. Mitchell, J.R. Johnson, B. Johnston, R. Curtiss, M. Mellata, Zoonotic potential of Escherichia coli isolates from retail chicken meat products and eggs, Appl. Environ. Microbiol. 81 (2015) 1177–1187, https://doi.org/10.1128/AEM.03524-14.

[64] M.L. Devane, E. Moriarty, L. Weaver, A. Cookson, B. Gilpin, Fecal indicator bacteria from environmental sources; strategies for identification to improve water quality monitoring, Water Res. 185 (2020) 116204, https://doi.org/10.1016/J.WATRES.2020.116204.

[65] A. Goudarztalejerdi, A. Mohammadzadeh, S.V. Najafi, F. Nargesi, S. Joudari, Serogrouping, phylotyping, and virulence genotyping of commensal and avian pathogenic Escherichia coli isolated from broilers in Hamedan, Iran, Comp. Immunol. Microbiol. Infect. Dis. 73 (2020) 101558, https://doi.org/10.1016/j.cimid.2020.101558.